Seroprevalence Of Crimean Congo Hemorrhagic Fever in Cattle in Thi-qar Province, Iraq

Douaa. A. Hashim[1], Tamadhir A. Al-Hamed[2]

[1]*Department of Internal and Preventive Veterinary Medicine, College of Veterinary Medicine,University of Basrah, Basrah, Iraq. Email: Tamadher67@gmail.com. Pgs.douaa.ameer@uobasrah.edu.iq*
[2]*Virus Department, Ministry of Agriculture, Veterinary Department, Baghdad Governorate, Iraq.*

KEYWORDS
Crimean -Congo hemorrhagic, ELISA Hemorrhagic fever virus (CCHV), Iraq

ABSTRACT
Crimean–Congo Hemorrhagic Fever is a zoonotic disease that transmission by infected ticks bites and from animals that are main carrier host without clinical septum so the current study was aimed to serological detection antibodies of Crimean–Congo Hemorrhagic Fever virus (CCHV) in cattle of Thi-qar province. Sera were analyzed for the presence of antibodies to CCHFV using the commercially available double-antigen ELISA kit. The study was conducted on 262 cases of cattle (87 male and 175 female) infected with CCHF based on Infected with ticks. Blood samples were collected from different part in Thi-qar province AL-Rifai, AL-Shatra, AL- Naser and Suq-ALshuhk. The wholly percentage of infected cattle with antibodies was 169(64.5%) positive from 262 cases. Highly prevalence record in Al-shatra positive from150 \115positive (76.66%) The prevalence of antibodies was higher in cattle at age 2 to 3 years old 55(73.33%) than in cattle aged less than 2 years 49 (58.33%). In addition, the percentage of cattle with antibodies in female was higher compare in male. These results suggest that is (CCHV) widespread in the cattle populations southern of Iraq.

1. Introduction

Hemorrhagic fever is world wide spread zoonotic disease discovered in 1944 in the Crimean area of the erstwhile Soviet Union and then afterward was secluded in Congo, from a child with similar symptoms [1]. Crimean–Congo Hemorrhagic Fever (CCHF) is an emerging disease because more than 1000 human cases presence informed every year from South-Eastern Europe and Western Asia [2]. The disease is endemic in Africa, the Balkans, the Middle East, and Asia, with an infected rate reach to 10,000 to 15,000 CCHF infections every year [3]. The Virus is part of the *Nairo* virus genus, and its lineage is *Nairoviridae*. It is a virus with RNA that is negatively oriented. [4]. Ticks considers as a main role vector for the transmission of several diseases [5],[6],[7]. According to geographic range of diseases that caused by ticks CCHF virus is most extensive among the tick-borne viruses that infect humans. Human Mortality 3 to 30% due to CCHF, and when it surges further than endemic level, it converts to a disastrous [8]. Sources of infection include tick bites, treated with infected animals' meat or by contact with an infected patient's blood through acute stage of infection [9]. About 30 distinct species of the virus has been found in ticks. Main means of transmission are hard-bodied *Hyalomma* ticks belonging to the *Ixodes* family [10]. Wild , domestic mammals and birds are the main hosts of CCHFV [11]. Sheep, goats, and cattle show high levels of virus in their blood, transporting the CCHF-across infected ticks, birds help spread the illness to great distances [12]. Rats, and tick larvae and nymphs also transmission CCHFV, human transmission comes into direct touch with another person's blood or body fluid, [13]The illness in human characterized by increasing bleeding, fever, and muscle soreness. Highly elevated aspartate aminotransferase, creatinine phosphokinase, alanine transaminase, and lactate dehydrogenase are found in biochemical testing [14]. In prothrombin assays, clotting time is delayed, the pathogenesis of disease closely related with indirectly releasing cytotoxic substances. These molecules result in activation of endothelial cells and lead to loss of function [15].

