Physiological study of Crimean-Congo haemorrhagic fever in Sheep in Thi-Qar province

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Abstract

Crimean – Congo hemorrhagic fever virus (CCHFV) causes a lethal tick – borne zoonotic disease with severe clinical manifestation in humans but it does not produce symptomatic disease in wild or domestic animals. It is endemic in a large region of the world. One of the main indicators that the disease is endemic in a region is the presence of antibodies specific to CCHFV in animal populations. The factors contributing to differential outcomes of infection between species are not understood. Serological studies have shown that sheep are important to the survival of CCHFV in nature. Nevertheless, need for more studies on this subject in Iraq.

A total of 186 samples were tested for anti-CCHFV IgG using ELISA, while only 100 samples were used for physiological tests. The study revealed a high seropositive rate of 78.53% (144 samples), while 21.46% (42 samples) were negative. Regions such as Al-Eakihah, Al-Rifai, and Al-Garma recorded high infection rates of 92.85%, 90%, and 90%, respectively, compared to lower rates in Al-Fadhliya (75%), Al-Tar (50%), and Al-Manar (60.71%).

Hematological analysis showed significant differences between positive and negative samples in WBC and RBC, while no significant differences were observed in PLT, HGB, and HCT. A significant difference was found in hematological parameters between male and female samples, except for RBC and HGB in females. In pregnant ewes, significant differences were observed in WBC, RBC, and HGB, whereas PLT and HCT showed no differences. In non-pregnant ewes, all parameters showed significant differences except HGB. Age also played a role, with significant differences observed in WBC, PLT, HGB, and HCT in sheep younger than one year, while RBC remained unchanged.

Liver and kidney functions were also analyzed, showing significant differences in urea, AST, and ALP between positive and negative carriers, while creatinine, UA, and ALP showed no differences. Gender-based analysis revealed significant differences in urea, creatinine, ALT, and ALP, while UA and AST remained unchanged except for UA in females. Pregnant ewes showed differences in urea, ALP, creatinine, and ALT, while AST and ALT in non-pregnant ewes showed no significance.

These findings suggest that sheep may play a crucial role as a reservoir in the epidemiology of CCHFV.

Keyword: Physiological, Crimean-Congo haemorrhagic fever , Sheep

I.INTRODUCTION:

Crimean-Congo hemorrhagic fever virus (CCHFV) is an enveloped, segmented, and negative sense single-stranded RNA virus in the *Orthonairovirus* genus in the *Nairoviridae* family within the order *Bunyavirales* (Lombe *et al., 2021;* Sana *et al., 2022*).

Crimean-Congo hemorrhagic fever (CCHF) is an important tick-borne zoonotic disease with a wide geographic distribution that affects people in contact with infected animals and ticks (Shayan *et al., 2015*). This disease is endemic in Africa, Asia, the Middle East, and southern Europe (Fillatre *et al., 2019*).

CCHFV was first described in the Crimean region in 1944–1945, and the causative virus was isolated for the first time in 1956 from a teenage boy in Kisangani in the Belgian Congo (now the Democratic Republic of the Congo) (Spengler *et al., 2019;* Fillatre *et al., 2019*). Since then, infectious CCHFV strains have been isolated from ticks and CCHF patients, and CCHFV-specific antibodies have been detected in birds (ostriches), domestic and wild animals such as sheep, goats, cattle, horses, donkeys, camels, pigs, hares, hedgehogs, and ground squirrels in different geographical regions, demonstrating scientific evidence on vector, reservoir species, and virus dynamics in nature (Chinikar *et al., 2010;* Spengler *et al., 2016*).

CCHF is highly contagious and has a fatality rate in human ranging between 10% and 50% (Flusin *et al., 2010*). Some researchers have recorded a fatality rate of 3–80% with acute and severe hemorrhagic

manifestations. However, the initial symptoms are generally nonspecific, such as fever, fatigue, myalgia, headache, and diarrhoea, followed by progressive haemorrhage, shock, and multiorgan failure in severe cases (Mazzola *and* Kelly, *2019*).

