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Serological and physiological study of Crimean - Congo haemorrhagic fever in Sheep in Thi-Qar province

A Thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

﴿يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۚ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ﴾

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I hereby declare that the dissertation is my original work except for quotations and citations which have been dully acknowledged. I also declare that it has not been previously and is not concurrently, submitted for any other degree at university of Shatra or other institutions.

Haneen Hussain Talib

2024

Certification

We certify that this thesis (**Serological and physiological study of Crimean-Congo haemorrhagic fever in Sheep in Thi-Qar province**) was prepared under our supervision at the Department of Microbiology, Faculty of Veterinary Medicine, University off AL - Shatrah, as a partial requirement for Master degree in Veterinary Medicine / Microbiology.

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Dedication

To my father, my soul and my comfort

To my mother, and I tell her every success in my lifetime have been due to your prayers

To my brothers and sisters my life support

To my husband and my children, the source of my happiness

To my supervisors who guided and supported me by their knowledge

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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.

Out of 186 samples collect from Thi- Qar province , the study period extend from October 2023 to February 2024 , these sample have been used for serological detection of anti CCHFV IgG by ELISA, only 100 samples were used for physiological tests. The present study has shown a high level about (144)78.53% of sero-positive carrier sheep while negative samples (42)21.46% in Thi – Qar province which include different regions such as Al-eakihah , Al- rifai and Al-garma region which recorded about 92.85% , 90% and 90 % respectively comparative with another region of Thi -Qar province that appear low level of infected such as Al-fadhliya , Al-tar and Al- manar which recorded about 75%, 50% and 60.71% respectively.

The current study has focused on and attempted to connect the different physiological states, sex and age with infected animals. The seropositivity in male animals revealed a high level (83.92%) compared with that were appeared low seropostivity (74.61%), while recorded high infected in non – pregnant about 75% comparative with pregnant about 73,80. Also, the present study showed ahigh level of infection in age from 5 to 9 years about 82.35% comparative with another age ranges which appeared about 71.11, 78.50.

The hemalogical result of the present study was appeared significant differences between positive and negative samples in White blood cell and red blood cell. While there is no significant differences between them in Platelets, Hemoglobin and Hematocrit. The statistical analysis was appeared significant differences between female and male sex of hematology parameter in positive and negative samples while no significant differences between female positive and negative samples in red blood cell and HGB parameter. The statistical analysis was appeared significant differences between positive and negative ewe pregnant in WBC, RBC and HGB while, no significant differences in PLT and HCT.in non pregnant, this study was appeared significant differences between positive and negative samples in all the hematology parameter except HCG was appeared no significant differences between positive and negative samples. Also, the statistical analysis was appeared significant differences between positive and negative ewe in WBC, PLT, HGB and HCT parameter at less than one year's age, while no significant differences in RBC parameter between them. The age between 1 to 5 years was appeared no significant differences in RBC, PLT, HGB and HCT, while significant in WBC parameter between positive and negative samples. Also, hematology tests were appeared significant differences between positive and negative samples at 5 to 9 years of age in WBC, RBC, PLT, and HCT while, no significant differences in HGB parameter between them.

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. 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 does not find significant

differences between them in UA and AST except in UA was appeared significant differences in female between positive and negative samples. Also, the present study was connected between pregnant and non-pregnant animals with kidney and liver enzyme of positive and negative carrier ewe, the statically analysis was appeared significant differences between pregnant and non- pregnant in positive and negative samples at urea , ALP and creatinine , ALT in pregnant, while this study do not find significant differences between them in creatinine in non-pregnant , AST in pregnant and ALT in non-pregnant, Age of the animals play an important role in the spread of some viruses because of some viruses have special receptors at a certain age, therefore, this study was interested and connected between age of animals and CCHF virus, the statically analysis was appeared significant differences between positive and negative samples in urea UA, ALT and ALP while no significant differences in creatinine and AST at less than one years of age. While it was appeared significant differences between them in urea, creatinine, ALT and ALP while no significant differences in ALT and ALP at one to five years of age. The age between five to nine years was appeared significant differences between positive and negative samples in creatinine, UA, AST, ALT and ALP except urea parameter did not find significant differences between them at this age.

Finding indicate that sheep may play an important role as a reservoir in the epidemiology of CCHFV.

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List of Abbreviations

Abbrev	Full name
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AST	Aspartate aminotransferase
BSL	Biosafety Level
Cbc	Complete blood count
CCHF	Crimean-Congo Hemorrhagic Fever
CFR	Case fatality rates
CFT	Complement fixation test
CFT	Complement Fixation Test
DIC	Disseminated Intravascular
DIC	Disseminated intravascular coagulation
ER	Endoplasmic reticulum
GPC	Glycoprotein precursor
Hb	Hemoglobin
HCT	Hematocrit
HGB	Hemoglobin
HRP	Horseradish peroxidase
IFA	Indirect Immunofluorescence assays

IgG	Immunoglobulin G
IgM	Immunoglobulin M
INR	International normalized ratio
LSD	Least Significant Difference
MCHC	Mean corpuscular Hemoglobin concentration
MCV	Mean corpuscular volume
MHC	Mean corpuscular Hemoglobin
MVB	Mean platelet volume
NP	Nucleoprotein
OD	Optical density
PCR	Polymerase Chain Reaction
PCV	Packed cell volume
PLT	Platelets
PT	Thromboplastin
PTT	Partical thromboplastin time
RBC	Red Blood Cell
RNA	Ribonucleic Acid
RT-PCR	Reverse transcription polymerase chain reaction
TNF-&	Tumor necrosis factor alpha
TNF-B	Tumor necrosis factor beta

UA	Uric acid
WBC	White Blood Cell

Chapter one

Introduction

1.1 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 (Messina *et al.*, 2015; Al-Abri *et al.*, 2017; 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).

Fatal outcomes are correlated to increased viral load and dissemination, intravascular coagulopathy, and multi-organ failure (Zivcec, 2016). CCHFV-specific vaccines and approved therapies are still unavailable, and supportive care remains the mainstay of treatment (Messina *et al.*, 2015).

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).

Some CCHF survivors have been reported to experience sequelae that may persist for longer than a year (Hoogstrall, 1979). 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).

1.2. Aim of the Study

The aim of this study is to investigate the seropositivity of CCHFV and assess its physiological impact on domestic animals in Thi-Qar province.

- 1- Conduct an epidemiological analysis of CCHFV within Thi-Qar province.
- 2- Perform serological testing using ELISA in areas of Thi-Qar province where human infections have been reported.
- 3- Evaluate of hematological parameters, liver enzyme levels, and kidney functions in CCHFV-infected sheep compared to non-infected (control) sheep.

Chapter two

Literature review

2. Literature review

2.1. Historical perspective

Earlier Congo Crimean Hemorrhagic Fever Virus was considered as a haemorrhagic disease characterized by haematuria, melena, haematemesis, bleeding gums and bleeding in abdominal cavity. External parasites such as louse or tick were suspected to cause infection (Hoogstraal, 1979), which was later identified as *Hyalomma* tick larvae, frequently found on blackbirds. Crimean hemorrhagic fever virus (CHFV) was reported as a clinical entity in early 1940s when Soviet military personnels got infected in Crimea (Chumakov, 1947).

Further viral etiology and tick-borne route of infection was suspected and later demonstrated by inducing a mild infection in healthy human volunteers. They were inoculated with filtered nymphal *Hyalomma marginatum* suspensions along with antibiotics to develop the disease (Chumakov, 1974). Later in 1967, Chumakov and his co-workers isolated Drosdov strain of CCHF virus using suckling white mice (Chumakov *et al.*, 1968) and this prototype virus was later used in 5 experiments for the production of antigens and anti-bodies required for serological surveys. It also paved way for the identification and classification of viral isolates from different geographic locations. This ultimately led to the conclusion that CHF virus was antigenically identical to the Congo virus due to which the name was modified as Crimean–Congo hemorrhagic fever virus (Hoogstraal, 1979).

2.2. Causative agent and Classification

RNA arbovirus named Crimean-Congo hemorrhagic fever virus (CCHFV). It belongs to the genus *Orthonairovirus* (family *Nairoviridae*, order *Bunyavirales*), within the realm *Riboviria* (a new megataxonomic taxon rank), according to the most recent classification based on the advances in viral genomic and metagenomic comparison analysis (ICTV, 2022). The viral genome consists of three RNA segments: small (S), medium (M) and large (L), which encode the viral nucleoprotein (NP), the glycoprotein precursor (GPC) that yields the structural glycoproteins (GN and GC), and the RNA-dependent RNA polymerase, respectively. CCHFV exhibits higher genetic diversity than other tick-borne viruses, which reveals a wide dispersion of the virus (Kuhn, et al., 2020).

Since increasing complete genomic sequences of CCHFV are becoming available, new viral classifications are appearing in the last years. Previously classified into six main geographical clades (Dennis, et al., 2013), up to nine genetically different clades are currently proposed for CCHFV, according to the phylogenetic analysis of the complete genetic sequence of the S RNA segment of the genome, and based on the geographical origin (Gruber et al., 2019).

Four of them are predominantly distributed in Africa, two in Asia and three in Europe, as follows: Africa-1 (genotype I), Africa-2 (genotype II), Africa-3, (genotype IIIa) and Africa 4 (genotype IIIb); Asia- 1 (genotype IVa) and Asia-2 (genotype IVb); Europe-1 (genotype V), Europe-2 (genotype VI) and Europe (genotype VII) (Gruber et al., 2019).

2.3. Virion Structure, Genome and Replicative Cycle

The CCHF virion is spherical and approximately 80–100 nm in diameter with a bilayered lipid envelope (figure 1). The viral glycoproteins GN and GC protruding

from the lipid envelop are likely associated with receptor recognition and are involved in the early steps of viral replication (Kong et al., 2022).

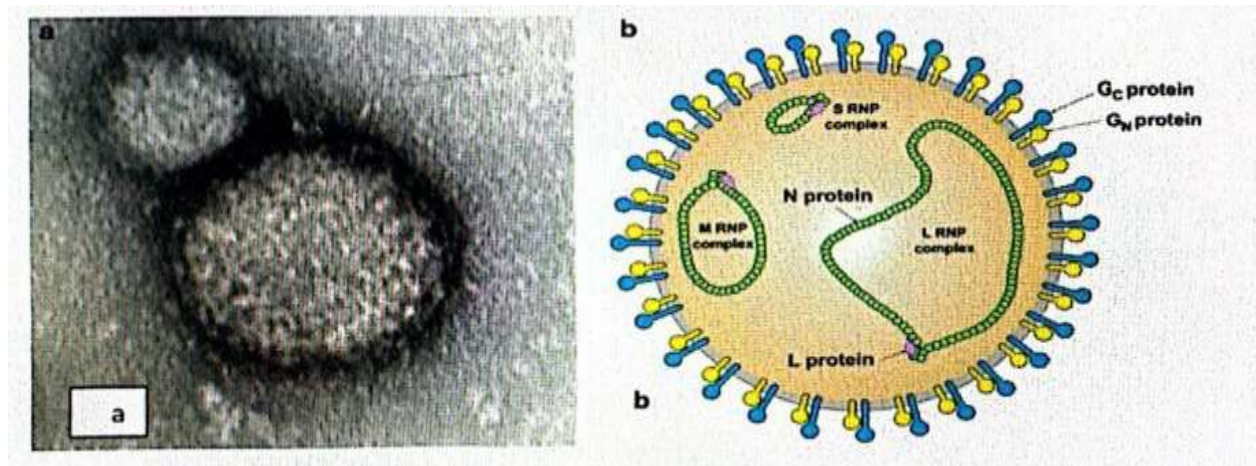


Fig. 1. (a) Transmission electron micrograph of a Crimean-Congo hemorrhagic fever virus particle. (b) Schematic illustration of nairovirus particle. Garrison *et al.*, 2020)

2.4. Proteins

Orthonairoviruses typically express three structural proteins. The most abundant structural protein in an orthonairovirion is N, which encapsidates the orthonairoviral genomic segments. The least abundant protein is L, which mediates viral genome replication and transcription. Two glycoprotein subunits, G_N and G_C, mediate virion entry. G_N and G_C and non-structural proteins (encoded by some, but not all *orthonairoviruses* (Freitas *et al.*, 2020)) are encoded as a precursor polyprotein.

