



Incidence and transmission dynamics of Crimean-Congo Hemorrhagic Fever Virus (CCHFv) in slaughterhouse environments: ELISA based detection and risk assessment

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ABSTRACT

This study investigates Crimean-Congo Hemorrhagic Fever Virus (CCHFv) transmission dynamics in a slaughterhouse environment. A total of 409 blood samples from indigenous animals and 61 butchers were analyzed using an Indirect enzyme-linked immunosorbent assay (I-ELISA). Slaughtered animal seroprevalence was 19.3%, higher in sheep (21.9%) than goats (13.8%). Butcher seroprevalence was 3.3%, with reasonable knowledge of zoonotic disease transmission. Significant association between butcher and animal infections was found, but logistic regression results were inconclusive, suggesting other transmission factors. Further research is needed to understand transmission mechanisms and develop targeted prevention measures.

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Introduction

Crimean-Congo Hemorrhagic Fever (CCHF) is a significant zoonotic viral disease with global health implications. The causative agent, the Crimean-Congo Hemorrhagic Fever Virus (CCHFv), is a member of the Nairovirus genus within the Bunyaviridae family. It is known to induce severe health implications in humans while often resulting in minor or no symptoms in animals (Chinikar et al. 2008; Telmadarraiy et al. 2008).

CCHFv is notable for its expansive geographical range, making it one of the most significant tick-borne viruses globally, with periodic outbreaks underscoring its importance (Ergönül 2006). Originally identified in Crimea in 1944 and later in Africa by 1956 (Simpson et al. 1967; Elevli et al. 2010), the disease has sporadically

emerged across Asia, Africa, East Europe, and the Middle East. Iraq, in particular, has become an endemic hotspot for CCHF since its first recorded case in 1979 (Vescio et al. 2012; Messina et al. 2015; Aziz et al. 2016). The dramatic increase in CCHF cases in Iraq during the first half of 2022 highlights the escalating public health concern (Alhilfi et al. 2023). Ticks, especially those of the Hyalomma genus, are the primary vectors responsible for CCHFv transmission (Grech-Angelini et al. 2016; Gargili et al. 2017). They infect a wide range of animals including sheep, goats, and cattle, pivotal to the virus's transmission cycle. Infected animals, predominantly livestock, often present no discernible clinical symptoms but harbor a viremia of enough intensity to infect adult ticks (Noroozifar & Shahroosvand 2010). Notably, the slaughterhouse environment, with its close proximity to

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potentially infected livestock, could serve as a major catalyst for CCHFv transmission (Alavi-Naini et al. 2006). The major route of infection for humans is represented by the bites of infected ticks, but also by the exposure to the blood of infected wild or domestic animals. In endemic regions, cases of people acquiring the infection through the contact and consumption of raw fresh or under-cooked meat immediately after slaughtering have been described (da Silva et al. 2015; Fazlalipour et al. 2016). Human-to-human transmission through close contact or nosocomial infections has been documented as well (Garrison, Smith & Golden 2019).

For the prompt and accurate diagnosis of CCHF, various methods have been employed. One such critical diagnostic tool is the enzyme-linked immunosorbent assay (ELISA), which has been highlighted as a highly sensitive and specific technique for detecting CCHFv. ELISA, especially when using antigens derived from virus cultures, provides a reliable means to detect CCHFv, even in complex environments like slaughterhouses (Garcia et al. 2006).

Despite the advances in our understanding of CCHFv, a glaring gap exists in understanding its incidence and transmission dynamics within slaughterhouses. These settings, with their inherent practices and the potential for close contact with infected livestock, pose a significant risk for virus amplification and subsequent transmission. Given the potentially catastrophic ramifications of a widespread CCHF outbreak, it's paramount to continually assess and understand the risks, especially in vulnerable environments like slaughterhouses. This is further underscored by global movements and religious events that might increase livestock slaughter, making the dissemination of the virus more profound and challenging to control (Mohammed, Mostafa, Ahmad, Yahya & Tayib 2022).

Given the substantial public health risks associated with CCHFv, particularly in environments like slaughterhouses, this study aims to shed light on the incidence and transmission dynamics of CCHFv in these settings. Utilizing ELISA-based detection, our goal is to deliver a thorough risk assessment, paving the way for informed preventive and mitigation strategies.

Materials and Methods

Study Design and Setting

A cross-sectional study was conducted at the Zakho slaughterhouse. Blood samples were collected from local breed animals that were free from ticks. The sampled animals included sheep and goats. In addition to the animal samples, blood samples were also obtained from

the butchers working at the same slaughterhouse. This study design allowed for an evaluation of the seroprevalence of CCHFv antibodies in both animals and humans within the same environment.

Sample collection

Total of 409 blood samples were meticulously collected from the indigenous animals, comprising 279 sheep and 130 goats. These animals were specifically chosen for their local breed characteristics and were verified to be free from ticks.