The virus is harboured by wild and domestic mammals. Transmission to humans can occur either through the bite of an infected tick vector, predominantly species of the genus *Hyalomma* (Papa *et al., 2015;* Shayan *et al., 2015*), or through direct contact with blood or other bodily fluids from infected individuals or livestock (Lani *et al., 2015*).

In humans, CCHFV infection can result in a range of disease outcomes (Bente *et al., 2013*). Most cases are asymptomatic or mild with non-specific symptoms, such as fever, headache, myalgia, dizziness, back and abdominal pain, nausea, vomiting, and diarrhoea. However, some cases quickly progress to severe, often fatal, hemorrhagic fever characterised by vascular dysfunction, hemorrhagic manifestations, multi-organ failure (including cerebral, liver, and kidney failure, as well as cardiac and pulmonary insufficiency), shock, and death (Whitehouse, *2004;* Bente *et al., 2013*).Laboratory findings associated with poor outcomes include high viral load (viremia), elevated serum levels of liver-associated enzymes (AST and ALT), disseminated intravascular coagulation (DIC), thrombocytopenia, prolonged clotting times, weak or absent antibody responses, and increased serum levels of inflammatory cytokines and chemokines (Ergonul *et al., 2006;* Pap *etal., 2016*). The primary aim of this study is to investigate the seropositivity of CCHFV and assess its physiological impact on domestic animals in Thi-Qar province.

II.MATERIALS AND METHODS:

Animal Sampling:

Two hundred sheep from different regions of Thi–Qar province were chosen for this study. The animals were collected from regions with hemorrhagic infection. Throughout the study period, which start from 10/ 2023 to 2/ 2024. The animals were clinically healthy, aged between less than one to nine years. The study was chosen sheep in different sex (male and female) and physiological status (pregnant and non – pregnant).

ELISA Assay For CCHFV:

The detection of anti-CCHFV to CCHFV in a total of 186 serum samples was investigated according to commercial ELISA Kit (SUNLONG BIOTECH CO, LTD, China, Sheep Crimean-Congo hemorrhagic fever IgG (CCHF-IgG) Procedure.

Hematological Parameters:

The hematological parameters were done in Laboratory of Internal Medicine/Collage of Veterinary Medicine University of Shatrah by using hematological autoanalyzer (Count 60) made in Genex company. The instrument can measure and calculate 22 different parameters. These instruments used two reagents only (Diluent and Lyse) and Maintenance reagent (Probe cleanser only) and it has a printer mechanical inside with thermal paper. The hematological parameters estimated by this instrument were (RBC, WBC, Hb, PCV, HCT, Platelet).

Measurements Alanine Aminotransferase (ALT and AST) (U/I):

Alanine aminotransferase and Aspartate aminotransferase are measured by monitoring the concentration of pyruvate hydrazone formed with 2,4-dinitrophenyl-hydrazine and by monitoring the concentration of oxaloacetate hydrazone formed with 2,4-dinitrophenyl-hydrazine (Schumann and Klauke, 2003).

Measurements Alkaline Phosphatase (ALP) (U/I):

This estimation was done by using the colorimetric determination of alkaline phosphatase activity (Biolabo-France).

Measurements of Urea:

Urea concentration was determined by using a special urea Kit (bioSystems, Spain) (Tietz, 1996).

Measurements of Creatinine:

Creatinine concentration was determined by using a special creatinine Kit (BIO-LABO. SA, Maizy, France).

Measurement of uric acid:

Uric acid concentration in plasma was determined according to method of Barham and Trinder (1972).

