



Seroprevalence of Crimean-Congo Hemorrhagic Fever among Cows and Their Owners in Nineveh Province, Iraq

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Abstract

CRIMEAN-CONGO Hemorrhagic Fever (CCHF) nowadays one of challengeable zoonotic disease both for breeding and human societies. The disease caused by CCHF virus. It is severe disease with high mortality rate to human. Our study was aimed to detect specific antibodies against CCHFV both in cows and their owners by ELISA technique in Nineveh province in Iraq. A total of 124 sera were collected from cows in various ages, genders, and breeding methods that distributed within the Nineveh province including Al-Gyarah, Makhmoor, Al-Hammam, Al-Shora, Zammar, in addition to Hatra cities. In addition to obtaining the same number of samples (124) in different ages and genders from the owners of these cows. The study results showed that overall seroprevalence of CCHFV in cattle was 60+/124 (48.4%). The prevalence of CCHF was highest in age group 5-7 years 26+/39 (66.6%) compared with other age groups 1-2 years 6+/18 (33.333) and 3-4 years 28+/67 (41.791). A 56 cow sera out of 117 were found seropositive (47.9%) while 4 ox sera out of 7 were seropositive (57.1%) against CCHFV. Our study showed that the seroprevalence of CCHFV in inbreeding system 35+/68 (52.5%) was higher than that in outbreeding system cows 25+/56 (44.6 %). The overall seroprevalence in owners was 6+/124 (4.8%) with sero positive rate of 3+/60 (5%) in male and 3+/64 (4.7 %) in female genders. It was concluded that CCHF was present in different areas of Ninawah province Iraq country both in cows and their owners with different seroprevalence rate in both of them with different genders and sex.

Keywords : Crimean-Congo Hemorrhagic Fever, I-ELISA, Orthonairoviridae, Seroprevalence, Cattle.

Introduction

Crimean-Congo Hemorrhagic Fever virus (CCHFV) is the virus that causes important viral disease which was called Crimean-Congo Hemorrhagic Fever disease (CCHFD). It was one of the zoonotic basic disease that infect both animals and human at the same time [1]. The causative agent belongs to the genus *Orthonairoviridae* under family *Nairoviridae* at the order *Bunyavirales* [2]. It is negative sense ssRNA virus their genome composed of three parts tripartite large and medium finally small (L,M,S) respectively, S segment encodes for glycoprotein GP, nucleoprotein NP and finally for RNA-dependent RNA polymerase [3]. Hard tick *Hyalomma* is the main vector that transmit the disease to human and the animals [4]. The wide separation of the tick *Hyalomma* lead to wide distribution of the disease all over the world, It can transmit the virus horizontally throw-out their life cycle also vertically from one generation to other in addition to the ability of the tick body as in *H. marginatum* to play as biological storage for the virus which can transmit the virus to

the vertebrate host after ten months of up taking the virus at 4 C° that give the ability of the disease to occur even in winter [5]. The disease is hazardous because it infects animals with a mild type and often without any symptoms, which allows the infected animals to act as a route for the infectious agent for transmission to humans, which are highly contagious, risky, and have a high mortality rate [6]. Cattle confirmed the primary global CCHFV reservoir in spite of the high seroprevalence among sheep, horse and goats [7]. Seroprevalence studies in domestic animals such as cattle, sheep, goats, can provide a clear idea about the distribution of the disease in certain human societies and these studies can be done by detection of specific antibodies against CCHFV in each of cattle, sheep, goats, camels, equines and even donkeys [8]. The disease present all over 50 countries in Asia, Africa, Europe [9]. Tick can play as reservoir and biological vector for the virus over all months of the year after up taking of it [10] [11]. The disease was discovered in Iraq first time at 1979 and from that time many

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(Received 06 February 2024, accepted 16 July 2024)

DOI: 10.21608/EJVS.2024.268377.1835

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episodes occur in 2018,2021,2022 [12], this study was aimed to detect specific antibodies against CCHFV in both of cows and their owners in Nineveh province in Iraq using ELISA technique.

Material and Methods

Blood collection

Animals

Blood collected from cows from jugular vein after shaving and disinfecting the area by 70 % of alcohol then it put in gel tube, the serum was separated by 3000 rpm for 10 min in the centrifuge then it put in Eppendorf tube and kept under deep freeze 20- C° until using it later.

Human

Blood collected from human from venipuncture vein after disinfecting the area by 70 % of alcohol then it put in gel tube , the serum was separated by 3000 rpm for 10 min in the centrifuge then it put in Eppendorf tube and kept under deep freeze 20- C° until using it later.