After obtaining informed consent from 61 butchers with minimum 6 work experience in the same slaughterhouse, a 5ml of blood with information were obtained from each participant, such as demographic characteristics, exposure to risk factors during work, usage of personal protective equipment, and their knowledge and attitude regarding zoonotic diseases. The blood samples were transferred to the Duhok central public health laboratory for serum separation, and kept in -20 °C for analysis.

iELISA-based Detection

For the detection of antibodies against CCHFV in the serum samples, we utilized the Indirect enzyme-linked immunosorbent assay (I-ELISA) Double antigen ELISA kit (IDVet, France). Adhering strictly to the manufacturer's guidelines, the absorbance was recorded at 450 nm using a Bio-Tek EL-800 microplate reader. The distinction between positive and negative samples was achieved using the manufacturer's recommended formula:

$$SP\% = (\text{mean OD of positive control} / \text{mean OD of sample}) \times 100.$$

Based on these calculations, samples presenting an SP% of 30 or less were classified as negative. In contrast, samples with an SP% greater than 30 were designated as positive.

Data Collection and Surveillance

Within the confines of the slaughterhouse, interviews were conducted with the butchers to assess their level of awareness about CCHFv transmission dynamics. Particular attention was given to their choice of clothing and their understanding of the pathways through which the virus could potentially spread.

Statistical Analysis

For the slaughtered animals, seroprevalence of CCHFv antibodies was ascertained by dividing the number of positive results by the total sample size (279 sheep and 130 goats), and results were presented as percentages. Chi-square statistics, using IBM SPSS Statistics version 25, were employed to determine associations between

infection and other study variables. A significance level was set at a P value of 0.05.

For the butchers, the seroprevalence rate was computed based on the number of positive tests out of the 61 samples. Pearson's Chi-squared was used to examine the relationship between the number of infected slaughtered animals and infected butchers.

To exploring Butcher Infection Risk Factors, a logistic regression model was implemented.

All results were presented with 95% confidence intervals. All tests ensured adherence to their respective assumptions for validity.

Results

Seroprevalence in Slaughtered Animals

Of the 409 animals tested in the Zakho slaughterhouse, 79 returned positive results for CCHFv antibodies (19.3%). Breaking down by species, 61 of the 279 sheep (21.9%) and 18 of the 130 goats (13.8%) tested positive. This difference in seroprevalence between the two species was statistically notable with a P value of 0.05

Seroprevalence Among Butchers

The study involving 61 butchers, the seroprevalence of CCHFv antibodies indicated that only 2 individuals tested positive, reflecting a 3.3% seroprevalence rate. The majority of these butchers were uninfected, with an average infection status score of 1.0328 (SD 0.17956 [95% CI 1.0085, 1.0571]). When evaluating their understanding of zoonotic transmission, the butchers presented an average knowledge score of 1.2623 (SD 0.44353 [95% CI 1.1835, 1.3411]), hinting at a predominant basic understanding of the topic. Age-wise, the cohort had an average age of 31.30 years (SD 9.289 [95% CI 29.1456, 33.4544]). Regarding the use of personal protective equipment (PPE), the mean score was 1.3934 (SD 0.49257 [95% CI 1.3099, 1.4769]), suggesting a balanced distribution between users and non-users, with a slight inclination towards non-usage. Overall, while the majority of butchers remained uninfected, the seroprevalence rate emphasizes the importance of consistent monitoring and adherence to protective measures.

Relationship Between Infection Rates in Slaughtered Animals and Butchers

Based on the Pearson's Chi-squared test with Yates' continuity correction, the results show a significant association between the infection status of butchers and that of animals in the slaughterhouse (X-squared = 8.4795, df = 1, p-value = 0.003592). This suggests that

the likelihood of butchers being infected might depend on the infection status of the animals they handle

Exploring Butcher Infection Risk Factors

In our attempt to decipher the correlation between the infection statuses of animals and butchers, we utilized the logistic regression modeling approach. Analyzing the data, the 'Animals' variable presented a coefficient estimate of -0.03613 (SE = 0.40521), a Z-value of -0.089, and a p-value of 0.929 ($p > 0.05$), indicating that the infection status in animals might not be a reliable indicator for the infection status in butchers.

Discussion

Crimean-Congo Hemorrhagic Fever (CCHF) is a viral disease that can be transmitted from animals to humans. Infected animals often show no symptoms, but the virus poses a significant public health threat. Livestock like cattle, sheep, and goats that carry the virus in their blood can serve as sources of transmission to humans and other animals (Kagunyu & Wanjohi 2014). Laboratory testing has played a crucial role in identifying CCHFV antibodies in domestic animals. This testing helps us understand where the virus is circulating, assess the risk to animal herders and other people, and gather initial evidence of its presence in specific areas (Ceianu, Panculescu-Gatej, Coudrier & Bouloy 2012).

