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Sero-Epidemiological Assessment and Molecular Analysis of Crimean-Congo Hemorrhagic Fever in Small Ruminants in Maysan Governorate, Southern Iraq

Article Info.

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Abstract

Crimean-Congo hemorrhagic fever (CCHF) is a severe zoonotic disease in which ticks serve as both the primary vector and natural reservoir for the virus. Small ruminants are considered amplifying hosts and a potential source of human infection, making them a relevant model for epidemiological studies. This study represents the first investigation of CCHF in Maysan Governorate, southern Iraq. A total of 174 serum samples (98 sheep and 76 goats) were analyzed using the enzyme-linked immunosorbent assay (ELISA) technique. The overall seroprevalence rate was 31.6%, with 34.7% in sheep and 27.6% in goats. Regional differences were observed, with the highest seropositivity in Al-Kahla (36.6%), followed by Al-Maimouna and Qalat Salih (33.3% each), Al-Amara (32.5%), Al-Majar Al-Kabir (29.5%), and the lowest in Ali Al-Gharbi (23.3%). A statistically significant association ($P < 0.05$) was found between seroprevalence and animal age, whereas no significant difference was observed based on sex ($P \geq 0.05$). In parallel, 810 ticks were collected from the same animals and grouped into 41 pools according to collection sites. Real-time PCR analysis revealed that 85% (35/41) of tick pools contained detectable CCHFV RNA, with Ct values ≤ 40 in most cases. These findings highlight a high level of viral circulation among tick populations in the study area and underscore their critical role as active vectors of CCHFV.

Keywords: zoonotic, CCHF, Tick-borne disease, ELISA, Nairoviridae.

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Introduction

Crimean-Congo hemorrhagic fever (CCHF) is a tick-borne zoonotic viral disease with a wide geographical distribution, spanning Africa, Eastern Europe, Asia, and the Middle East. The causative agent, the Crimean-Congo hemorrhagic fever virus (CCHFV), belongs to the Nairoviridae family (1, 2).While animals such as sheep, goats, and cattle are asymptomatic carriers, CCHFV causes severe illness in humans, characterized by fever, hemorrhagic manifestations, and multi-organ failure, with case fatality rates ranging from 5% to 60% (3). Due to the absence of effective antiviral treatments or vaccines, CCHF remains a significant public health threat in endemic regions (4).

The virus transmits from animals to humans when infected ticks (especially from the *Hyalomma spp*.) are crushed with a wound in the host's skin or through the tick's bite. In addition to tick vectors, the bodily secretions and blood of infected patients or animals may also serve as sources of infection(5).

In Iraq, CCHF has been considered endemic since the first recorded outbreak in 1979 (6). In recent years, Iraq has witnessed a resurgence of cases with an increasing trend in morbidity and mortality rates (7). In 2022 alone, Iraq reported 1,112 suspected cases, 295 of which were laboratory-confirmed, with 53 fatalities (8, 9).

During the year 2023, a noticeable increase in the number of infections was recorded during the Eid al-Adha period, with 118 cases reported during the Eid compared to 63 cases before the holiday and 23 cases after the holiday. The increase in cases was closely linked to the rise in the rate of animal slaughter during the Eid days (10). Although several sero-epidemiological studies have been conducted in different Iraqi governorates, there remains a paucity of data regarding the circulation of CCHFV in livestock in Maysan Governorate, a region with active livestock trade and high tick abundance.Without baseline data on CCHFV circulation in small ruminants in Maysan, the assessment of zoonotic risk and the planning of effective control strategies remain inadequate. Since there was no information about Crimean-Congo hemorrhagic fever in animals, this study was conducted to investigate the seroprevalence and molecular detection of CCHF in sheep and goats in Maysan Governorate, southern Iraq.

Materials and Methods

Animals and Area of Study

The present study comprised 174 animals, including 98 sheep and 76 goats (44 males and 130 females) in different age groups. All the animals were infested with ticks. Data documentation included the type of animal, age, and gender. The study included various rural and urban areas in Maysan Province, southern Iraq, covering the regions of Al-Amarah, Al-Kahla, Al-Majar Al-Kabir, Al-Maimouna, Ali Al Gharbi, and Qalat Salih (Figure 1).