Statistical Analysis:

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Least significant difference-LSD was used to significant compare between means. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

III.Result:

Distribution of all studied samples according to region:

The results of the current study show significant difference (p≤0.01) in seropositivity between samples that distributing according to regions**,** where the results showed that the highest total numbers of samples in Al-Batha , Al-Manar, Al- Nasiriyah and then Al-Shatrah with percentage 18.27, 15.05 , 15.05 and 11.82 respectively compare with Al-Fadhiliyah, Souq Al-Shuyoukh, Al-Eakikah, Al-Tar , Garmat Bani Saeed and Al- Rifai with percentage 8.6, 7.52 , 7.52 , 5.37,5.37 and 5.37 respectively . There is a significant increase (p≤0.01) of sheep which have seropositivity for CCHF compare with negative samples, The highest positive percentage recorded was (92.85, 90, 90) for Al-Eakikah, Garmat Bani Saeed and Al- Rifai receptively compared to the other sample. The results of the current study also indicate the presence of significant differences between positive and negative samples in all regions.

Table (1): Distribution of all studied sheep (positive and negative) according to region:

Sample Region	Total N	Total %	Positive N	Positive %	Negative N	Negative %	P.Value
Suq ash shuyukh	14	7.52	12	85.71	2	14.28	0.000
Al-Fadhliyya	16	8.6	12	75	4	25	0.000
AL-Tar	10	5.37	5	50	5	50	1.000
AL-Batha	34	18.27	25	73.52	9	26.47	0.000
AL-Manar	28	15.05	17	60.71	11	39.28	0.032
AL-Shatrah	22	11.82	18	81.81	4	18.18	0.000
AL-Eakikah	14	7.52	13	92.85	1	7.14	0.000
Livestock al-nasiriyah	28	15.05	24	85.71	4	14.28	0.000
AL-Garma	10	5.37	9	90	1	10	0.000
AL-Rifai	10	5.37	9	90	1	10	0.000
Total	**186**	**100**	**144**	**78.53**	**42**	**21.46**	
P.Value	**0.0001****		**0.0001****		**0.0001****		
**(p≤0.01).							

Distribution of all studied samples according to sex:

As illustrated in table (2), show a distribution of the sample into two distinct groups according to gender: females and males. The sample consists of a total of 186 sheep, with 130 (69.89%) being females and 56 (30.10%) being males, out of the 130 females, 97 tested positive (74.61%) and 33 tested negative (% 25.38) , Out of the total of 56 male, 47 tested positive (83.92%) and 9 tested negative (16.07%). The overall proportion of positive outcomes in the complete sample is 79.26% (144 out of 186), whereas the overall proportion of negative results is 20.72% (42 out of 186). The study also showed a significant difference p≤0.01 between males and females, as Males have a greater proportion of favorable outcomes compare with female.

Table (2): Distribution of all studied sheep (positive and negative) according to sex

Sample Sex	Total N	Total %	Positive N	Positive %	Negative N	Negative %	P.Value
Female	30	9.89	7	4.61	3	5.38	.000
Male	6	0.10	7	3.92		6.07	.000
Total	86	00	44	9.26	2	0.72	.000
P. Value	.0001*8		.0007**		.0041**		
*(p≤0.01).							

Result study hematology parameter of Positive CCHF and Negative CCHF

The results in table 4-5 represent the comparison of blood parameters in the positive and negative sheep groups and T-test was used to determine whether there were any statistically significant differences between the two groups. Two groups have been compared, with one group having 72 sheep that tested positive for seropositive of CCHF and the other group having 28 sheep who tested negative .

Page 96

The result indicates a significant increase in the count of red blood corpuscles and white blood cells between the positive and negative samples. There were no significant changes in the percentage of platelets, concentration of hemoglobin, and percentage of hematocrit for both the positive and negative groups.

Table 3: Hematology parameter according into + CCHF and - CCHF

parameter / Sample	Positive N(72)	Negative N(28)	T-test	P.value
WBC(10^9/L)	.58	2.31	.056*	.0268
RBC(10^12/L)	.81	.46	.502*	.0389
PLT(R 10^9/L)	22	26	2.71 NS	.694
HGB(g/Dl)	.6	.92	.893 NS	.866
HCT(%)	7.3	8.7	.021 NS	.857
(P≤0.05), NS: Non-Significant.				

Urea, Creatinine, Uric acid ALT, AST and ALP, concentration in carrier and non – carrier sheep.

