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Seroprevalence of Crimean Congo Hemorrhagic Fever in cows by ELISA in Mosul city

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Abstract

Crimean-Congo hemorrhagic fever (CCHF) is described as a tick-borne viral zoonosis highly prevalent in Africa, Asia, Russia and the Balkans within the distribution range of ticks that belong to the genus *Hyalomma*. This research aimed to verify the seroprevalence of CCHF in cows employing Indirect enzyme-linked immunosorbent assay (I-ELISA) in Mosul city / Iraq, to examine some epidemiological risk factors related to the incidence of CCHF. From October 2019 - September 2020, one hundred eighty-four blood samples were taken from 3-8-year-old cattle of both sexes, from several management systems and origins, from various parts in Mosul city, 10 ml of blood was taken from the jugular vein in test tubes without anticoagulant to obtain the sera for I-ELISA test, while epidemiological data were obtained by interviewing the farm-owners. I-ELISA was applied to detect the antibodies of CCHF in the serum. The overall seroprevalence of CCHF in cows was 40/184 (21.7%). As for the risk factors associated with increased seroprevalence of the disease, an increase in seroprevalence rates was observed at ages 6, 7 and 8 years 30.5%, among females 26.3% and imported animals 27.5%. which showed that the serological prevalence in indoor feeding and outdoor feeding, was not significantly different ($p<0.05$). This study concluded that there is a higher seroprevalence of CCHF in Mosul city along with many risk factors related to its incidence.

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Introduction

Crimean-Congo Hemorrhagic Fever (CCHF) is associated with the Nairovirus genus of the Bunyaviridae family. It is responsible for this zoonotic disease in Africa, Asia, Europe and the Middle East (1). The prevalence of CCHF is simultaneous with the prevalence of the major vector ticks of the genus *Hyalomma*. The CCHF is asymptomatic in infected cattle whereas, inhuman the disease is more prevalent as high as 80% and more obvious clinical manifestations (1-3). The disease is transmitted to humans by tick bites, contact with ticks, being exposed to blood or tissues of viremia animals, or actual contact with blood and body fluids of diseased cattle (4-6). Ticks are the main vectors for transmitting CCHF; however, secondary cases resulting from nosocomial contamination and direct contamination have been often found among health care and slaughterhouse personnel (7). The CCHF was initially detected in the Crimean region of Russia in the 1940s. Nonetheless, but has since spread globally to places like Africa, Europe and Asia (8). It is worsened by the asymptomatic infection or mild fever that the disease causes in domestic and wild vertebrates with a detectable viremia of up to 14 days (9,10). In the same way, the diseased ostriches show merely low and short-lived viremia without any clinical manifestations (11). As such, new infections in animals are usually undiagnosed and approaches like polymerase chain reaction (PCR), isolating the virus in cell culture, and IgM detection using enzyme-linked immunosorbent assay (ELISA) are mostly applied in

diagnosing CCHF in humans or when an animal is required to be considered CCHF-free. Should there be any chance or inkling that diagnostic samples may be tainted with CCHF, careful handling according to appropriate biosafety standards and anyone handling the samples must know about the potential risk and use personal protective equipment (PPE) to prevent human infections (12).

The gravity of CCHF in humans emphasizes the effect of this zoonotic illness on public health. Even though CCHF does not economically affect the production of livestock animals, the serological screening of animal serum samples for CCHF- Specific antibodies are of great importance Since prevalence in animals often indicates the presence of circulating viruses, such research helps to identify high-risk circumstances for human infection, so slaughterhouse personnel, veterinarians, stockmen and others engaged in the livestock sector must have awareness of the disease (12).

As a result of the inadequate information about the seroprevalence of CCHF in Mosul city, Iraq, this research endeavoured to establish the seroprevalence of the disease in cows and to investigate if there are some epidemiological risk factors related with the disease.

Material and methods

Animals and Samples Collection

From October 2019 to September 2020 the study was conducted on 184 cows, three to eight years old, from different farms in Mosul city, north Iraq.

Information regarding age, gender, origin, and husbandry system was recorded during samples collection. Serum was separated from the tube without anti-coagulants after obtaining 10 ml of blood samples from each individual cow via jugular vein puncture using 18G needle into sterile vacutainers® the serum aspirated carefully by pipette into dry, sterile and labeled test tubes, storage at -20ºC before they are used (13,14) for detection of CCHF antibodies by I-ELISA.

Indirect Enzyme-Linked Immunosorbent Assay (I-ELISA)

Double antigen ELISA for the detection of antibodies against the CCHF in serum or plasma from cattle, sheep, goats or other susceptible species was performed according to the techniques described by the manufacturer (ID Vet, France). The absorbance was calculated at 450 nm utilizing BioTek EL-800 micro plate reader.