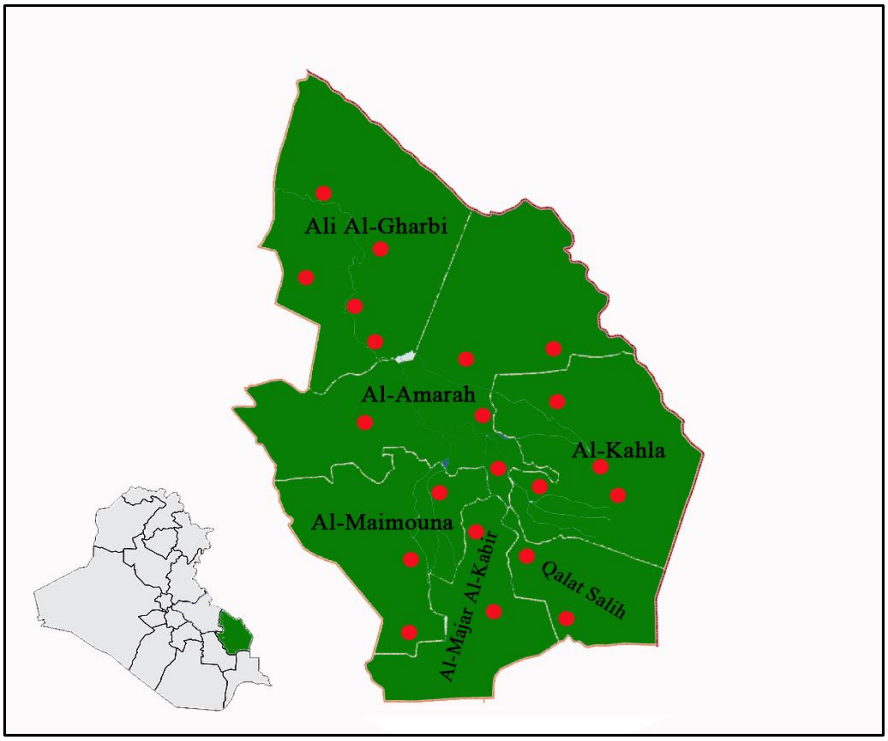

Figure 1: The locations of collected samples in Maysan Governorate

Sample Collection

In the present study, the blood samples were collected during the period between June and September of 2024. Five milliliters were drawn using a syringe from the jugular vein directly under sterile conditions, then placed in a tube containing activated gel to separate the serum. The serum was kept in the deep freeze.

Tick collection

Tick samples were obtained from the identical animals that provided blood samples. The samples were carefully extracted with surgical tweezers and subsequently put into a tube filled with 70% ethanol. The specimens were categorized into 41 pools according to the specific collecting locations. The specimens were sent to the Central Research Laboratory of the Veterinary Directorate to check for the CCHF virus using "Real-Time-Polymerase Chain Reaction" methods.

Tick Identification and Preparing

Each tick was rinsed with phosphate-buffered saline (PBS) and thereafter put into an Eppendorf tube containing stainless steel beads and 200-300 µL of PBS. The samples were then mashed using a tissue homogenizer for four minutes. After homogenization, the tubes were subjected to centrifugation, and the supernatant from each tube was collected for RNA extraction (11).

RNA Extraction

RNA extraction was performed according to the manufacturer's guidelines from QIAGEN (Germany), utilizing the QIAamp® Viral RNA Kit and following the prescribed protocols in the kit's instructions.

Real-Time Polymerase Chain Reaction

The kit utilized in this investigation was a real-time, one-step PCR assay, produced by Micrboss Hightech GmbH (Duisburg, Germany), for the detection of CCHFV. It employs two fluorogenic dyes: 6-carboxytetramethylrhodamine (quencher) and Yakima Yellow (VIC/HEX; reporter). According to the manufacturer's instructions, a Ct value of up to 40 is considered positive, while values between 41 and 45 are regarded as doubtful.

Serological analysis

In the present study, the ELISA kit ""ID Screen® CCHF Double Antigen Multi-species – CCHFDA"" was made by ID. Vit, Grabels, France, was employed. All serum samples were examined to detect IgG antibodies. The serological examination has been done in the Central Research Laboratory, Veterinary Directorate, Iraq. The test was conducted in line with the manufacturer's directions. The HumaReader HS ELISA microplate reader was used for the purpose of testing the microplates with an optical density of 450 nanometers.