2.2. CCHFV Genetic Structure

The small (s), medium (m), large (l) RNA segments make up the nairovirus known as CCHFV genome. The L- segment encodes an RNA dependent RNA polymerase (RdRP; the L-protein), the L- segment encodes viral glycoproteins, and the S-segment encodes the nucleocapsid protein (N). The genome of sNSVs are formed ribonucleoprotein complex after being encapsidated by the viral N protein. Rather than existing a naked RNAs Snippe *et al.*,2007).

RNA synthesis is crucial for viral reproduction and so provides a possible therapeutic target. Baghdad -12-Crimean-Congo Hemorrhagic fever virus strains N-protein was described as having a 1.2-crystal structure. Comparing the Crimean – Congo hemorrhagic fever virus N- Structure to the most recent report on the CCHFV strains N-structure (GenBank accession number: YL04057), considerable variations in domain positions may be seen (Carter *et al.*, 2012; Guo *et al.*,2012).

All these discrepancies might have significant effective repercussions, which detailed below, and are geographically distant from china. The key RNA binding

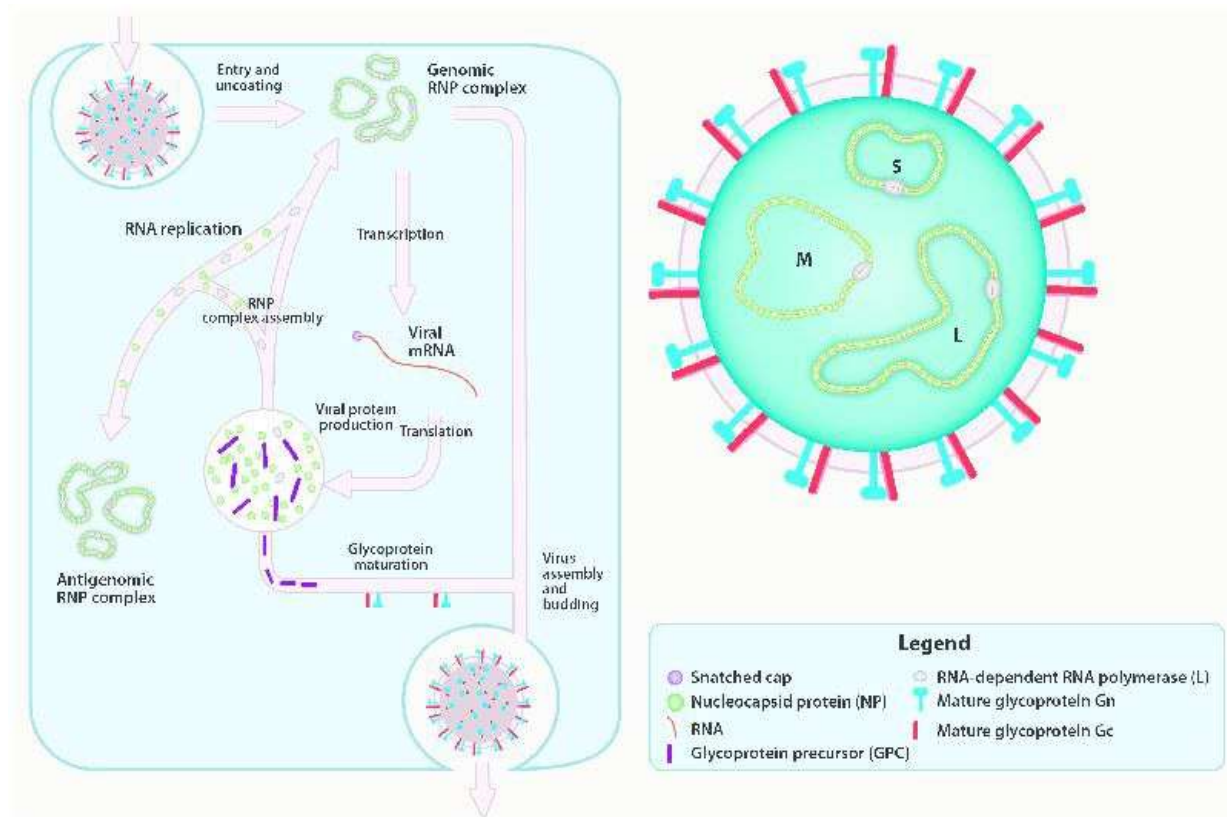


Figure3: CCHF virus genomic structure (Marko, et al.,2016)

and N-protein functions of N-N oligomerization are revealed by the structure, and these activities have been investigated in a minigenome assay in mammalian cells.

As a result of the strong and unexpected structural similarity between the N-structure and the N-protein belong Arenavirus Lassa Virus LASV , it is also possible that the present classification of sNSVs has to be revised (Carter *et al.*,2012)

2.6. Replication Cycle

The CCHFV GN and/or GC are involved in early step of replication cycle of CCHFV. It has been suggested that GC is responsible for binding to the cellular

receptors and mediates fusion in endosomes (Fig. 2). The processes of internalization, virion assembly and egress are dependent on host-cell skeleton. The cellular receptors for CCHFV entry have not been identified. A functional interaction has been suggested between CCHFV GC and cell surface nucleolin. Following adsorption of CCHFV at plasma membrane, the CCHFV is endocytosed through a clathrin-mediated endocytosis mechanism. (Neogi *et al.*,2022) CCHFV entry has also been suggested to be dependent on cholesterol and a low pH. Following endocytosis, CCHFV particles are transported to early endosomes and subsequently to multivesicular bodies (MVB), where the CCHFV membrane is fused to the endosomes membrane. Following this process the genomic RNPs are released into the cytosol and transcription and replication process will be initiated. The replication of the RNPs minimally require the L protein and N. Since CCHFV is a negative strand RNA virus, genomic RNPs are used as template to synthesize capped mRNA.

These processes are followed by transcription and viral protein processing and subsequent maturation. The assembly and egress process for CCHFV is most probably like other bunyaviruses. (Morikawa *et al.*,2007) and penetrate into the cells in a clathrin-dependent manner. After fusion, the viral genome is released to the cytoplasm. The viral mRNAs are translated into the NP and L proteins at cytoplasm, and the glycoprotein precursor (GPC) translated into endoplasmic reticulum (ER). The NP and L protein helping to replicate the genomic RNA. The GPC is processed and mature to Gn and Gc in the ER and the Golgi. The new CCHFV particles most probably assemble in the Golgi followed by virus release in Golgi derived vesicles. (Burt *et al.*, 2013).

The CCHFV contain the negative-sense small (S), medium (M) and large (L) genome segments, encapsidated by the nucleoprotein (N), and the RNA Fig. 1. (a)

Transmission electron micrograph of a Crimean-Congo hemorrhagic fever virus particle. (b) Schematic illustration of nairovirus particle. (Garrison et al., 2020) b

adependent RNA polymerase (RdRp), which are responsible to initiate transcription and genome replication in the host cell. The terminal complementary sequences 50-UCUCAAGA and 30-AGAGUUUCU are conserved in all nairoviridae members (Bamford and Zuckerman, 2021).

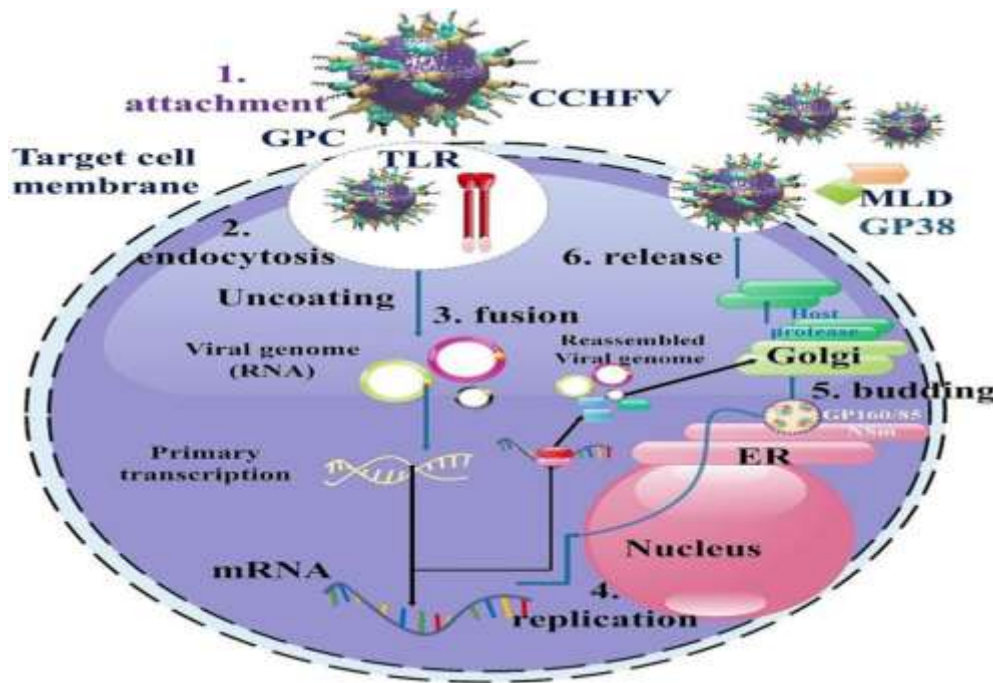


Figure 2: CCHFV replication cycle. (Muzammil *et al.*, 2024)

2. 7. Transmissions

Humans are infected by the bite of or by crushing an infected tick of the *Hyalomma* spp., against bare skin. The infection can also be acquired by percutaneous and permucosal route by contact with animal blood or tissues and drinking unpasteurized milk. The possibility of aerosol transmission is suspected in few cases in Russia, but no definite evidence exists (Watts, et al., 1988). The

endemicity of CCHFV is closely associated with the geographical distribution of the main arthropod vector and reservoir, *Hyalomma* ticks. (Gargili et al.,2017).