2. Material and Methods

Study Animals

A total of 262 animals included 87 male and 175 female from many areas in Thi-qar province / Iraq, collected blood samples from animals clinically infected with ticks during July 2023 to January2024. Underneath sterilized circumstances, drained Transfer 5ml of venous blood using a disposable syringe into a gel tube without anticoagulant. In the lab, every blood sample was centrifugate at 3000 rpm for

5 minutes, with the serum being separated and stored in labeled Eppendorf tubes before being frozen at -4°C for serological testing.

Serological Testing

Hemorrhagic fever: In accordance with the manufacturer's guidelines from **ID Vet & FRANCE**, an indirect-ELISA kit containing the specific antigen of Hemorrhagic fever virus was utilized to detect and measure specific IgG levels in serum samples .The kit and serum samples were prepared, diluted. Following the addition of Stop Solution, the optical density **(OD)** of the tested samples was measured at a wavelength of 450 nm using the Microplate ELISA reader **ID Vet & FRANCE**. Analysis of **OD**'s findings, for every individual sample.

Determine The S\P Percentage (S\P %):

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

Samples presenting an 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 independent sample test using statistical software program **(SPSS for windows version 22, USA).** Differences were considered significant at ($P \leq 0.05$).

3. Results and Discussion

Of 262 serum samples were subjected to an indirect ELISA test to identify specific IgG antibodies, the final outcomes were subsequently disclosed. 169 (64.5%) animals testing positive for antibodies CCHV antibodies virus. Among seropositive, there were 37 (42.5%) male and 132((75.42%) female. According to statistical data, there were no significant differences (P) observed between male and female. Seropositive higher than male also adult animals showed rising in CCHFV antibodies .

Table 1. Total Infected Animals In Thi-Qar

Rate	Negative	Positive	Total	Province
64.5%	**93**	**169**	**262**	**Thi-qar**

P ≤0.01

Table 2. According To Gender This Study Refers To Highly Females Infected Compare To Males' Gender. Seroprevalence Study Of Cchf For Cattle In Thi-Qar Governorate Regarding Gender For Positive And Negative Elisa Test.

Gender	Positive	Negative	Total	X^2
Male	37(42.53%)	50 (57.475%)	87	27.471
Female	132*(75.43%)	43 (24.5%)	175	
	169	93	262	

** Significant (P<0.05).*

Figure (1). ELISA assessment for infected cattle and buffalo in Thi-qar Governorate regarding gender. AB Different letters among groups indicates significant differences (P<0.05). Ab Different letters within group indicates significant differences (P<0.05).

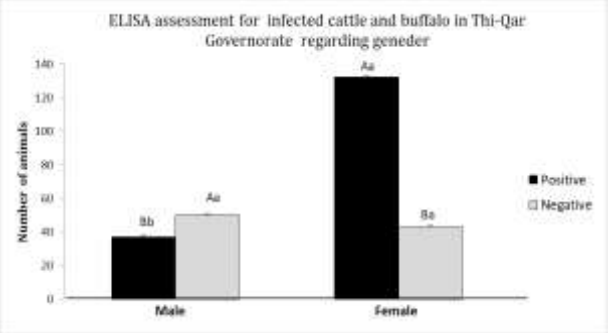


TABLE 3. Infected animals according to age group in Thi-qar showed infected animals high significant in 3 age compared other ages less than 2 years in Thi-qar Governorate. Seroprevalence study of CCHF for cattle in Thi-qar Governorate regarding age for positive and negative ELISA test.

Age	Positive	Negative	Total	X^2
1	49 (58.33%)	35 (41.66%)	84	
2	65 (63%)	38 (36.89%)	103*	4.038
3	55* (73.33%)	20 (26.66%)	75	
	169	93	262	

**Significant(P<0.05).*

Figure (2). Sero-prevalence of infected cattle and buffalo in Thi-qar regarding to age. AB Different letters among groups indicates significant differences (P<0.05). abc Different letters within group indicates significant differences (P<0.05).