Enzyme Linked Immuno Sorbent Assay ELISA

ELISA kits (iD-vet/ France) ID Screen® CCHF Double Antigen Multi-Species were obtained from the market was used in the study. to detection of specific antibodies against CCHFV both in cows and human. This kit was prepared directly to detection specific antibodies against Nucleo Protein NP of CCHF virus in both animal species and human at all. The kit was used according to manufacturer's instructions as follow:

The micro titer palate was coated with NP antigens of CCHFV, the serum were added to the wells, after incubation and washing the specific conjugate was added, after incubation and washing the substrate TMB was added, after incubation and washing stop solution was added the results were read by ELISA reader (biosan) using 450 nm then the values calculated as follow:

OD of positive control $OD_{Pc} > 0.350$

Ratio of OD_{Pc} and $OD_{Nc} = OD_{Pc} / OD_{Nc} > 3$

Then the final results were interpreted as follow

$S/P \% \leq 30$ was negative

$S/P \% > 30$ was positive

Statistic

Statistical analysis was performed using IBM-SPSS V.27 software and to identifying the difference between groups QI square analysis was achieved followed by bonferroni correction at significance $P \leq 0.05$.

Results

The results showed that 60 out of the 124 (48.4%) bovine sera tested positive for specific antibodies

against CCHFV by indirect ELISA. The prevalence of CCHF was significantly highest in age group 5-7 years (66.6%) compares' with other age groups (Table1).

A 56 cow sera out of 117 were found seropositive (47.9%) while 4 ox sera out of 7 were seropositive (57.1%) against CCHFV (Table 2). Our study showed that the seroprevalence of CCHFV in inbreeding system (52.5%) was higher than that in outbreeding system cattle (44.6 %),(Table 3).

The overall seroprevalence in owners was 4.8%. Out of 60 and 64 people, three men and three women were found to be seropositive (4.7%) and (5%) against CCHFV, respectively (Table 4).

Discussion

Crimean-Congo Hemorrhagic Fever (CCHF) one of tick-born viral disease which is caused by CCHF virus. The disease is characterized by its ability to infect a wide range of animals, such as cows, sheep, goats, birds, wild animals in addition to human [13]. Animals, especially cows, are susceptible to a mild form that can occasionally show no symptoms [13]. These animals serve as a conduit for the disease's transfer to humans, infecting them with a severe variant that has a high mortality rate [14]. The disease was initially identified in Iraq in 1979 [15], and since then, it has continued to recur periodically with a constant frequency. This could be because ticks have long served as the primary vector and reservoir for the virus. Our result revealed that 60/124 cows presented positive results for CCHFV that form 48.4 % infection rate meaning present of the disease in high percent in Nineveh province, this result was different from another study in Mosul city conducted by [16] which they showed that percent of seropositive cows was 21.7 % and the difference between us may be due to the nature of our study which we focused in our study only to suspected animals that carried ticks in their body whereas in that study they take their samples randomly from cows with or without carrying ticks and despite the different results, while in another study in Basrah city / southern of Iraq conducted by [17] which they revealed that 18+/48 of cows under their study were presented positive result for CCHFV which form 37 % seropositive results, the difference in these results between our and their study may be due to the nature of their study in Basrah which they focused to human samples rather than animals also climate differences between Mosul and Basrah may play an important reason for this difference in results. Ruminants in general, including cows, play a crucial role in the epidemiology and dynamics of CCFV by amusing ticks with the prospect to transmit and maintain CCFHV and also by serving as amplifier host for it [18]. The CCHF is important zoonotic disease and one of important risk factor of CCHF disease distribution was occupational persons that contact

continuously with mild or asymptomatic infected animals. Human can be acquired the infection of CCHFV by three ways: direct bite with adult infected tick, direct contact with diseased animals either by their secretions or blood, and finally by direct contact between infected human with intact one [19]. So we revealed in our results that 6/124 samples collected from the owners of cows under study were presented positive result that form 4.838 % prevalence rate, this result agreed with [17] which they showed 27/627 presented positive result with prevalence rate reached to 4.3 % that confirm the presence of the disease in Iraq from north to south indicating continuous frequency of disease in the country both in animals and human. Also a study conducted by [20] to describe the epidemic wave of CCHF disease attacked the country at 2018 showed that only 10/143 were presented positive results with prevalence rate reached to 7% in Iraq. Regionally, studies have shown that the prevalence rate in human in some countries was as follows: In Turkey Erzincan province 14.0% [21]. Iran 16.49% [22]. In Greece 4.2 % [23]. Also in Romania 2.8 % [24]. In Mauritania 11.8% [25]. In an analytical study conducted by [26] which they focused in a systemic review by meta-analysis they recorded data from some countries explained via pooled seroprevalence of CCHFV from 2006-2022 in occupational peoples they revealed prevalence rate in some countries also as: (Turkey 20.43% , Iran 15.15% , Greece 10.37% , Pakistan 7.45% , South Africa 7.07% , India

24.53% , Mainland China 8.21% , Spain 2.56% , Saudi Arabia 0.40%). All these results are agreed somewhat with our study, regardless in the difference of infection rates between one country and another, one place to another, all the results confirm that there is a strong correlation between infection in animals and the people those living in rural areas in close vicinity with animals.