2.7.1. Vectors of CCHF by Ticks

Ixodid (Hard) ticks are characterized by the presence of scutum (dorsal shield) and the capitulum (head) is terminal. Ticks are the obligate blood feeding arthropods from animals and man and all stages of life cycle viz., larvae, nymph and both sexes of adults are blood feeders. Ticks transmit a greater variety of infective agents, than any other group of haematophagous arthropods many of them are zoonotic diseases which are inter transmissible between animals and man. Toxins and most infectious agents are transmitted to vertebrate to salivary fluids inoculated while the parasite feeds. (Sharm et al., 2020).

Vectors of CCHF CCHFV are primarily transmitted via hard-bodied *Hyalomma* ticks of the family Ixodes, particularly *Hyalomma marginatum*. (Anagnostou and Papa, 2009). The geography of CCHF infection reflects the distribution of *Hyalomma* ticks, which have a northern geographic limit of 48° north latitude (Papa, 2010). CCHFV has also been isolated from *Rhipicephalus*, *Boophilus* and *Dermacentor* spp., which may also transmit the virus (Hoogstraal,1981).

Infected animals may also act as reservoir during the period of viremia. Most important source for acquisition of the virus by ticks is believed to be infected small vertebrates on which immature *Hyalomma* ticks feed. The larvae and nymphs mainly feed on the lower vertebrates such as rodents, rabbits and hares. Adults feed on higher vertebrates such as cattle, goat and sheep etc as well as man.

2.7 .2. Transmission by another sources

Ground-feeding birds are often hosts of CCHF virus-infected ticks, but birds apparently do not become viraemic. Unusually severe winter-spring weather, resulting in decimation of *Hyalomma* populations and also of hosts of immature stages, appears to have been largely responsible for virus circulation to revert from epizootic (epidemic) to enzootic intensity. Humans become infected, when bitten by infected ticks, or when crushing these ticks in their bare hands or shearing tick infested sheep. Household and nosocomial cases resulting from contamination by bloody discharges from CCHF patients have been especially numerous and severe, often with great mortality in villages and hospitals, where the disease was unrecognized (Sharma et al., 2020).

2. 8. Epidemiology

2. 8. 1. Global Distribution

The virus from an outbreak of Crimean haemorrhagic fever in soliders and peasants in the Crimean Peninsula in 1944 was not isolated or characterised until 1967. Congo haemorrhagic fever virus, isolated from a patient in the former Zaire (now Democratic Repulic of the Congo) in 1956, was shown in 1969 to be the same virus. As a consequence the name of both countries have been used in combination to describe the disease (Hoogstraal, 1979) Distribution of the virus reflect the broad distribution of *Hyalomma* ticks , the predominant vector of the virus (Avsic-Zupanc,2007 ,Grard *et al.*, 2011, papa *et a.l*,2011, Swanepoeland,2011)

With regard to the geographic distribution .most of the countries reporting the disease as present are localized in Asia and to a minor extent in Africa. Indeed the

recognized geographic distribution of CCHFV includes several areas :Asia,Eastern Europe. Middle East and most of Africa (Spengler *et al.*, 2019) in Africa the countries reporting CCHF as present to the OIE are fewer than the ones where scientific studies demonstrated the circulation in animals on the contrary.the Democratic Republic of the Congo reported CCHF as present during up to 25%of the study period. While no scientific study on animals' population in the country is available.

The differences between the official data and the scientific literature may be explained within context of the veterinary service in conducting accurate surveillance on animals' populations at country level. (Fanelli *et al* 2024) CCHF infection has been reported in more than 30 countries of Asia, the Middle East, South-eastern part of Europe and Africa (Whitehouse 2004). By the year 2013, more than 8000 human cases were reported in South eastern Europe (Albania, Bulgaria, Greece,and Kosovo)and Turkey .

An epidemic characterized by fever and bleeding with a history of tick exposure in wide geographical area including Tokat, Sivas, Corum, Amasya, Yozgat, Gumushane ,Bayburt, Erzurum, Erzincan and the surrounding area;Northern parts of the Central and Eastern Anatolia and southern part of the Black Sea region (Kelkit Valley)in spring and summer of 2002 in Turkey has attracted the attention .in 2003,the disease diagnosed as CCHF and it spread to other provinces of Turkey as well (Elaldi *et al.*, 2009 ,Ergonul 2006) Due to rising human population in the Eastern Mediterranean region (Al-Abri *et al* 2017) the favourable circumstances for vector ,and the migration of animals and wild birds transporting infected ticks from Syria, Iraq, and Iran ,CCHFV has become endemic in the south west Turkiye.(Saltik 2022) This disease was considered as one of the most important hemorrhagic viral fever in human owing to the high mortality ranging from 10%to

40% as well as possible nosocomial transmission (Masoudy *et al.*, 2016)The disease was endemically broke out in Africa, Asia, Middle-East, and Eastern Europe .some 914 patents with CCHF were reported in 2017 with the rate of 106 subjects in India(Swain *et al* 2017) CCHFV is prevalent in west Africa with documented human fatal cases occurring in Senegal and Mauritania (Nabeth *et al* 2004 ,Tall *et al* 2004).

CCHFV endemic foci are expected to broaden in the face of climate change, (Estrada *et al* 2010) human movement as well as the movement of animals infested with a tick (Spengler *et al* 2019) Furthermore, virus behaviour and replication characteristic 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). CCHFV infection affected 10.562 cases between 2002 and 2017 with 4.74% mortality in turkiye (Halk 2022)

2.8.2. CCHF prevalence in Iraq

As early as 1979, when ten people were initially diagnosed with the illness, CCHF became known in Iraq. Then, between 1990 and 2009, a total of six instances was documented, in 2010, eleven instances; in 2018, three fatal cases; and most recently, 33 confirmed cases, including 13 deaths (case fatality rate 39%), were reported in 2021 (Ahmed *et al.*,2022). It has previously been reported that Iraq has been affected by CCHF, first in 1979, then in 1992, 1996,2012, and until 2023. (Al-Shauwreed *et al.*, 2023).

Despite CCHFV has recently spread throughout Iraq, causing high mortality and morbidity rates in humans, the prevalence and distribution of CCHFV in Iraq are

poorly understood, making it impossible to quantify the risk of infection (Nubgan and Al-Saadi, 2023)

CCHFV is an emerging and re – emerging virus with extensive geographical distribution. The virus has a constant presence in Africa, the middle East, Asia, and Eastern Europe. African countries from which CCHF has been reported include Burkina Faso, Africa Republic, Demo-cratic Republic of Congo, Egypt, Kenya, Mauritania, Namibia, Nigeria, South Africa, Senegal, sudan, Tanzania and Uganda (Kautman *et al.*, 2017) .

The presence of CCHFV in Somalia was suggested after evidence of the CCHFV in Hyalomma ticks obtained from somali cattle and sheep exported to the United Arab Emirates (Khan *et al.*, 1997). In the middle East, the disease has been described in Iran, Iraq, Saudi Arabia, Oman, and the United Arab Emirates (Mohamed 1997; Chinikar *et al.*, 2005). East European countries with described CCHF disease include Albania, Bulgaria, Greece, Kosovo, Turkey, Georgia, and Russia. (Onishchenko, 2001; Papa *et al.*, 2002). Portugal, Hungary, France, and Romania (Fillipe *et al.*, 1985; Ceianu *et al.*, 2012) are at risk of CCHF based on serological evidence of viral circulation. Asian countries with described CCHF disease include Afghanistan, Kazakhstan, Pakistan, China, India, Tajikstan, and Uzibekistan (Mustafa *et al.*, 2011; Mishra *et al.*, 2011).

CCHFV cases have over the past few years been described in new regions while the disease has re-emerged in some countries after periods of inactivity. CCHF was first described has the highest incidence of CCHF cases per annum with a mortality rate slightly below 5% over 1660 cases were reported by Russian Pro-Med between 2005 and 2007 (Rakhmanova *et al.*, 2018). The first human case in Iran was reported in 1999, and an increase in incidence has been observed (Papa

et al., 2015; Shahhosseini *et al* 2017). A nosocomial outbreak of CCHF at a hospital in India in 2011 stands as the principal case in the country (Mishra *et al*, 2011) and from there on, sporadic cases have been reported (Yadav *et al.*, 2016). The first recorded CCHF cases in Spain were described in 2016 from an adult male who most likely acquired infection through a tick bite and a nosocomially acquired infections by a health professional who nursed the index case (Negredo *et al.*, 2017). Even though CCHF has not been described in some countries, the presence of CCHFV in ticks and CCHFV – specific antibodies in wild and domesticated ruminants have been demonstrated (Bente *et al.*, 2013).

CCHFV endemic foci are expected to broaden in the face of climate change, (Estrada *et al.*, 2010) human case movement as well as the movement of animals infested with a tick (Spengler *et al.*, 2019). However, in some regions, the observed increase in reported cases could be a product of improved awareness and diagnostic capacity. CCHFV poses a zoonotic risk with public health implications and as such CCHF is a notifiable disease. Nonetheless, with the exception of Turkey reporting more than 50 cases per year (WHO, 2017), CCHF cases are currently sporadic in endemic zones.

Role of animals in CCHF outbreaks CCHF virus, similar to other zoonotic agents, appears to produce little or no disease in its natural hosts but causes severe disease in humans. CCHF virus infection has been detected in numerous domestic and wild vertebrates through virus isolation and majority by sero-epidemiological survey to detect antibodies to CCHF virus. Hares, hedgehogs, cattle, sheep, goats, horses, yaks and swine were reported as the main natural hosts of the CCHF virus (Camicas *et al.*, 1990). Birds were considered resistant to CCHFV infection until the mankind witnessed death of a worker slaughtering ostriches on a farm in Cape Province of South Africa. Later on low titer of virus was recorded from guinea

fowl also but it was concluded that birds are asymptomatic carrier of infection (Shepherd *et al.*, 1987).

Migratory birds are often transport the ticks and their associated pathogens across the barriers such as rivers, deserts and mountains, oceans and continents. In domestic animals, the infection is usually sub-clinical and lasts from a few days to a few weeks. So, shepherds, campers, agricultural workers, veterinarians, abattoir workers, and other persons in close contact with livestock and ticks are at risk of infection (Gonzalez *et al.*, 1998; Ergonul, 2006; Zakhshvili *et al.*, 2010).

2. 9. Pathogenesis

Very little is known about the pathogenesis of CCHF. The most significant feature of pathogenesis is ability of CCHFV to disable immune system of host and immune cells as well as endothelial cells (EC) are the key contributors. Virus either interacts with immune cell indirectly concomitant with the release of mediators or interacts with ECs directly. The upregulation of proinflammatory cytokines and soluble molecules activate ECs, which in severe cases may produce toxic effects vasodilation, hypotension, increased vascular permeability, shock, multiple organ failure, and death Several studies exhibit that clearance of CCHFV is delays. (Akinci *et al.*, 2013)

CCHFV impair innate immune response and delays adaptive immune system and cause systematic spread as well as uncontrolled replication of virus throughout host body accompanied with the induction of interferon, increased level of IL-6, IL-1, TNF-alpha, apoptosis of lymphocytes. partial activation of macrophages and dendritic cell, lympho-histiocytosis, hemophagocytosis, and weak antibody response (Xiao *et al.*, 2011; Akinci *et al.*, 2013; Rodrigues *et al.*, 2012).