Statistical analysis

The information gained in this study was examined using biostatistical methods, with significance established at ($P < 0.05$), employing "IBM SPSS Statistics version 27". Conducting the tests of Chi-square to ascertain the correlation between variables and infections. Additionally, 95% confidence intervals were calculated using the Wilson score method to estimate the precision of the observed proportions and associations.

Results

The present investigation revealed that 31.6% of the animals examined for CCHF illness in Maysan Governorate tested positive. Among 174 examined sheep and goats, 34.7% of sheep and 27.6% of goats tested positive. However, the difference between species was not statistically significant ($p \geq 0.05$) (Table 1).

Table 1: Seroprevalence of CCHF in small ruminants Maysan Governorate

Animal	No	CCHF +	95% confidence intervals
Sheep	98	34 (34.7%)	25.7% – 44.9%
Goats	76	21 (27.6%)	18.5% – 39.0%
Total	174	55 (31.6%)	24.9% - 39.1%

P value = 0.186

The areas within Maysan Governorate displayed differing rates of seroprevalence with no statistical significance ($p \geq 0.05$): Ali Al-Gharbi (23.3%), Al-Amarah (32.5%), Al-Maimouna (33.3%), Al-Kahla (36.6%), Al-Majar Al-Kabir (29.5%), and Qalat Salih (33.3%) (Table 2).

Table 2: Distribution of Seroprevalence of CCHF in small ruminants according to regions of Maysan Governorate.

Region	No	CCHF +	95% confidence intervals
Ali Al-Gharbi	30	7 (23.3%)	11.0% - 41.5%
Al-Amarah	40	13 (32.5%)	19.3% - 48.5%
Al-Maimouna	30	10 (33.3%)	18.0% - 52.0%
Al-Kahla	30	11 (36.6%)	20.5% - 55.7%
Al-Majar Al-Kabir	17	5 (29.5%)	12.8% - 54.9%
Qalat Salih	27	9 (33.3%)	17.2% - 53.4%

P value = 0.920

Additionally, the present investigation found that the seroprevalence of CCHF in the Maysan Governorate was statistically significantly higher ($p < 0.05$) in older animals than in younger ones (Table 3).

Table 3: Seroprevalence of CCHF in Maysan Governorate, according to age of animals.

Age	No	CCHF +	95% confidence intervals
Less than 1 year	45	4 (8.9%)	3.5% - 20.7%
1 – 2 years	36	11 (30.5%)	17.0% - 47.2%
2 – 3 years	56	23 (41.1%)	28.3% - 55.1%
3 – 4 years	20	10 (50%)	29.9% - 70.1%
4 years and more	17	7 (41.1%)	20.0% - 65.5%

P value = 0.00001

The findings of the current investigation indicate that no significant variation (P value ≥ 0.05) was observed in the CCHF seroprevalence based on sex.

In the current investigation, a total of 810 ticks were collected from sheep and goats in Maysan Governorate. RT-PCR was employed to detect CCHFV RNA in pooled tick samples. The results showed that 85% (35 out of 41) of the tested tick pools exhibited clear positive amplification, with Ct values ≤ 40. These samples typically demonstrated sharp amplification curves beginning around cycle 20, indicating a strong presence of viral RNA. Additionally, 10% (4/41) of the pools showed weak or marginal amplification at later cycles (Ct range: 41–45). Meanwhile, 5% (2/41)

of the pools were completely negative, with no detectable amplification signals, as shown in Figure 2.

Table 4: Seroprevalence of CCHF in small ruminants in Maysan Governorate based on sex.

Gender	No	CCHF +	95% confidence intervals
Male	44	16 (31.8%)	19.6% - 46.9%
Female	130	39 (30%)	22.8% - 38.4%
Total	174	55 (31.6%)	24.9% - 39.1%