According to table (4) that refers to the measurement the level of liver enzymes and kidney function testes in both groups of sheep (positive and negative) for the seropositive of CCHF. the number of positive sheep (72) while negative sheep (28).

Significant differences P ≤0.01 in urea, AST, and ALT of the Positive and Negative groups and the current study found no significant variation in the levels of creatinine, uric acid, and ALP among the groups. However, there was a statistically significant variation observed in both groups. The negative group exhibited elevated levels of AST, whereas the positive group displayed higher levels of ALT.

Table (4): Urea, Creatinine, Uric acid ALT, AST and ALP, concentration in carrier and non – carrier sheep.

Samples Parameter	Positive N (72)	Negative N (28)	T-test	P - Value
Urea (mg/dL)	37.09	29.85	5.038	0.0377
Creatinine (mg/dL)	0.40	0.37	0.064 NS	0.795
UA (mg/dL)	0.18	0.17	0.048 NS	0.902
AST(U/L)	87.73	97.95	8.812*	0.0485
ALT(U/L)	53.09	28.07	9.226**	0.0001
ALP(U/L)	75.03	76.44	7.519 NS	0.913
* (P≤0.05), ** (P≤0.01).				

IV. DISCUSSIONS:

Geographically, Iraq is an eastern Mediterranean country where CCHFV is endemic, and outbreaks are becoming more frequent. Several CCHFV outbreaks were reported between 1989 and 2009 (Al Salihi *et al.,* 2024).

Crimean- Congo hemorrhagic fever virus causes a tick-bore viral disease with a geographical distribution in certain endemic areas such as eastern and southern Europe, Asia, the Middle – East, and Africa (Spengler *et al.,* 2015).it possesses a high mortality rate (up to 40%), and there is no licensed vaccine available to combat the disease (Mertens., et al., 2013). When infection is acquired by tick bite, incubation time ranges from 1 to 9 days, however, if the infection is contracted from infected tissues or blood, incubation time is 5 to 13 days (Serretiello *et al.,* 2020; Eslava *et al.,* 2024)

Furthermore, virus behavior and replication characteristics are difficult to study due to the requirement of high containment laboratories. The virus has a high potential for emergence and introduction in new areas and remains a high health risk worldwide (Elliott, 2014; Zivcec *et al.,* 2015). A serological survey in Iran from 1975 to 1999, confirmed that 25–80% of the sheep were CCHFV seropositive (Chinikar *et al.,*2010).

Seroepidemiological studies of CCHFV in animals provide evidence of the virus circulating in endemic regions and also help to identify the risk areas. Anti-CCHFV IgG has been detected in both domestic and wild animals in various endemic regions. Seroprevalence results without symptomatic infection from various animals show that animals can be infected with CCHF. Serum IgG positivity in animals can last longer than asymptomatic viremia (7-15 days) (Reed **et al.,** 2002; Spengler **et al.,** 2016)

The CCHFV diagnosis can be achieved by RT-PCR, antigen-capture ELISA (cELISA), IFA, ELISA, immunohistochemical staining of infected tissues, or isolation of virus (Anonymous, 2020), In this study, a total of (186) specimens of serum sheep that were chosen for examination by ELISA test, the results of examination were appeared a high level about (144)78.53% of positive carrier sheep while negative samples (42)21.46% in Thi -Qar province, these result higher comparative with another study such as Huguette **et al.,** (2023) was recorded from 147 sheep 23 specimen appeared positive result to ELISA and 124 specimens appeared negative result , also these results lower from Huguette et al., 2023 in cattle (441 cattle) which appeared positive result (433) 98.18% and (8) negative results. also, the present study appeared higher infected percentage comparative with Tchetgna **et al.,** (2023) in goat (168) which recorded 11(6.55%) positive result while 157 goat appeared negative result.