Infection in human leads to a spectrum of liver damage that can range from mild injury to fulminant hepatic failure (Negredo *et al.*, 2017, Conger *et al.*, 2009;

Conger *et al.*, 2015; Ergonul *et al.*, 2006))The prevalence of liver injury has led to the clinical characterization of disease severity to be partially based on elevated liver enzymes (Ergonul *et al.*, 2006). CCHFV can be neurotropic in humans and neuropsychiatric disorders occurring during CCHFV have been reported (Whitehouse, 2004 ; Ergonul, 2006) Both cardiac and pulmonary sequelae can occur during CCHF (Dogan *et al.*, 2011 ,Bilgin *et al.*, 2014 ,Engin *et al.*, 2009, sannikova *et al.*, 2007)Respiratory distress includes the development of acute respiratory disease syndrome(ARDS)with parenchymal and alveolar infiltration ,pleural effusion ,coughing that includes hemoptysis, dyspnea and pulmonary haemorrhage .Additionally, a retrospective study identified the presence of pulmonary artery enlargement ,indicative of pulmonary hypertension, in several CCHF patient (Aktas *et al.*, 2016)

2.9.2. CCHF virus pathogenesis in sheep

It is noteworthy that the given was specific to herds that belong to the local market and that been raised under local veterinary health conditions. Consequently, appear slightly different from those mentioned in textbooks. (Swanepoel, *et al.*, 1983).

Only a transient but constant thermal peak has been found as a clinical symptom associated with a period of onset in adult CCHF virus-infected sheep. The persistence of fever reflects the effectiveness of virus infection and replication. Among five pregnant infected ewes that gave birth, four had normal delivery and one had an abortion. However, the lack of virus isolation from the foetus did not support an association with the virus infection. Although concomitant CCHF virus isolation has been reported elsewhere with ewe abortion in the same herd, no data supported the abortive effect of the virus (Woodall *et al.*,1967).

A self – limited increase in blood enzyme to AST is not specific to liver dysfunction, and could be related to any type of cell damage from various tissue;

such findings cannot be clearly associated with virus infection without further investigation. In another experiment done elsewhere, no liver dysfunction was noticed when sheep were infected with the CCHF virus (Swanepoel, *et al.*, 1983).

Neutrophilia observed in two sheep might be related to a concurrent infectious germ, and more likely to a parasite (Gonzalez *et al.*, 1998).

Livestock are infected when bitten by infected ticks, and develop asymptomatic transient viraemia lasting 7-15 days. (Atim *et al.*, 2023) In experimental inoculation, sheep and cattle become infected with the CCHF virus, but they develop only transient mild fever. Viremia levels and duration are relatively low and short, and antibodies are detectable shortly after cessation of viremia. (Ksiazek 2021) In contrast to humans, domestic animal does not show clinical sing of illness, and act as accidental host, which amplify and disseminate the virus. Sero-epidemiological studies in endemic regions indicate that various domestic animals (sheep, goats, and cattle) could be asymptotically infected with CCHFV. Therefore. they can act as sentinels, providing initial evidence of the silent circulation of the virus, and allow identification and localization of CCHFV foci, which could be a risk for human health .(Ceianu *et al.*, 2012, Raileanu *et al.*, 2015).

2.10. Clinical feature

CCHFV infections are asymptomatic in animals and birds are thought to be resistant. Humans are the main victims to this disease. The course of the disease can be divided into four phases – incubation, pre hemorrhagic, hemorrhagic and convalescence (Hoogstraal, 1979). The incubation period depends on the mode of infection. Infections acquired via tick bites usually become apparent after 1-3 days

(Whitehouse, 2004). Exposure to blood or tissues results in longer incubation period. In Indian cases, the incubation period ranged from 7 – 12 days through the later mode (Whitehouse, 2004; Patel *et al.*, 2011; Mourya *et al.*, 2012).

Sporadic outbreaks are common in places with poor clinical pathology facilities and improper understanding of viral pathogenesis. In addition, it requires advanced laboratory facilities (biosafety level 4) and better disease investigation using susceptible animal models. Understanding the pathogenesis from blood parameter changes and liver biopsies of the affected are often inadequate. Complications due to cerebral haemorrhage, anemia, recurring episodes of diarrhoea and the associated dehydration and shock, myocardial infarction, pulmonary oedema and pleural effusion often result in fatality. Multiple organ failure, cardiac and pulmonary insufficiencies are the main reasons associated with death due to CCHF (Whitehouse, 2004)

Liver lesions may range from several necrotic foci to massive necrosis with little or no inflammatory response. Capillary fragility is a common feature of CCHF, suggesting endothelial infection causing the characteristic rash. In fatal cases, thrombocytopenia and extremely low platelet counts are noticed even at an early stage. Disseminated intravascular coagulopathy (DIC) is also evident in the disease process of CCHF (Whitehouse, 2004)

No clear pathogenesis is described for CCHF. Endothelial damage is a common feature leading to capillary fragility and accounts for the characteristic rash and contributes to hemostatic failure by stimulating platelet aggregation and degranulation (Whitehouse, 2004). Thrombocytopenia occurs and dysregulation of the coagulation cascade lead to DIC. Proinflammatory cytokines are important in pathogenesis and the il-6 and TNF- α level are significantly higher in fatal CCHF (Ergonul, *et al.*, 2006). A study shows that viral genome patient (Bodur *et al.*,

2009). In CCHF there is increased serum ferritin level which can be used as a marker for disease activity and prognosis (Barut *et al.*, 2010).

2.11. CCHFV immunity

Antibody production against CCHFV is one of the important factors for survival. Fatal CCHF cases rarely established an antibody response, and a significant reverse correlation between viral load and anti-body levels was (Duh *et al.* 2007; Wolfel *et al.*, 2007; Saksida *et al.*, 2010).

The association between a weaker or undetectable antibody response and a higher viral load in fatal cases indicates that an impaired immune response leads to uncontrolled replication of the virus. Although it is not exactly known, the underlying mechanism could be as a result of inadequate activation of the adaptive immune response by DCs because of partial maturation of CCHF-Infected DCs (Bent *et al.*, 2010, Peyrefitte *et al.*, 2010).

Human IgM and IgG Antibody responses can be detected against the glycoproteins {GP38, GN and Gc}, and nucleocapsid (N) Protein in CCHF Survivors {Golden *et al* 2019, Fritzen *et al* 2018, Emmerich *et al* 2018}. There is some correlation with the levels of the inflammatory cytokines inter-leukin {IL} - 6, IL-8 and tumor necrosis factor {TNF}- α With fatal/severe CCHF (Ergonul *et al.*, 2006, Ergonul *et al.*, 2017, Papa *et al.*, 2006).

In particular, higher levels of IL-6 and IL-8 by day 5 post –disease onset are strongly associated with fatal outcomes and can predict poor outcomes (Ergonul *et al.*, 2017) Furthermore, high levels of the monocyte chemokine MCP-1 {CCL2} also correlated with severe human disease (Ergonul *et al.*, 2017) Other studies have identified the presence of secreted trigger receptor expression on myeloid cell-1

{Strem-1}, which is an amplifier of inflammatory responses, in the serum of those with severe CCHF (Altay *et al.*, 2016) .

Genetic variation in human type I interferon {IFN-I} responses may partially influence CCHF severity. Retrospective analyses of CCHF human cases have identified polymorphisms in toll-like receptors {TLR}, TLR8/9 and TLR3 are reported to play a role in acute disease (Engin *et al.*, 2010, Engin *et al.*, 2016) This includes development of neutralizing antibodies responses for which Gc is the only know target (Zivcec *et al.*, 2016, Bertolotti-Ciarlet *et al.*, 2005).

Antibody responses can be maintained long term ,suggesting life-long protection against CCHFV (Burt *et al.*, 1994 ,Yadav *et al.*, 2019)No immunodominant epitope was identified and most responses targeted peptides from N Protein, however at least two survivors had CD8+ T-cell responses against Gc.Thus, long –live CD8+ T-cell responses targeting CCHFV are produce during infection and present in survivors .Although some studies indicate that antibody (convalescent human plasma) can protect against severe disease (vassilenko *et al.*, 1990).

Interferons (IFNs) are important compound for the antiviral response of the innate immune system and have important role in limiting the spread of infection. Production of type 1 IFNs (IFN- α and β) yields a rapid and efficient host response against the viruses (Weber and Mirazimi, 2008).

Activation of the innate immune system by the molecules produced during viral replication lead to induction and secretion of type I IFNs and subsequently upregulation of interferon –stimulated genes (ISGs) (Samuel 2001; Andersson *et al.*, 2008).

Natural killer (NK) cell is part of the innate immune system response against viruses and play a role in the detection and lysis of infected cell (Yilmaz *et al.*, 2008). T and B lymphocytes also have important function in the immune response. In a recently developed mouse model for CCHF, it was shown that the lymphocytes in the spleen were activated within 3 day. the numbers of NK, B and T cells increased on the first day of infection, but they decreased in numbers significantly on the third day, regardless of activation (Bent *et al.*, 2010). possibly, these cells increased because of the early production of cytokines, but later the uncontrolled apoptosis of lymphocytes contributes to a depletion in lymphocyte count both in the blood and the spleen, which is presented as lymphopenia (Geisbert *et al.*, 2000, Bent *et al.*, 2010, Connolly-Andersen 2010).

Early host antibody responses may also be a predictor of disease outcome. Antibody responses to CCHFV may be rapid, with CCHFV –specific IgM detectable as soon 2-3 days after symptom onset and CCHFV-specific IgG within 5-6 day (Ergunay *et al.*, 2014). Clearance of viraemia correlates with early IgM responses (Ergunay *et al.*, 2014), whereas in fatal cases, there is little evidence of an antibody response against (Kaya *et al.*, 2014).

2. 12. Diagnosis

Early diagnosis is essential to save the life of the patient and to prevent nosocomial infections. It will also help in implementing appropriate precautionary measures to check disease spread. Clinical manifestations and anamnesis are the first indicators of CCHF. Diagnosis is confirmed considering the epidemiologic factors and laboratory tests. The disease must be differentially diagnosed from tick typhus, leptospirosis, borreliosis, malaria, yellow fever, dengue fever and KFD *etc.* (Sunitha *et al.*, 2021)

Clinical diagnosis is practically impossible during the initial stages due to non-specific initial symptoms and sporadic onset nature of the disease. Therefore, laboratory diagnosis is considered Clinical diagnosis is practically impossible during the initial stages due to non-specific initial symptoms and sporadic onset nature of the diseases. (Sunitha *et al.*,2021)

Therefore, laboratory diagnosis is considered more definitive and reliable where serum or plasma is used for isolation, serological and molecular techniques. Saliva and urine samples of CCHF infected patients also may serve the purpose, but their diagnostic application is yet to be confirmed. Virus could be isolated from liver, kidneys, lungs, spleen, bone marrow and brain during autopsy.