Optical density (OD) values were acquired from the readings and uploaded to a Microsoft Excel spread-sheet. Samples presenting (SP%) was calculated following the manufacturer's guidelines and samples were designated as positive or negative. When both duplicates showed an SP% > 30 an animal was designated as positive. %SP= mean OD sample/ mean OD positive control*100. Were SP≤30 = negative and SP>30 = positive.

Statistical analysis

Statistical analysis was performed employing computed 2*2 tables in Epi-InfoTM® 7 software version 7 (15).

Results

In this study, the general seroprevalence of CCHF was obtained from the outcomes of the I-ELISA analysis. Altogether, 40 out of the 184 sampled animals revealed positive results indicating seroprevalence of 21.7% (Table 1). This study found that the seroprevalence of CCHF was substantially higher in cattle aged 6-8 years' Relative risk (R.R)2 times, Confidence interval (C.I) 1.011-6.116 in comparison with other age ranges (Table 2). The present research shows that the seroprevalence was considerably raised in female than in the males (P<0.014) RR: 1.8times, CI: 0.938-4.7152. The seroprevalence was also substantially higher among imported cattle (RR: 2.06 times, CI: 1.123-5.424) in comparison with local animals (P<0.0000) (Table 2). The investigation also showed no significant difference in the seroprevalence between indoor feeding 21.3% and outdoor feeding 22.11% cattle (Table 2). Besides, this research indicated that the seroprevalence was substantially elevated in Spring and Summer 30% and 28.2%, respectively (RR: 2.5 and 2.4 times, respectively in comparison with Winter and Autumn (Table 3).

Table 1: The seroprevalence of CCHF in cows in Mosul city

Anti CCHF VIRUS IgG (Antibody) in cows	
Positive N (%)	40 (21.7)
negative N (%)	144(78.2)
Total N (%)	184(100)

Discussion

CCHF is described as a tick-borne viral Zoonosis widely distributed in Africa, Asia, Russia and the Balkans within the distribution range of ticks belonging to the genus *Hyalomma* (16,17). This is a pioneering seroprevalence investigation of CCHF in Mosul city. It determined that the general seroprevalence rate of the disease in Mosul is 21.7% by using I-ELISA in serum. A reduced and/or approximately similar seroprevalence was indicated in previous researches of the CCHF in Iraq and elsewhere. The seroprevalence was 37% in cattle employing I-ELISA in Basrah (18). In Iran, it was 25 % (19); in Iran Isfahan province, 20% (20); in Turkey maramara region, it was 13% (21); in the Sultanate of Oman, it was 17.5% (22); in north Kordufan state, Sudan, 7% (23); in Nigeria, it was 24% (24). The seroprevalence of the CCHF may differ from country to country and even in different areas within a country, probably attributable to the diversity of samples, incidence of competent vectors, management practices, climatic variations, the effectiveness of control initiatives, the extent of the cattle trading sector,

Table 2: Relative risk factors of cattle related to the seroprevalence rate of the CCHF in cows

Factors	No. tested cattle	I-ELISA technique			
		No of positive (%)	RR	95% CI	P
Age					
3 years	60	9 (15%)[a]	1		
4-5years	65	13 (20%)[a]	1.3	0.557-3.603	0.40
6 -8 years	59	18 (30.5%)[b]	2	1.011-6.116	0.040
Gender					
Male	70	10 (14.2%)[a]	1		
Female	114	30 (26.3%)[b]	1.8	0.938-4.715	0.054
Origin					
Native	75	10 (13.3%)[a]	1		
Imported	109	30 (27.5%)[b]	2.06	1.123-5.424	0.021
Husbandry system					
Outdoor feeding	95	21 (22.1%) [a]	1		
Indoor feeding	89	19 (21.3%)[a]	1.03	0.518-2.108	0.900

Values significantly different P<0.05 labeled with different letters a and b.