The current study was appeared higher percentage of infected with CCHFV in different animals in another study such as Nurettin **et al.,** (2022), that founded seropositivity rates in cattle, goats, sheep, hare and wild boars (10.81%, 15.15%, 19.23%, 23.81 and 2.5% respectively. Albayrak **et al.,** (2012) reported an antibody prevalence of 85% in goats, these results higher than results with this study which appeared 78.53% in sheep but the present study higher than Albayrak **et al.,** (2012) in sheep which recorded 66%. Furthermore, Telmadarrairy **et al.,** 2010, was recorded the seropositivity rate of 39%in livestock. Also in recent related study conducted in Mosul north of Iraq , the CCHF infected rate was found 14%, which was calculated using indirect IgG and comprised 19.16 % of sheep and 6.25% of goat (Altaliby **et al.,** 2021).

Complete blood count (CBC) which is one of the most commonly ordered blood tests in medicine providing an overview of an animal general health status as well as information for infection, inflammation and inflammatory disease, deficiencies in the immune system, bone marrow disease and other health-related conditions.

The results of the present study are in line with those obtained by (Barznji **et al.,** 2014) they found that the hematological in sheep infested with Hyalomma species were lower in RBC, PCV, HBG, MCH, MCV and MCHC than non-infested ones and indicated that the normocytic normochromic type of anemia. While the Tyler and Cowell (1996) and Pfaffle **et al.,** (2009) classified the type of anemia as macrocytic normochromic depending on values of MCV, to lesser extent on (MCH) values in tick infestation. Prognostic variables for CCHF mortality include PLT, PT, PTT, and INR (Onguru **et al.,** 2010). The decreased in hemoglobin levels, may be be result from the beginning of anemia caused by bleeding. Hong zhao **et al.,** (2023) was not found hematological changes in total white blood cells, monocytes, red blood cells or platelets.

 The higher total leukocyte counts in the infested sheep than non-infested sheep; may be due to inflammation caused by tick bite which leads to migration of white blood cells as a response toward the tick bite (Barznji **et al.,** 2014).

The liver and kidney affected by CCHF virus therefore the current study was connected between the liver and kidney enzyme with positive and negative carrier animals, the statical analysis was appeared significant difference between them in urea, AST and ALP while no significant differences between positive and negative carrier samples in creatinine, UA and ALP in table 9. In CCHF infected African sheep, a slight but significant increase in AST was found, but no change in ALT was observed (Gonzalex **et al.,** 1998) .

Rathore and Sondhi, (2021) were found that AST and ALT elevations were a considerable burden in patients with Crimean-Congo hemmorrhagic fever. The Overall pooled prevalence of elevated at least on liver injury marker, AST, and ALT, was 77.95%, 85.92% and 64.30% respectively. It was also perceived that the incidence of raised AST levels was higher than the levels of ALT.

This study also focused on the connect between sex of animals with kidney and liver enzyme of positive and negative carrier ewe, the statical analysis was appeared significant differences between male and female in positive and negative samples at urea, creatinine ALT and ALP, while this study do not find significant differences between them in UA and AST except in UA was appeared significant differences in female between positive and negative samples. The elevation in serum ALT, AST may be result from that a Crimean-Congo hemorrhagic fever virus has the potential to induce significant liver a damage of liver

Page 98

cells and cellular degeneration or destruction occurs in this organ and the increase in the level of ALP in serum could be attributed to the increased permeability of plasma membrane or cellular damage . This elevation could potentially be attributed to the release of these enzymes from the cytoplasm into the blood circulation and indicating a necrosis and inflammatory reactions (Sharma *et al.,* 2020), although the mechanism(s) responsible for this harm are not well understood (Lindquist et al., 2018).The results were matched with the results obtained by (Aktas and Aktas, 2019; Najafi *et al.* 2024).

V.CONCLUSION:

This study highlights the high seroprevalence of Crimean-Congo hemorrhagic fever virus (CCHFV) in sheep within the Thi-Qar province, with a significant variation in infection rates across different regions. Males showed a higher seropositivity rate compared to females. Hematological analysis revealed significant differences in RBC and WBC counts between infected and non-infected sheep, while liver and kidney function tests indicated notable changes in urea, AST, and ALT levels. These findings emphasize the role of sheep as potential reservoirs for CCHFV, contributing to its epidemiology and transmission risk in endemic regions.

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