Molecular diagnostic techniques and real-time polymerase chain reaction (RT-PCR), aids the rapid diagnosis of disease and for molecular epidemiology studies. However, poor surveillance, harmonisation of case identification and availability of validated sero-diagnostic kits for animal testing reduce the efficiency of surveillance systems. Rapid diagnostic tests (in clinic or field conditions) are necessary in remote or low-resource areas. ELISA or immuno fluorescence assay, IgM-capture ELISA and IgG-sandwich ELISA are used for the detection and quantification of antibodies against the CCHFV. In spite of its lower sensitivity when compared to PCR, it is much sensitive, specific, rapid and reproducible than CFT and immunofluorescence. Indirect Fluorescent Antibody (IFA) technique could also be employed in viral detection.

Viral concentration can be detected by either cell cultures or intra-cerebral inoculation of suckling mice especially during the initial stages of disease. The latter, though slower, is more sensitive and detects the virus for a longer period. Virus isolation require BSL-4 biosafety level laboratory. Supportive diagnostic techniques include biochemical tests and complete blood count. Thrombocytopenia

along with leucopenia, increased serum aspartate alanine aminotransferase, lactate dehydrogenase, and keratinizes phosphokinase are observed in CCHF. (Sunitha *et al.*, 2021).

2.12.1. Diagnostic Techniques

CCHFV infection causes only a mild fever in domestic and wild vertebrate animals with a detectable viraemia of up to 2 weeks (Gonzalez *et al.*, 1998; Gunes *et al.*, 2011). Similarly infected ostriches develop only low and short-lived viraemia and no clinical signs (Swanepoel & Burt, 2004). Therefore, recent infections in animals are rarely diagnosed and methods such as polymerase chain reaction (PCR), virus isolation in cell culture and IgM detection by enzyme-linked immunosorbent assay (ELISA) are mainly used in human CCHF diagnostics or in the special case that an animal has to be classified as CCHFV free. For prevalence analysis and for determination of whether CCHFV is circulating in a country, methods for the detection of IgG antibodies are. If there is any possibility or suspicion that diagnostic samples could be contaminated with CCHFV, they should be handled under an adequate biosafety level and all persons dealing with those samples should be aware of the possible risk and should use personal protective equipment to avoid human infections.

2.12.2. Detection and identification of the agent

For testing animals for viraemia, rapid diagnosis can be achieved by detection of viral nucleic acid in serum or plasma using conventional (Burt *et al.*, 1998) or real-time reverse transcription (RT-) PCR (Sas *et al.*, 2018),

or by demonstration of viral antigen (Shepherd et al., 1988). Specimens to be submitted for laboratory confirmation of CCHF include blood and liver samples. Because of the risk of laboratory-acquired infections, work with CCHFV should be conducted in appropriate biosafety facilities.

The virus can be isolated from serum and organ suspensions in a wide variety of cell cultures, including Vero, LLCMK2, SW-13, BSR-T7/5, CER and BHK21 cells, and identified by immunofluorescence using specific antibodies. Isolation and identification of virus can be achieved in 1–5 days, but cell cultures lack sensitivity and usually only detect high concentrations of virus present in the blood.

2.12.3. Virus isolation in cell culture

CCHFV can be isolated in mammalian cell cultures. Vero cells are commonly used, usually yielding an isolate between 1- and 5-days post-inoculation. CCHFV is poorly cytopathic and thus infectivity is titrated by demonstration of immunofluorescence in infected cells (Shepherd et al., 1986). SW-13 cell line has also been used extensively for virus isolation, producing plaques within 4 days. Identification of a CCHFV isolate has to be confirmed by immunofluorescence or molecular techniques (Burt et al., 1998; Shepherd et al., 1986).

2.12.4. Nucleic acid detection

Molecular-based diagnostic assays, such as RT-PCR, serve as the front-line tool in the diagnosis of CCHF, as well as other viral haemorrhagic fevers (Drosten et al., 2003). The benefit of molecular diagnostic assays is their rapidity compared to virus culture, often allowing

a presumptive diagnosis to be reported within a few hours after receiving a specimen (Burt et al., 1998).

The RT-PCR is a sensitive method for diagnosis, but because of the genetic diversity of CCHFV, there might be some challenges with regard to design of primers or probes that allow detection of all circulating strains of the virus. Indeed, based on geographical origin and phylogenetic analyses of the S gene segment, CCHFV has previously been classified into nine geographical clades – four predominantly diffused in Africa, three in Europe, and two in Asia.

Several real-time RT-PCR assays that detect strains from different geographical locations have been evaluated (Gruber et al., 2019). While some assays have been shown to be highly sensitive, detecting as little as 10 viral RNA copies per ml of plasma, it is necessary to combine at least two molecular assays to ensure detection of the different CCHFV clades (Gruber et al., 2019).

2.12.5. Serological tests

Virus neutralisation assays, generally considered to be highly specific, are rarely used for CCHFV diagnosis. Members of the Orthonairovirus genus generally induce a weaker neutralising antibody response than members of other genera in the family *Nairoviridae*. Another drawback is the necessity to perform this assay in high biosafety containment because it uses live virus (Burt et al., 1994; Rodriguez et al., 1997). Currently, there are only a few CCHFV commercial kits for IgM or IgG by ELISA or immunofluorescence (IFA). These are all designed for the human diagnostic market. However, it is possible to adapt these commercial ELISAs and IFAs

for serological testing in animals. In addition, some in-house ELISAs have been published for the detection of CCHFV-specific antibodies in animals. Diagnostic performance for humans have been compared between the methods using sensitivity, specificity, concordance and degree of agreement with particular focus on the phase of the infection (Emmerich et al., 2021).

Available serological test systems detect anti-CCHFV IgM and IgG antibodies accurately, but their diagnostic performance varies with respect to the phase of the infection. In the early and convalescent phases of infection, the sensitivity for detecting specific IgG antibodies differed for the ELISA. Both test systems based on immunofluorescence showed an identical sensitivity for detection of anti-CCHFV IgM antibodies in acute and convalescent phases of infection.

IgM antibodies in livestock (sheep, goat and cattle) can be detected by using an IgM-capture ELISA. IgG antibodies can be detected by an IgG-sandwich or indirect ELISA, and total antibodies can be detected by competition ELISA. The benefit of competitive ELISA is the capacity to investigate different animal species, because they are host species independent. Commercial kits for the detection of CCHFV-specific antibodies or the detection of viral antigen are available.

The limiting factor for the replication of these protocols in other laboratories is the availability of antigens and (where relevant) specified monoclonal antibodies. Most of the tests described for livestock and wild animals have not undergone a formal validation process (Mertens et al., 2013). One of the biggest challenges for such validation studies is the availability of an adequate number of positive well characterised control samples.

2.13. Prevention and control

Preventing CCHFV infection involves addressing the many risk factors for CCHFV exposure, for farmers, wearing appropriate clothing such as long sleeves and pants, reducing activities in tick-infested areas and use of integrated pest management strategies to reduce tick population in farm environment can minimize risk of CCHFV infection via tick bites (Kumar *et al.*, 2020) While handling livestock or domesticated animal, appropriate acaricidal agents should be used to control tick population .protective clothing and gloves should be used whenever there is chance of contact with skin or mucous membranes of viremic animals ,particularly when blood and tissue are handled Consumption of unpasteurized milk and uncooked meat should be avoided.

Human-to-human transmission of CHF virus is seen when direct contact with blood and body fluids occurs (Mishra, 2011) Furthermore, using protective equipment when slaughtering tick-invested livestock either in backyard slaughter processes or in abattoirs may reduce exposure via contaminated animal products in the health –care setting ,personal protective equipment is essential to prevent transmission during care of patients infected with CCHFV (Leblebicioglu *et al.*, 2016; Gozel *et al.*, 2013) In case of penetrating injury ,the area should be washed with soapy water and ethanol and the person should be followed up for 14 day in terms of CCHF.

Other exposures should be placed under medical surveillance and individuals should be instructed to record their temperatures twice daily, If body temperature of 38.3 C or higher is measured, ribavirin should be initiated promptly as a presumptive treatment (Borio *et al.*, 2002) The universal precautions (glove, gowns, goggles, masks)should be instituted

during contact with the patient and their secretion, and invasive procedures should be minimized as soon as possible (Whitehouse, 2004)

2.14. Vaccination

Vaccine against CCHF is not available in most of the countries. However, a formalin inactivated vaccine derived from suckling mouse brain has been used in Bulgaria and former Soviet Union (Papa *et al.*, 2011) There is no vaccine available for animal use (WHO. 2013).

The major hindrance in developing vaccine against CCHF virus is the great genetic variation noted in different strains. Despite this genetic variability, Ahmed *et al.*, have shown that some epitopes are conserved, and CCHF vaccines may have to be either immunogens derived from several CCHFV strains, or can target the immune response on conserved neutralizing epitopes. (Ahmed *et al.*, 2005) A naked conventional CCHF m RNA vaccine has been described. The vaccine expresses the NP of the CCHF AnK-2 strain flanked by a 5 cap (anti-reverse cap analog), 3-poly A tail, 3 and 5 untranslated regions to enhance stability and translation of the construct.

Intramuscular vaccination of IFNAR-/- using a prime boost approach provided 100% protection following viral challenge while a single dose of the vaccine construct conferred 50% protection (Aligholipouretal2019). Recombinant viruses have been extensively investigated as vectors in gene therapy and gene delivery for vaccine development. Viral expression systems explored for CCHF vaccine development comprise the modified Vaccinia Ankara virus (MVA) (Buttigieg *et al.*, 2014; Dowl *et al.*, 2016).

The CCHFV Turkey-Kelkit 06 strain was propagated in cell culture, harvested, and inactivated by formaldehyde to prepare an inactivated vaccine. The vaccine was mixed with the Imject Alum adjuvant and delivered intraperitoneally using a

prime ,boots, and boots strategy in IFNAR $-/-$ mice (Canakoglu *et al* .,2015) DNA vaccine do provide an attractive alternative for emerging and re-emerging pathogens such as CCHFV .