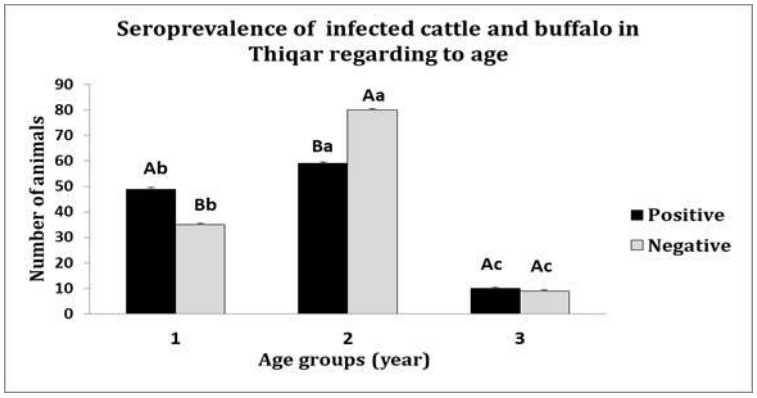


TABLE 4. Results according to regions in Thi-qar high rate of infected animals report in AL -Shatra and AL-Rifai. Seroprevalence study of CCHF for Infected Animals by Regions in Thi-qar for positive and negative ELISA test. AB Different letters among groups indicates significant differences (P<0.05).

Region	Positive	Negative	Total
AL-Rifai	12(60%)	8 (40%)	20
Al-Shatra	115 * (76.66%)	35 (32.33%)	150
Al-Shatra	10(28.57%)	25 (71.24%)	35
AL-Naser	9(47.37%)	10 (52.63%)	19
AL-Naser	6(22.22%)	21 (77.77%)	27
Suq-ALshuhk	1(9%)	10 (90.90%)	11
	162	100	262

X^2 = 59.946

** Significant P ≤0.01.*

Table 5. Animal Types In Thi Qar Governorate.

Animal	Positive	Negative	Total
Cow	**143**	**84**	**227**
Buffalo	**10**	**25**	**35**
Total	**153**	**109**	**262**

Figure (3). Seroprevalence study of CCHF for cattle in Thi-qar Governorate regarding animal type for positive and negative ELISA test. ^{AB} Different letters between groups show significant distinctions (P<0.05). ab Different letters within the same group indicates significant differences (P<0.05).

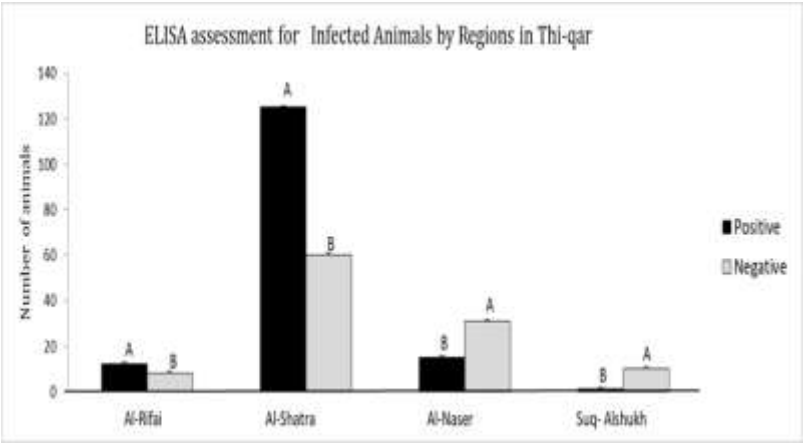
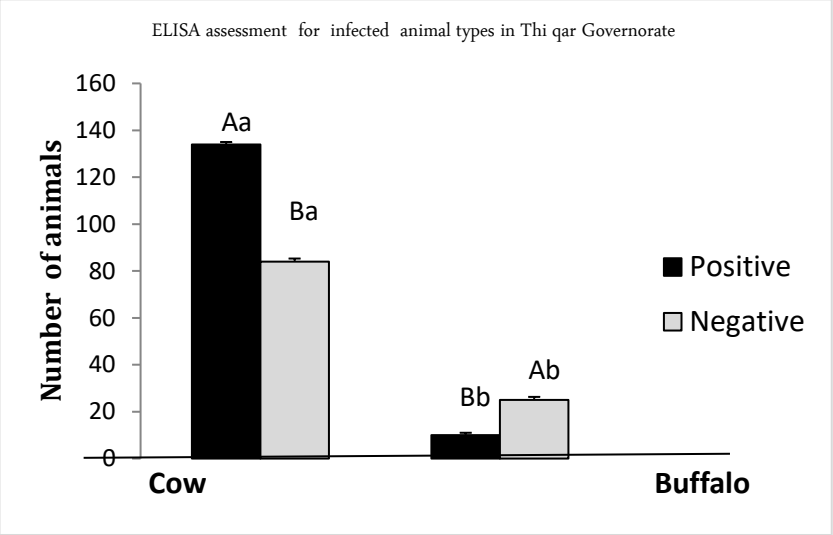