P value = 0.433

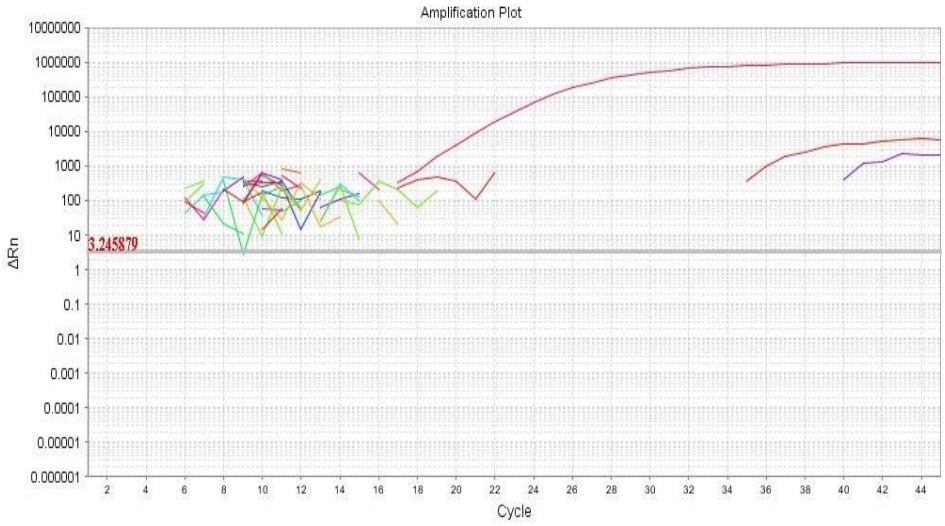

Figure 4.17: Graph displaying the results of RT-PCR

Discussion

Crimean-Congo hemorrhagic fever (CCHF) is a life-threatening viral disease that is endemic in many parts of the world, including Africa, Asia, Eastern Europe, and the Middle East (12). While infection in animals is typically subclinical or asymptomatic, CCHFV infection in humans can lead to severe illness characterized by high fever, hemorrhagic manifestations, and multi-organ failure, with case-fatality rates ranging from 5% to 60% (13, 14). The lack of effective vaccines or specific antiviral treatments remains a major challenge in the control and management of the disease (15). Sheep and goats work as amplifying hosts for the virus, and they are essential in the

transmission cycle (16). The present investigation showed that the overall seroprevalence of CCHF in Maysan governorate was 36.8% (55/174), the sheep rate was 34.7%, and the goats were 27.6%. This study serves as a preliminary evaluation of the status of disease in small ruminants in the Maysan governorate, southern Iraq.

Other research documented that in Basrah governorate, a study was carried out that showed the rate of infection in sheep was 76.9%, while in goats, it was 23.1% (17). Furthermore, another investigation conducted in Thi-Qar governorate revealed that the seropositive rate in sheep and goats was 52.9% and 35.5%, respectively (18).

The present study showed a difference in seroprevalence rate within the regions of collected samples depending on geographical distribution, as well as revealed a slight increase in the areas adjacent to the border of Iran, with no statistical significance. In Iraq and neighboring regions. Rising temperatures and extended summer seasons have been associated with increased tick activity and prolonged transmission periods; a study in Eastern Turkey (2012–2021) demonstrated clear seasonality in CCHF cases with peaks in temperature and humidity correlating with higher incidence ($p < 0.05$) (19).

Cross-border livestock trade between Iraq and endemic countries (Turkey and Iran) facilitates the movement of infected animals and ticks, raising the risk of introducing CCHFV into new areas (20).Inadequate tick-control strategies (limited use of effective acaricides) and low public awareness of protective measures contribute to persistent exposure risks (21). As well as occupational exposure, particularly among breeders, butchers, and slaughterhouse workers in close contact with animals, further increases the risk of human infection (22).In this investigation, older animals demonstrated a significantly higher seroprevalence of CCHF infection compared to younger animals ($P < 0.05$). This finding is consistent with previous studies conducted in Basrah and Thi-Qar governorates, where a similar age-related increase in seropositivity was observed (17, 18). The higher seroprevalence in older animals may be attributed to cumulative exposure to tick bites over time, increasing the likelihood of contact with CCHFV-infected ticks, particularly in endemic regions where tick infestation pressure remains high (23, 24).In the present study, no significant association was observed between gender and the seroprevalence of CCHF infection ($P \geq 0.05$). This finding is consistent with previous reports from Iraq and other endemic regions, which similarly found no gender-related differences in CCHF seropositivity (17, 18, 25). The lack of association suggests that biological sex does not play a role in susceptibility to infection. Instead, exposure to infected ticks remains the primary determinant of infection risk, which is influenced by factors such as animal management practices, environmental conditions, and the level of awareness and implementation of tick control measures (5, 25, 26).In this study, RT-PCR assay results from pooled tick samples in Maysan governorate showed a notably high positivity rate, with 85% of tested pools showing distinct amplification curves at Ct values ≤40. This strongly indicates widespread presence of CCHFV RNA among tick populations in these regions, highlighting the significant role of ticks, particularly those from the Hyalomma genus, as both

vectors and reservoirs for CCHFV in endemic and emerging areas (27, 28, 29).Such high rates of viral RNA detection suggest that the environmental burden of CCHFV is substantial, potentially increasing the risk of transmission to livestock and humans, especially in settings where agricultural activity and human-animal interaction overlap (29, 30).