DNA vaccines are temperature stable and can be designed to incorporate specific immune-genic viral proteins desired for immunization. Depending on the immune correlates of protection, genetic vaccines can be tailored to raise either type 1T-helper (Th)or type 2 Th cell responses (Robinson and Pertmer. 2000)

Chapter three

Materials and

Methods

Materials and Methods

3.1 Materials:

The materials used in this study are listed in table (3-1):

Table (3-1): The materials used in the study:

Devices and Materials	company	Origin
Clot Activator/ Gel Tube 6ml	BIOTIGH	China
EDTA Tube 2ml	BIOTIGH	China
Eppendorf tube	BIOTIGH	China
Freezer	Concord	Lebanon
GLOVES	CRV	China
Micropipettes	Eppendorf	Germany
Micropipettes tips	Eppendorf	Germany
Disposable syringe 20ml	IGG company	China
Disposable syringe 10ml	IGG Company	Iraq
CBC Automated 5 Differential	Kjmr-iv Rotator mixer	China
Disposable syringe 5ml	Medical Equipment Co.Ltd.	China
Centrifuge	Nuve	Turkey
RX(Daytona)	RANDOX Laboratories Ltd	British

3.2 Kits:

3.2.1: ELISA kit (Sunlog, China) that were used in this study which composed of the following reagents

Table (3-2): Materials provided with the kit:

No.	Material	Quntitiy(96 Determinations)
1-	Chromogen solution A	6 ml x 1 bottle
2-	Chromogen solution B	6 ml x 1 bottle
3-	HRP-Conjugate Reagent	6 ml x 1 bottle
4-	Microelisa Stripplate	1
5-	Negative Control	0.5 ml X bottle
6-	Positive Control	0.5 ml X bottle

7-	Samples Diluent	6 ml x 1 bottle
8-	Sealed Bags	1
9-	Stop Solution	6 ml x 1 bottle
10-	Wash Solution	20 ml(30X) X 1 bottle

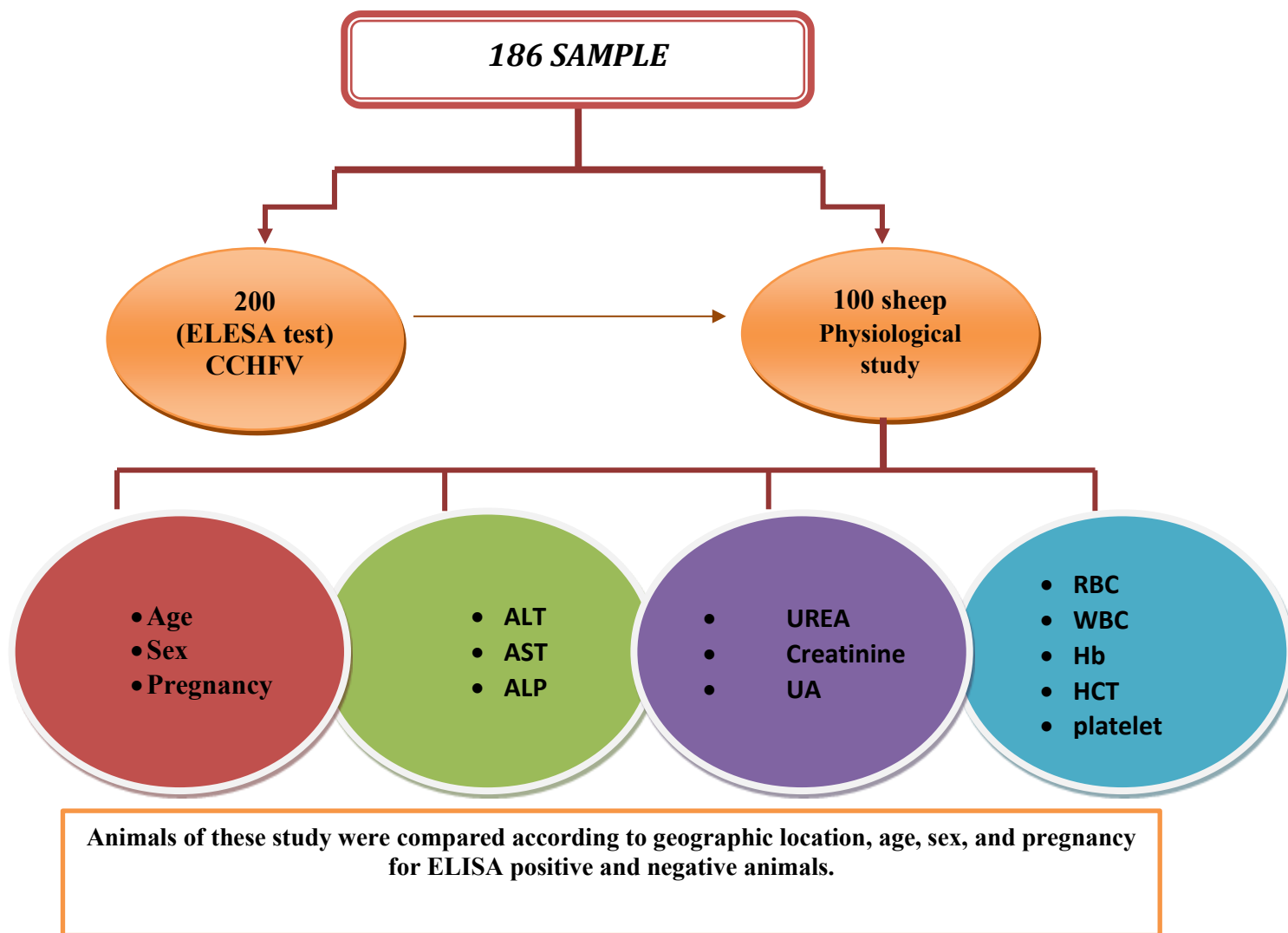
3.2.2. Kits for Physiological study

ALP	Randox	UK(Britain)
ALT	Randox	UK(Britain)
AST	Randox	UK(Britain)
Creatinine	Randox	UK(Britain)
Devices and Materials	company	Origin
UA	Randox	UK(Britain)
Urea	Randox	UK(Britain)

3.3 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).

Experimental Design



3.4. 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. The diagnostic sensitivity and specificity of the ELISA Kit by procedure:

In the Microplate, number the corresponding micropores of the sample in sequence, leave two well as negative control, two well as positive control and one empty well as blank control. (Blank control hole don't add samples and HRP-Conjugate reagent, the rest step operation is same), Negative and positive control in a volume of 50 ul are added to the negative and positive control well respectively. In sample well, 40 ul sample dilution buffer and 10 ul sample are added (dilution factor is 5). Sample should be loaded onto the bottom without touching the well wall. Mix well with gentle shaking after that incubate 30 min at 37C after sealed with Closure plate membrane, dilute the concentrated washing buffer with distilled water (30 time for 96T).

carefully peel off Closure plate membrane, aspirate and refill with the wash solution. Discard the wash solution after resting for seconds. Repeat the washing procedure for 5 times then, add 50ul HRP-Conjugate reagent to each well except the blank control well after that incubate at the same time and temperature degree in the prior step, then wash the closure plate membrane at the same method the prior step.

Chromogen Solution A (50ul) and Chromogen solution B (50ul) were added to each well, mix with gently shaking and incubate at 37C FOR 15minutes. please avoid light during coloring and add 50ul stop solution to each well to terminate the reaction. The color in well should change from blue to yellow. The final step was

Read absorbance O.D at 450nm using a Microtiter plate Reader. The OD value of the blank control well is set as zero. Assay should be carried out within 15 minutes after adding stop solution.

3.5. Determination of Results:

The average value of positive control ≥ 1.00 ; the average value of negative control ≤ 0.10 . The critical value (CUT OFF) calculation: critical value = the average value of negative control +0.15. For determinate Negative and positive judgement: if the $OD < \text{CUT OFF}$, the sample is Sheep CCHF-IgG negative and if OD value $\geq \text{CUT OFF}$, the sample is Sheep CCHF-IgG positive.

3.6. Hematological Parameters:

The hematological parameters were done in Laboratory of Internal Medicine/Collage of Veterinary Medicine University of Shutrah 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).

3.7. Measurements Alanine Aminotransferase (ALT) (U/I):

Principle:

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



Procedure: Measurement against reagent blank:

Reagents	Reagent Blank	Sample
Sample	-----	0.1ml
Buffer (R1)	0.5ml	0.5ml
Distilled Water	0.1ml	-----
Mixed test tubes and incubated for exactly 30 min. at 37°C		
2,4 Dinitrophenylhydrazine (2,4 DNP) (R2)	0.5ml	0.5ml
Mixed test tubes and allow to stand for exactly 20 min. at 20 to 25°C		
Sodium Hydroxide (R3)	0.5 ml	0.5 ml
Mixed the test tubes and then read the absorbance of sample (A_{sample}) against the reagent blank after 5 min. at wave length 546nm.		

Calculation: From a table in paper of kit can obtain the activity of ALT in the serum by plotting the measured absorbance against the transaminase activities in U/I.

3.8. Measurements of Aspartate Aminotransferase (AST) (U/I):**Principle:**

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



Procedure: Measurement against reagent blank:

Reagents	Reagent Blank	Sample
Sample	-----	0.1ml
Buffer (R1)	0.5ml	0.5ml
Distilled Water	0.1ml	-----
Mixed test tubes and incubated for exactly 30 min. at 37°C		
2,4-DNP (R2)	0.5ml	0.5ml
Mixed test tubes and allow to stand for exactly 20 min. at 20 to 25°C		
Sodium Hydroxide (R3)	0.5 ml	0.5 ml
Mixed, read the absorbance of the sample (A sample) against the reagent blank after 5 min. at wave length 546nm.		

Calculation: From a table in the paper of kit the activity of ALT in the serum can be obtained by plotting the measured absorbance against the transaminase activities in U/I.

3. 9. Measurements Alkaline Phosphatase (ALP) (U/I):

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

Principle:

The following equation could be an estimation of ALP activity (Tietz,1999):

**Procedure:**

Reagents	Serum sample	Serum blank	Standard	Reagent blank
Reagent 1	2 ml	2ml	2ml	2ml
Incubated for 5 min. at 37°C				
Serum	50μl	-----	-----	-----
Reagent 2	-----	-----	50μl	-----
Incubated for exactly 15 min. at 37°C				
Reagent 3	0.5ml	0.5ml	0.5 ml	0.5 ml
Mixed test tubes well				
Reagent 4	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Serum	-----	50μl	-----	-----
Distal water	-----	-----	-----	50μl
Mixed the test tubes and incubated at room temperature for 10 min. in the dark room and then reading the absorbance of the sample (A_{sample}) against the reagent blank after at wave length 510nm.				

Calculation:

$$\text{ALP (U/I)} = \frac{\text{Abs Serum sample} - \text{Abs serum blank}}{\text{Abs standard}} \times N$$

N= 141,8 U/I

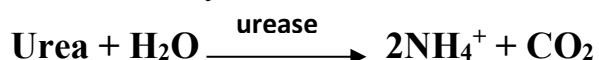
Abs = Absorption

3.10. Measurements of Urea:

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

Principle of the test

In this assay system the reaction involved is as follows:



Procedure

Solution	Blank	Standard	Sample
Urea Standard (S)	-	10µl	
Sample	-	-	10µl
Reagent (A)	1.0 mL	1.0 mL	1.0 mL
The tubes were mixed thoroughly and incubated for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.			
Reagent (B)	1.0 mL	1.0 mL	1.0 mL
Mxed thoroughly and incubated for 10 minutes at room temperature (16-25°C) or for 5 minutes at 37°C.			
The absorbance of the standard and samples against the blank were read at 600 nm, the colour is stable for at least 2 hours.			

Calculation

The urea concentration in the sample is calculated using the following general formula:

A sample

$$\frac{A \text{ Standard}}{\text{C Standard}} \times \text{Sample dilution factor} = C \text{ Sample}$$

A Standard

3.11. Measurements of Creatinine:

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

Principle: Colorimetric reaction (Jaffe reaction) of creatinine with alkaline picrate measurement kinetically at 490 nm (490-510), without any per treatment step. This reaction has been improved (specificity, speed and adaptability) by the development of an initial-rate method (Tietz, 1999).