Figure (4). Seroprevalence study of CCHF for cattle in Thi -qar Governorate regarding animal type for positive and negative ELISA test. AB Different letters among groups indicates significant differences (P<0.05). ab Different letters within group indicates significant differences (P<0.05).



Figure(5). Thi-qar map explain distribution of disease in Thi-qar Gonvernorate.

CCHF infection in humans is a lethal, with high fatality rates of up to 40% (WHO) Mainly transmission by ticks' bites during suck on infected animals. Infected animals have not notability signs only short or low viremia [16], [17]. in recent years detection of CCHFV RNA even in peri-domestic rodents [35].current study aimed to investigate a CCHFV antibodies in cattle and buffaloes This work is a novel since it addresses for the first time the presence of CCHFV antibodies in cattle in Thi-qar one of big southren province of Iraq that have highly possess cattle and buffaloes. present study showed that the level of IGg antibodies detection in serum samples indicates the seroprevalence of CCHF,IGg-antibodies in cattle was 169(64.5%) positive from 262 cases. The results closely with study in Africa in cattle Overall, 964 samples 66% of the samples that were tested showed positive results for CCHFV-specific IGg antibodies [18]. Meanwhile, the results were with agreed with study in Uganda in cattle Twenty four out of the 37 (64.8 %) were seropositive for CCHFV [19]. Compare in other study in Uganda out of 117 cattle, 110 (94.0%) had antibodies for CCHFV present. which it was higher than current study [20]. Also, agreement with study in Iran that found showed that the disease's occurrence rate varied from 9.5% to 40% [21],[22],[23],[24]. **Nevertheless,** in this current research, it was noted that female animals displayed a noticeably greater seroprevalence compared to male animals, which goes against what was seen in Senegal and Sudan [25]. This result agrees with the findings in previous studies by [26] in Pakistan **,** [19],[27],[17]. in the tropics of Colombia refers This higher

seroprevalence in female ruminants could be related to the stress due to pregnancy and lactation which may reduce the immunity, [16]. Refers to the higher seroprevalence due to females being kept longer on the farms than males increase their lifetime risk to tick bites and hence CCHF infection [28],[29]. Moreover study refers that adult has high infected result compared to young age animals this was the same us study in Kordufan State, Sudan that showed older cattle were eight times more likely to be infected with the virus, [**7**],[30],[31]. Another Previous epidemiological surveys indicated that higher risks of CCHFV was notice in older cattle including in Iran and Egypt and Turkey [32],[33],[34]. and is consistent with [35], findings the increased CCHFV seropositivity in older livestock. [16], believe that the CCHFV IgG antibodies increase in older cattle compared to younger cattle due to the increased time for possible exposure of CCHFV In addition, the presence of CCHFV IgG antibodies in young animals may imply a recent infection, which may not have been detected due to lack of awareness .Moreover in this study titer antibodies were found highly in cattle compare with buffaloes this may related to live nature different between buffaloes and cattle.

4. Conclusion and Recommendations

The disease is highly endemic in Thi-qar Governorate and there is variation in the incidence rate of infection in different regions. The highest infection rate was recorded in Thi-qar Governorate, at 64.5% among cows, buffalo and calves.

Funding Statement

The novelty of the study is the detection of Crimean-Congo viral hemorrhagic fever to identify and conduct a serological and epidemiological study of the focus of the disease and the areas in which it is endemic, and to limit its spread and prevent it.

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