Table 3: Relative risk of seasonal factors related to the seroprevalence rate of the CCHF in cows

Factors	No. cattle tested	I-ELISA test			
		No. positive (%)	RR	95% CI	P
Autumn (Oct.-Nov.-Des.)	43	5 (11.6%)[a]	1		
Winter (Jan-Feb- March)	45	7 (15.5%)[a]	1.3	0.459-3.89	0.5
Summer (July-Aug- Sept.)	46	13 (28.2%)[b]	2.4	1.02-1.65	0.04
Spring (April-May- Jun)	50	15 (30%)[b]	2.5	1.0216-6.515	0.03

Values significantly different (P < 0.05) are labeled with different letters a, b and c.

population size, biosecurity, and uncontrolled animal movement (25). The present investigation reported a significant variance in the seroprevalence of CCHF related to age, showing a higher seroprevalence rate in cattle aged 6-8 years old, which agreed with Shabani *et al.* (26). This may be attributed to the increase in exposure to ticks at older ages than at young ages and females rather than in males. The present study revealed a higher seroprevalence of CCHF in females, which agreed with Shabani *et al.* (26) and may be due to fact that older ages have a greater chance for exposure to ticks than younger ages and female than in male (27). The findings indicated that the seroprevalence was considerably increased among imported cattle compared to local, most likely because the majority of the cattle population in Mosul city are Iranian, Turkish and Syrian imports and these countries of origin are known to be CCHF-prevalent countries (26). It is also important to note that some animals were imported into Mosul city with no proper border controls or quarantine. The findings also indicated that the seroprevalence was non-significant among cattle fed indoor in comparison with animals fed outdoor, this finding comes in to agrese with Williams *et al.* (28), and the reason could be poor management and control of the viability of ticks in both indoor feeding and outdoor feeding cattle, which play a large role in transmitting this disease.

It has been earlier revealed in this paper that the seroprevalence was substantially elevated in spring and summer in comparison with the other seasons, most likely due to the climate, with spring and summer being high tick population seasons thus encouraging the spread of the disease in both seasons (29). The ELISA technique has proven its efficiency in diagnosing diseases, including Crimean Congo hemorrhagic fever, and viral and parasitic diseases (30).

Conclusion

This first study of CCHF in cows in Mosul city north Iraq has revealed relatively high seroprevalence of the disease in Mosul city and discussed are many risk factors relate to the occurrence of the disease such as age, gender, origin and seasons. Hence these factors should be monitored for strategic control of the disease in Mosul city as well as in the country, it has been concluded that the CCHF in Mosul. Iraq might form a focal point for the infection that infects ticks and helps transmit the disease and thus spread it to humans. Therefore, ticks control is generally necessary and may limit disease spread.

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Conflict of interest

The authors declare no conflicts of interest.

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الانتشار المصلي لحمى القرم والكونغو النزفية في الأبقار بواسطة الاليزا في مدينة الموصل

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الخلاصة

توصف حمى القرم والكونغو النزفية بأنه مرض فيروسي حيواني المنشأ ينتقل عن طريق القراد وينتشر بشكل كبير في إفريقيا وآسيا وروسيا والبلقان ضمن نطاق توزيع القراد الذي ينتمي إلى جنس *Hyalomma*. يهدف هذا البحث إلى التحقق من الانتشار المصلي لحمى القرم والكونغو النزفية في الأبقار باستخدام اختبار الممتز المناعي غير المباشر المرتبط بالإنزيم في مدينة الموصل / العراق، ولفحص بعض عوامل الخطر الوبائية المتعلقة بحدوث حمى القرم والكونغو النزفية من تشرين الأول ٢٠١٩ - أيلول ٢٠٢٠ ، تم الحصول على مائة وأربع وثمانون عينة دم من أبقار عمرها ٣-٨ سنوات من كلا الجنسين، من أنظمة إدارة وأصول متنوعة، من مناطق مختلفة في مدينة الموصل، وتم جمع ١٠ مليلتر دم من الوريد الوداجي خالية من مضادات التخثر لاختبار الممتز المناعي غير المباشر المرتبط بالإنزيم، تم الحصول على البيانات الوبائية من خلال مقابلة أصحاب المزارع. تم تطبيق اختبار الممتز المناعي غير المباشر المرتبط بالإنزيم للكشف عن الأجسام المضادة لحمى القرم والكونغو النزفية في المصل. كان معدل الانتشار المصلي الكلي لحمى القرم والكونغو النزفية في الأبقار ١٨٤/٤٠ (٢١,٧٪). أما بخصوص عوامل الخطر المرتبطة بزيادة الانتشار المصلي للمرض فقد لوحظت زيادة في معدلات الانتشار المصلي في الأعمار ٦ و ٧ و ٨ سنوات ٣٠,٥٪، بين الإناث ٢٦,٣٪ والحيوانات المستوردة ٢٧,٥٪. والتي أظهرت عدم وجود فرق معنوي في نسب الانتشار المصلي للمرض بين نظامي التغذية الداخلية والتغذية الخارجية. خلصت هذه الدراسة إلى أن حمى القرم والكونغو النزفية ينتشر بنسبة عالية في مدينة الموصل إلى جانب وجود العديد من عوامل الخطر المرتبطة بحدوثه.