3.11. 1.Manual procedure:

The procedure used in this measurement for non-icteric specimen using working reagent as follow:

Solution	Blank	Standard	Sample
Working reagent	1 ml	1ml	1ml
DW	100µl	-----	-----
Standard	-----	100µl	-----
Serum	-----	-----	100µl
Mix well. Then record absorbance A1 at 490 nm(490-510) against reagent blank or distal water . Exactly 2 minute after the first reading, record absorbance A2.			

Calculation:

Calculate the results as follows:

$$\text{Creatinine Concentration} = \frac{(A2 - A1) \text{ sample}}{(A2 - A1) \text{ Standard}} \times n$$

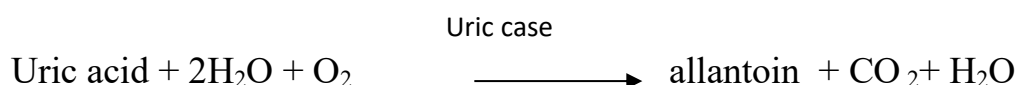
n= Standard Concentration

3.12. measurement of uric acid

Uric acid concentration in plasma was determined according to method of Barham and Trinder (1972). From the point of view of specificity and sensitivity the enzymatic determination by uricase is undoubtedly the method of choice (Curtius and Roth, 1974).

3.12.1. Principle

Uric acid present in the sample is determined enzymatically according to the following reaction:



3.12.2. Procedure:

Firstly, prepare the working solution by reconstitute the contents of one vial of reagent 3 with 25 ml of reagent 2. Prepare three test tubes labeled as blank, standard and serum.

	Reagent blank	Standard	Serum
Reagent1 (standard)	-	20 µ L	-
Serum	-	-	20 µ l
Working solution	1 ml	1 ml	1 ml

Mixed well and put in water bath at 30 °C for 5-10 minutes. Then read the results at 520nm.

3.12.3. Calculation:

$$\text{Serum uric acid (mg / dl)} = \frac{\text{O.D serum}}{\text{O.D standard}} \times 8$$

3.13. 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.

Chapter Four

Results

4. Results:

4-1. Distribution of all studied samples according to region:

The results of the current study show significant difference ($p \leq 0.01$) 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 , Garimat 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 ($p \leq 0.01$) of sheep which have seropositivity for CCHF compare with negative samples, The highest positive percentage recorded was (92.85, 90, 90) for Al-Eakikah, Garimat 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 (4-1): Distribution of all studied sheep (positive and negative) according to region:

Sample Region	Total		Positive		Negative		P.Value
	N	%	N	%	N	%	
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).							

4-2. Distribution of all studied samples according to sex:

As illustrated in table (4-2), 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 $p \leq 0.01$ between males and females, as Males have a greater proportion of favorable outcomes compared with female.

Table (4-2): Distribution of all studied sheep (positive and negative) according to sex

Sample Sex	Total		Positive		Negative		P.Value
	N	%	N	%	N	%	
Female	130	69.89	97	74.61	33	25.38	0.000
Male	56	30.10	47	83.92	9	16.07	0.000
Total	186	100	144	79.26	42	20.72	0.000
P. Value	0.0001*8		0.0007**		0.0041**		
**(p≤0.01).							

4-3. Distribution of all studied samples according to pregnancy and non pregnancy ewe:

The results as presented in table (4-3) demonstrates that a distribution of ewes according to the status of their pregnant for the purpose of comparison. The total number of 130 ewe was comprised of 42 pregnant, which constitutes (32.30% of the total). On the other hand, 88 ewes did not have pregnancies, which constitutes (67.69% of the total). Out of the 42 pregnant ewes, 31 tested positive (73.80%) and 11 tested negative (26.19%). Out of the 88 ewes who are not pregnant, 66 of them

tested positive (75.00%) and 22 of them tested negative (25.00%). Of the 130 ewes in total, 97 (74.4%) tested positive and 33 (25.59%) tested negative.

The study indicates that there is a significant difference $p \leq 0.01$ between positive and negative samples in pregnant and non-pregnant ewes.

Table 4-3: Distribution of all studied samples according to pregnancy and non-pregnancy ewe:

Sample Female	Total		Positive		Negative		P.Value
	N	%	N	%	N	%	
Pregnant	42	32.30	31	73.80	11	26.19	0.000
Non pregnant	88	67.69	66	75	22	25	0.000
Total	130	100	97	74.4	33	25.59	
P.Value	0.0001**		0.0091**		0.068 NS		
**(p≤0.01).							

4-4. Distribution of all studied samples according to age.

Table (4-4) displays data regarding a significant differences $P \leq 0.01$ between 186 sheep that divided according to age, with results categorized as either positive or negative for seropositivity of CCHF. The sample is divided into three age groups: (<1 year), (1-5 years), and (5-9 years).

The "Less than 1 year" group consists of 45 sheep, which accounts for 24.19% of the total. The "1-5 years" group comprises 107 sheep, representing 57.52% of the total. Lastly, the "5-9 years" group includes 34 sheep, making up 18.27% of the total.

In the "Less than 1 year" group, 32 out of 45 sheep had positive results (71.11%), and 13 had negative results (28.88%). On the other hand, In the "1-5 years" group, 84 out of 107 sheep had positive results (78.50%), and 23 had negative results (21.49%) however in the "5-9 years" group, 28 out of 34 sheep had positive results (82.35%), and 6 had negative results (17.64%). While among the total

186 sheep, 144 had positive results (77.32%), and 42 had negative results (22.67%).

Table 4-4: Distribution of all studied samples according to age.

Sample	Total		Positive		Negative		P.Value
Age	N	%	N	%	N	%	
Less than 1years	45	24.19	32	71.11	13	28.88	
1-5 years	107	57.52	84	78.50	23	21.49	
5-9 years	34	18.27	28	82.35	6	17.64	
Total	186	100	144	77.32	42	22.67	
P.Value	0.0001**		0.0001**		0.0097**		
**(p≤0.01).							

4-5. 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 .

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 4-5: Hematology parameter according into + CCHF and - CCHF

paramet	Positive	Negative		
Sample	N(72)	N(28)	T-test	P.value
WBC($10^9/L$)	9.58	12.31	2.056*	0.0268
RBC($10^{12}/L$)	7.81	9.46	1.502*	0.0389

PLT(R 10 ⁹ /L)	322	326	42.71 NS	0.694
HGB(g/Dl)	8.6	8.92	0.893 NS	0.866
HCT(%)	27.3	28.7	4.021 NS	0.857
* (P≤0.05), NS: Non-Significant.				

4-6. Hematology parameter according into sex +CCHF and -CCHF

The obtained from the present study revealed a comparison of various blood parameter readings categorized by sex and outcome (positive vs. negative). The sample number was 100, consisting of 20 males and 80 females, among the male , 17 tested positive for the CCHF, while 3 tested negative. Out of the female sheep, 55 ewe showed positive test results and 25 showed negative test results table (4-6).

The result appeared a significant differences $P \leq 0.01$ between those groups for RBC, WBC, platelets, hemoglobin, and hematocrit parameter in positive and negative samples while, no significant differences between female positive and negative samples in red blood cell and hemoglobin parameter.

Table 4-6: Hematology parameter according into sex + CCHF and - CCHF

Sample parameter	Male N (20)		Female N (80)		LSD Value
	Positive (17)	Negative (3)	Positive (55)	Negative (25)	
WBC(10 ⁹ /L)	10.10± 0.72 ab	9.23± 0.66 b	9.03±0.61 b	10.97±0.86 a	1.267*
RBC(10 ¹² /L)	9.21±0.69 b	12.05± 0. 86 a	8.58± 0.54 b	8.85± 0.61 b	1.506*
PLT(R 10 ⁹ /L)	427± 31.8 a	295± 16.9 c	292± 21.5 c	345± 26.8 b	41.702*
HGB(g/Dl)	9.03± 0.63 b	10.6±0.69 a	8.4± 0.57 b	8.3± 0.60 b	1.294*
HCT(%)	28.8± 1.7 ab	31.6± 2.5 a	26.8± 1.4 b	27.74± 1.8 ab	3.137*
Mean having with the different letters in same column differed significantly. (p≤ 0.01)					

4-7. Comparison of Hematology in pregnancy and non-pregnancy female:

Table (4-7) exhibits blood parameter data for two groups: pregnant and non-pregnant ewe, as well as their associated test results (positive and negative). Out of a total of 42 pregnant ewes, 32 ewes tested positive for the test, whereas 10 ewes tested negative. Out of the 38 non-pregnant ewes, 23 tested positive and 15 tested negatives for the seropositivity of CCHF.

The result indicating significant differences $P \leq 0.01$, particularly between non-pregnant groups and pregnant groups of all hematological parameters while no significant changes in HCT between pregnant and non-pregnant ewe. In addition, Our result appeared significant differences $P \leq 0.01$ between positive and negative samples in all the hematology parameter except HCG was appeared no significant differences between positive and negative samples

Table (4-7): Hematology results according to pregnancy and non-pregnancy female

Sample parameter	Pregnant N (42)		Non – Pregnant N (38)		LSD Value
	Positive (32)	Negative (10)	Positive (23)	Negative (15)	
WBC ($10^9/L$)	8.86±0.57 b	9.8±0.71 b	9.19±0.68 b	12.14±0.84 a	2.007*
RBC ($10^{12}/L$)	8.0±0.60 b	8.05±0.54 b	9.15±0.68 ab	9.63±0.73 a	1.445*
PLT ($R 10^9/L$)	278±20.6 b	343±27.1 a	306±22.8 ab	346±30.6 a	41.94*
HGB (g/Dl)	8.0±0.55	8.04±0.61	8.86±0.58	9.2±0.63	1.35 NS
HCT (%)	25.7±1.4 b	26.57±1.2 ab	27.9±1.7 a	28.9±2.4 a	2.985*
Mean having with the different letters in same column differed significantly. ($p \leq 0.05$)					

4-8. Distribution of Hematological Parameters According to Age:

A significant difference in blood parameters are apparent among various age groups and between positive and negative of seropositive for CCHF within these age groups. There were a total of 15 ewes that were less than one year old. Out of

these, 8 were positive and 7 were negative. The group consisted of 51 ewes, ranging in age from 1 to 5 years. Among them, 36 were classified as positive and 15 as negative. The group consisted of 34 ewes, ranging in age from 5 to 9 years. Among them, 28 ewes tested positive and 6 tested negative. The statistical analysis in table (4- 8) revealed significant differences $P \leq 0.01$ between positive and negative ewe in the WBC, PLT, HGB, and HCT parameters at less than one year of age. However, no significant changes were found in the RBC parameter between them. There were no significant differences in red blood cell count (RBC), platelet count (PLT), hemoglobin (HGB), and hematocrit (HCT) across ewe aged 1 to 5 years. However, there was a significant difference in white blood cell count (WBC) between samples that tested positive and negative. Furthermore, the hematological tests revealed significant differences in WBC, RBC, PLT, and

Age parameter	Less than 1 years N (15)		1 – 5 years N (51)		5 – 9 years N (34)		P
	Positive (8)	Negative	Positive	Negative	Positive	Negative	

HCT levels between positive and negative samples among individuals aged 5 to 9 years. However, there were no significant variations observed in the HGB parameter.