In light of our study's aim to comprehensively investigate the incidence and transmission dynamics of Crimean-Congo Hemorrhagic Fever Virus (CCHFv) within slaughterhouse environments, our findings have unveiled critical insights into the seroprevalence of CCHFv antibodies in both animals and human butchers working within the same setting. The overall seroprevalence of 19.3% among slaughtered animals at the Zakho slaughterhouse was notably higher than expected, raising concerns about the potential risk of CCHFv transmission within this environment. Furthermore, stratifying the data by species revealed a substantial difference in seroprevalence, with sheep exhibiting a significantly higher rate (21.9%) compared to goats (13.8%). These disparities in infection rates between animal species may be attributed to varying levels of susceptibility or exposure to ticks, the primary vectors of CCHFv. Such findings are consistent with previous studies conducted in other regions. For instance, studies in Iran and Iraq have reported a wide range of prevalence rates among sheep (ranging from 12.6% to 77.5%) and goats (ranging from 6.25% to 40%), indicating substantial variability in CCHFv seroprevalence (Saidi, Casals & Faghieh 1975; Telmadarraiy et al. 2010; Altaliby, Esmaeel & Hussain 2023). These variations in prevalence rates across

different regions may be attributed to the endemic nature of CCHF, the diversity of tick populations, and variances in laboratory testing methods used for antibody detection. Nonetheless, the heightened seroprevalence observed in our study highlights the pressing need for targeted prevention and control measures within the Zakho slaughterhouse environment.

In contrast to the animals, the seroprevalence of CCHFv antibodies among butchers was notably lower, standing at 3.3%. This finding is encouraging, as it suggests that the majority of butchers remained uninfected despite their close proximity to potentially infected animals and ticks within the slaughterhouse. The average infection status score of 1.0328 further corroborates this, indicating a predominantly uninfected cohort of butchers. Additionally, the butchers exhibited a reasonable level of knowledge (average knowledge score of 1.2623) regarding zoonotic disease transmission, which may have contributed to their lower infection rates. For instance, a study in Sistan and Baluchestan province, Iran, reported a much higher seroprevalence rate of 16.49% among butchers and slaughterhouse workers, emphasizing the high-risk nature of these occupations for CCHFv transmission (Mostafavi et al. 2017). Furthermore, studies in Iran have reported varying seroprevalence rates among similar occupational groups, such as 5% in Isfahan province (Shabani et al. 2018), 7.4% in Yasuj city (Hadinia, Ilami, Mousavizadeh, Akbartabar Tori & Khosravani 2012), and 14.8% in northeastern Iran (North Khorasan, Razavi Khorasan, and south Khorasan provinces)(Chinikar et al. 2012). Similarly, a survey conducted in Turkey identified a seroprevalence rate of 16.6% among individuals with a history of livestock slaughtering (Gunes et al. 2009).

The significant association between the infection status of butchers and that of animals in the slaughterhouse, as revealed by Pearson's Chi-squared test with Yates' continuity correction, indeed suggests a potential link between animal and human infections. This finding aligns with the concept that animals can serve as reservoirs or amplifying hosts for CCHFv, and it underscores the need for increased vigilance in managing the risks associated with such environments. However, the results of our logistic regression analysis, aimed at exploring the correlation between animal and butcher infection statuses, presented a different perspective. The 'Animals' variable was found to be an unreliable predictor of butchers' infection status, as indicated by the non-significant coefficient estimate, Z-value, and p-value.

These contrasting findings may be attributed to several factors and warrant further discussion. First, the presence of antibodies in animals does not necessarily imply active viral shedding, and the mere coexistence of infected

animals with butchers may not directly lead to human infections. Other routes of transmission, such as direct contact with infected animal blood or ticks, might play a more substantial role in human infections. Additionally, variations in personal protective equipment (PPE) usage among butchers could influence their risk of infection. It is plausible that individuals who use adequate PPE are better protected, regardless of the infection status of the animals they handle.

Research limitations

It is essential to acknowledge the limitations of our study. While we identified a significant association between animal and butcher infection rates, our logistic regression analysis did not provide a clear understanding of the underlying factors. Future research should delve deeper into the potential mechanisms of transmission, considering variables beyond the 'Animals' variable. Additionally, a larger sample size and a more comprehensive assessment of butcher practices and tick exposure could provide more insights.

Conclusion

Our study contributes to the growing body of evidence on CCHFv transmission in slaughterhouse environments. While the association between animal and human infections is significant, our logistic regression analysis suggests that additional factors may influence human infections. Further research is needed to comprehensively understand the intricate pathways of transmission and to develop targeted prevention strategies for at-risk populations.

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Ethical Statement

For the collection of samples from animals, this study was fully compliant with the ethical requirements for animal welfare. Regarding human samples, the study protocol was approved by the Ethical Committee of the Directorate General of Health in Duhok Governorate. All procedures were conducted in accordance with the approved guidelines, and all necessary precautions and

considerations were taken to ensure the welfare and rights of both human and animal subjects.

Declaration of competing interest

All authors of this manuscript declare there are no financial and personal relationships with other people or organizations that could inappropriately influence the intellectual work presented in this paper.

Conflict of interest

The authors declare that they have no conflict of interest. All authors have approved the manuscript for submission.

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