Conclusion

It was concluded that CCHF was present in Ninawah province in Iraq and distributed all over the province both in animals and human. The seroprevalence rate both in animals and human were different also according to different age and gender for each of them.

Acknowledgment

The researchers extend their thanks and gratitude to the Presidency of Tekrit University and the Deanship of the College of Veterinary Medicine for their continued support in completing this study.

Conflict of Interest

The authors declare that there is no conflict of interest.

Funding statement

The study was conducted by the researchers' self-funding, and there is no financial support for this research from any entity

TABLE 1. Seroprevalence of CCHF in different age group of Cows

| Age of the animal | No. of examined sera | No. of positive sera | % |
|-------------------|----------------------|----------------------|--------|
| 1-2 years | 18 | 6 | 33.3 A |
| 3-4 years | 67 | 28 | 41.8 A |
| 5-7 years | 39 | 26 | 66.6 B |
| Total | 124 | 60 | 48.4 |

Different capital letters mean presence of significance between groups at $P \leq 0.05$

TABLE 2. Seroprevalence of CCHF in Cattle based on gender .

| Sex | Number of examined sera | Positive sera | % |
|-----|-------------------------|---------------|--------|
| Cow | 117 | 56 | 47.9 A |
| Ox | 7 | 4 | 57.1 A |

Different capital letters mean presence of significance between groups at $P \leq 0.05$

TABLE 3. Seroprevalence rate of CCHF according to type of breeding

| Type of breeding | No. of samples | Positive result | % of infection |
|--------------------|----------------|-----------------|----------------|
| Inbreeding system | 68 | 35 | 51.470 A |
| Outbreeding system | 56 | 25 | 44.642 A |

Different capital letters mean presence of significance between groups under $P \leq 0.05$

TABLE 4. Seroprevalence of CCHF in human according to sex

| Sex | No. of examined sera | No of Positive sera | % |
|-------|----------------------|---------------------|-------|
| Man | 60 | 3 | 5 % A |
| Women | 64 | 3 | 4.7 A |
| Total | 124 | 6 | 4.8 |

Different capital letters mean presence of significance between groups at $P \leq 0.05$

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الانتشار المصلي لحُمى القرم-الكونغو النزفي بين الأبقار ومربيها في محافظة نينوى، العراق

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الملخص

يعد حُمى القرم-الكونغو النزفي (CCHF) أحد الأمراض المشتركة بين الانسان والحيوان والذي يعتبر هذا المرض في الوقت الحاضر تحديا كبيرا للحيوانات والمجتمعات البشرية. وهو مرض شديد ويمكن ان يسبب نسبة وفيات عالية عند الانسان. هدفت دراستنا إلى الكشف عن الأجسام المضادة المتخصصة ضد فيروس CCHFV في الأبقار وأصحابها باستخدام تقنية ELISA في محافظة نينوى في العراق. تم جمع مائة وأربع وعشرون (124) عينة دم من الأبقار في مختلف الأعمار والجناس وطرق التربية ومن مناطق مختلفة من محافظة نينوى توزعت بين مدينة الموصل مركز محافظة نينوى والقيارة ومخمور والحمام والشورى وزمار بالإضافة إلى مدينة الحضر. بالإضافة إلى الحصول على نفس العدد من العينات (124) من مربي واصحاب هذه الحيوانات بمختلف الجناس والأعمار. أظهرت نتائج الدراسة أن إجمالي عينات الامصال الموجبة لفيروس CCHFV في الأبقار كان 124/+60 (48.4%). وكان اعلى معدل انتشار في الفئة العمرية 5-7 سنوات 39/+26 (66.6%) مقارنة مع الفئات العمرية الأخرى 2-1 سنة 18/+6 (33.3%) و 3-4 سنوات 67/+28 (41.791%). كذلك 56 عينة مصل من أصل 117 عينة مأخوذة من الأبقار كانت موجبة (47.9%) في حين 4 عينات مصل من أصل 7 عينات مأخوذة من الثيران كانت موجبة (57.1%) ضد فيروس CCHFV. أظهرت دراستنا أن الانتشار المصلي الموجب لفيروس CCHFV في نظام التربية الداخلية 68/+35 (52.5%) كان أعلى منه في نظام التربية الخارجية 56/+25 (44.6%). ان معدل الانتشار المصلي الموجب الإجمالي لدى مربي هذه الحيوانات كانت 124/+6 (4.8%) مع معدل انتشار مصابي موجب 60/+3 (5%) في الذكور و 64/+3 (4.7%) في الإناث ، استنتج من هذه الدراسة ان مرض حُمى القرم-الكونغو النزفي CCHF كان موجودا في مناطق مختلفة من محافظة نينوى العراق في كل من الأبقار ومربيها مع اختلاف في نسبة الانتشار المصلي الموجب في كل منهما وباختلاف العمر والجنس.

الكلمات الدالة : حُمى القرم-الكونغو النزفي ، فحص الاليزا الغير المباشر ، Orthonairoviridae الانتشار المصلي ، ابقار.