Marginal positive results at high Ct values could reflect suboptimal sample preservation, resulting in partial RNA degradation, or an inherently low viral load in some tick populations. Factors such as environmental conditions, the time between collection and processing, and the methods used for RNA stabilization can critically influence the integrity of viral RNA and thus RT-PCR outcomes (31, 32).Therefore, conducting laboratory tests regularly to assess the epidemiological status of diseases affecting animals is of utmost importance, in addition to evaluating the severity and spread of ticks, developing control strategies, and taking immediate preventive measures, including selecting appropriate medications and ensuring their effectiveness, spraying barns, and dipping animals (30-32).

Conclusions

The findings of the study showed a high seroprevalence of Crimean-Congo hemorrhagic fever (CCHF) in small ruminants in Maysan Governorate, with an overall seropositivity rate of 31.6% and notable regional differences. There was a statistically significant relationship between infection rates and animal age, but no significant correlation with sex. Furthermore, no CCHFV RNA was found in the studied tick pools via RT-PCR, emphasizing the necessity for additional investigation to identify the circulating virus strain in Iraq. The report suggests raising public knowledge about the disease's danger through educational seminars and adopting effective tick vector control programs.

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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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التقييم المصلي الوبائي والتحليل الجزيئي لحمى القرم–الكونغو النزفية في المجترات الصغيرة في محافظة ميسان، جنوب العراق

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الخلاصة

تعد حمى القرم-الكونغو النزفية (CCHF) من الأمراض الخطيرة حيوانية المنشأ، حيث يُعد القراد الناقل الرئيسي والمستودع الطبيعي للفيروس. شهد العراق في السنوات الأخيرة ارتفاعاً ملحوظاً في عدد حالات الإصابة. وتُعد المجترات الصغيرة مضيفاً مضخماً للفيروس ومصدراً أساسياً لانتقال العدوى إلى الإنسان، مما يجعلها نموذجاً مناسباً لتقييم الوباء. تمثل الدراسة الحالية أول بحث يُجرى في محافظة ميسان حول حمى القرم-الكونغو النزفية، حيث شملت 174 عينة مصلية (98 من الأغنام و76 من الماعز)، تم فحصها باستخدام تقنية اختبار المناعة المرتبط بالإنزيم (ELISA). بلغ معدل الإيجابية المصلي الكلي 31.6%، بواقع 34.7% في الأغنام و27.6% في الماعز. كما أظهرت النتائج وجود تباين في معدلات الإصابة بين مختلف المناطق، حيث سجلت أعلى نسبة في قضاء الكحلاء (36.6%)، تلتها الميمونة وقلعة صالح (33.3% لكل منهما)، ثم العمارة (32.5%)، والمجر الكبير (29.5%)، بينما سُجلت أدنى نسبة في علي الغربي (23.3%). وكشفت الدراسة عن وجود فروق إحصائية معنوية ($P < 0.05$) في معدل الإصابة بين الحيوانات كبيرة السن مقارنة بالحيوانات الصغيرة. في المقابل، لم يكن هناك تأثير معنوي للجنس على معدل الإصابة ($P \geq 0.05$). ولم يُعثر على الحمض النووي الريبي لفيروس CCHF في عينات القراد المفحوصة باستخدام تقنية RT-PCR. يشير ارتفاع معدلات الإصابة إلى الحاجة المُلّحة لإجراء المزيد من الدراسات للتعرف على السلالات الفيروسية المنتشرة في العراق. كما توصي الدراسة بأهمية تنظيم ندوات إرشادية للتوعية بخطورة المرض، إلى جانب اعتماد برامج فعّالة لمكافحة نواقل المرض.

كلمات مفتاحية: مرض حيواني المنشأ، حمى القرم-الكونغو النزفية، مرض منقول بالقراد، اختبار المناعة المرتبط بالإنزيم، فايروسات النايرو.