Table (4- 8): Distribution of Hematological parameters according to Age

		(7)	(36)	(15)	(28)	(6)	
WBC ($10^9/L$)	9.14 $\pm$ 0.65 c	10.43 $\pm$ 0.75 bc	9.04 $\pm$ 0.59 c	10.88 $\pm$ 0.82 b	9.55 $\pm$ 0.74 bc	12.3 $\pm$ 0.89 a	1.612 *
RBC ($10^{12}/L$)	10.26 $\pm$ 0.77 a	11.46 $\pm$ 0.86 a	8.41 $\pm$ 0.55 b	8.46 $\pm$ 0.61 b	8.53 $\pm$ 0.69 b	9.03 $\pm$ 0.72 ab	1.703 *
PLT (R $10^9/L$)	473 $\pm$ 34.8 a	313 $\pm$ 22.5 b	285 $\pm$ 19.6 b	318 $\pm$ 23.7 b	327 $\pm$ 24.1 b	422 $\pm$ 29.7 a	52.78 *
HGB (g/dl)	9.9 $\pm$ 0.74 ab	10.4 $\pm$ 0.77bc	8.29 $\pm$ 0.62 b	8.3 $\pm$ 0.70 b	8.4 $\pm$ 0.56 b	8.6 $\pm$ 0.61 b	1.855 *
HCT (%)	29.8 $\pm$ 1.7 ab	31.4 $\pm$ 2.4 a	26.8 $\pm$ 1.3 b	26.9 $\pm$ 1.8 b	26.9 $\pm$ 1.4 b	28.4 $\pm$ 1.8 ab	3.052 *
Mean having with the different letters in same column differed significantly. ($p \leq 0.05$)							

4-9. Urea, Creatinine, Uric acid ALT, AST and ALP , concentration in carrier and non – carrier sheep.

According to table (4-9) 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 $P \leq 0.01$ 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 (4-9): Urea, Creatinine, Uric acid ALT, AST and ALP, concentration in carrier and non – carrier sheep.

Samples Parameter	Positive	Negative	T-test	P - Value
	N (72)	N (28)		
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).				

4.10. Distribution of Urea , Creatinine, Uric acid ALT, AST and ALP , concentration according to sex:

Table (4-10) shows the blood biochemical parameters classified by sex and test results, with separate groups for males and females, also divided by positive (+) and negative (-) results .The total males were 20 sheep (17 positive, 3 negative) while the females were 80 (55 positive, 25 negative).

A significant differences $P \leq 0.01$ were seen between males and females in both positive and negative samples, with the most significant differences observed between positive males and other groups. Males frequently exhibited higher urea levels compared to females. Regarding creatinine, it was found that there were significant variations ($p \leq 0.05$) between the groups. Specifically, males, those who were positive, had larger amounts of creatinine than females. Furthermore, a significant differentiation ($p \leq 0.05$) was observed between the negative sheep and the other groups, as the negative males had elevated levels of AST. Throughout the study, the positive groups had slightly elevated levels of ALT, although the observed increases were only small enough to be statistically significant while, ALP level of sheep who displayed a negative test for CHHF showed significantly elevated ($p \leq 0.05$) levels of ALP compared to the other groups.

Table (4-10): Distribution of Urea, Creatinine, Uric acid, ALT, AST and AL, concentration according to sex:

Sample parameter	Male N (20)		Female (80)		LSD Value
	+(17)	-(3)	+(55)	-(25)	
Urea (mg/dL)	45.30±2.74 a	36.8±2.04 b	34.84±1.45 b	39.91±2.08 ab	6.705*
Creatinine (mg/dL)	0.53±0.09 a	0.49±0.06 ab	0.36±0.04 c	0.40±0.07 bc	0.127*

UA (mg/dL)	0.18±0.05 b	0.15±0.03 b	0.96±0.27	0.19±0.04 b	0.122*
AST(U/L)	93.49±4.81 b	111.64±8.02 a	86.04±4.17 b	92.96±4.93 b	9.604*
ALT(U/L)	28.69±1.67 a	25.5±1.30 b	28.84±2.06 a	26.44±1.56 ab	2.966*
ALP(U/L)	66.39±3.52 bc	125.68±8.44 a	75.22±3.79 b	55.32±2.41 c	12.750*
Mean having with the different letters in same column differed significantly. *(p≤ 0.05)					

4-11. Distribution of Urea, Creatinine, Uric acid ALT, AST and ALP, concentration according pregnant state

The results illustrated in table (4-12) showed that data comparing of various blood biochemical parameters between pregnant and non-pregnant women, further subdivided into positive and negative test result groups: The pregnant ewes were: 42 ewes, with 32 positive and 10 negative cases, while the non-pregnant ewes were 38, with 23 positive and 15 negatives for the hemorrhagic fever test.

The current study showed a significant higher ($p \leq 0.05$) of urea levels in negative pregnant ewe as compare with other group while higher creatinine levels in positive pregnant ewe compared to non-pregnant ewe, While the level was uric acid , showing much higher ($p \leq 0.05$) of the levels in non-pregnant positive ewe as compare with other group. Pregnant ewe with negative test for CCHF shows higher AST and ALP levels compared to other groups but no significant changes in the ALT levels across groups.

Table (4- 11): Distribution of Urea, Creatinine,Uric acid ALT,AST and ALP , concentration according pregnant state :

Sample parameter	Pregnant N (42)		Non - Pregnant N (38)		LSD Value
	Positive (32)	Negative (10)	Positive (23)	Negative (15)	
Urea (mg/dL)	36.72±1.92 b	53.88±2.66 a	32.96±1.42 b	25.94±1.27 c	6.705*
Creatinine (mg/dL)	0.53±0.09 a	0.49±0.06 ab	0.36±0.04 c	0.40± 0.07 bc	0.127*
UA (mg/dL)	0.18±0.05 b	0.15±0.03 b	0.96±0.27 a	0.19±0.04 b	0.122*
AST(U/L)	93.49±4.91 b	111.64±8.02 a	86.04±4.17 b	92.96±4.93 b	9.604*

ALT(U/L)	28.69±1.67 a	25.5±1.30 b	28.84±2.06 a	26.44±1.56 ab	2.966*
ALP(U/L)	66.39±3.52 bc	125.68±8.44 a	75.22±3.79 b	55.32±2.41 c	12.750*
Mean having with the different letters in same column differed significantly. *(p≤ 0.05)					

4-12. Distribution of Urea, Creatinine, Uric acid ALT, AST and ALP, concentration according age:

The data as illustrated in the Table (4-12) demonstrated that comprehensive data of blood biochemical parameters for distinct age groups, categorized by positive or negative test findings within each age group.

There is a significant difference ($p \leq 0.05$) in urea levels, with the highest levels typically found in the 1 – 5 years age group, particularly in the negative subgroup. However, there are no significant changes in creatinine levels across age groups and test results. On the other hand, there is a significant difference ($p \leq 0.05$) in uric acid levels, especially notable in the Less than 1 year age group. On the other hand, both the positive and negative groups exhibit higher levels of AST compared to older age groups. Additionally, the levels of ALT are significantly higher ($p \leq 0.05$) in the 1–5 years positive group and the 5–9 years negative group. There were notable variations in ALP levels, with the highest levels observed in the group of sheep under 1 year old who tested negative.

Table (4-12): Distribution of Urea, Creatinine, Uric acid ALT, AST and ALP , concentration according age:

Age parameter	Less than 1 years N (15)		1 – 5 years N (51)		5 – 9 years N (34)		P
	Positive (8)	Negative (7)	Positive (36)	Negative (15)	Positive (28)	Negative (6)	
Urea (mg/dL)	15.54±0.95 d	29.43±1.8 c	42.45±2.5 b	51.63±2.8 a	33.22±1.6 c	27.24±1.5 c	7.694*
Creatinine (mg/dL)	0.48±0.09 a	0.46±0.06 a	0.38±0.04 ab	0.48±0.07 a	0.47±0.07 a	0.28±0.05 b	0.104*
UA (mg/dL)	0.30±0.02 a	0.15±0.04 b	0.20±0.04 ab	0.19±0.03 ab	0.17±0.02 b	0.18±0.05 ab	0.125*
AST(U/L)	128.70±7.4 a	129.11±8.1 a	86.62±4.5 c	75.26±3.9 c	81.02±4.2 c	104.15±6.8 b	17.926*
ALT(U/L)	16.08±0.72 c	27.84±1.5 b	32.15±2.5 a	22.05±1.4 bc	24.66±1.8 b	36.15±2.6 a	7.541*
ALP(U/L)	38.42±2.4 d	116.60±8.3 a	87.53±5.2 b	62.68±3.7 c	56.20±2.9 cd	63.91±3.1 c	19.025*
Mean having with the different letters in same column differed significantly. (p≤ 0.05)							

Chapter Five

Discussions

5. Discussions:

5.1. Geographical Distribution:

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 Tchegnna *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, Telmadarairy *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).

Other studies such as Atim *et al.*, 2023 recorded CCHFV antibodies were detected in 110 (94%) out of 117 cattle and 83(89.3%) out of 93 goats, these result higher infection comparative with this study was recorded 78.53%.

The current study higher than (Saltik, 2022) was found to be seropositive (50/97) (51.54%), including 53.48% (46/86) donkeys and just 36.36% (4/11) horses.

The different regions of Thi- Qar such as Al-eakikah , Al- rifai and Al- garma region which recorded about 92.85% , 90% and 90 % respectively comparative with another region of thi qar province that appear low level of infected such as Al-fadhliyya , Al-tar and Al- manar which recorded about 75%, 50% and 60.71% respectively . Furthermore, in recent related study conducted in mousl, the CCHF seroprevalence rate of was recorded 14% using indirect IgG ELISA and comprised 19.16% of sheep and 6.25% of goats (Altaliby *et al.*, 2021).

This results disagreement with (Dakhil,2024) was recorded 52.9% and 19% in sheep of Thi- Qar and Basrah province respectively while, in goat was recorded 20.8% and 10.3% in Thi-qar and Basrah province respectively. Albayrak *et al.*, (2012) reported that anti-CCHF rate in sheep and goats were 85% and 66% respectively.

5.2. Epidemiological Study:

The current study focused on and attempted to connect the physiological states, sex and age with infected animals. The male sex appears high level of infected about 83.92% comparative with female was appeared low level of infected by

CCHF virus about 74.61% , the high level of infected in male may be due to small number of males in this study or reasons related to the virus receptors in males.

This result agreement with other research (Dakhil,2024) which recorded high level of infected female 48.4% and 20.9% comparative with male which recorded about 21.9% and 8.8% in Thi- Qar and Basrah province respectively, in all epidemiological diseases, the gender is not role in the infection rate unless presence a specific condition. Therefore, the high incidence rate in males may be an artifact caused by increase male sampling comparative with female samples.

The current study was showed significant differences between positive non pregnant ewe about 75% and positive pregnant ewe about 73.80 at ($P \leq 0.01$) while the present study was appeared no significant differences between negative specimens in pregnant and non -pregnant about 26.19 and 25 respectively at ($P \leq 0.01$)

Also, the present study was showed a high level of infected in age from 5 to nine years about 82.35% comparative with another age ranges which appeared about 71.11 and 78.50 the high level of infected in this age may be because of increase of animal number at this age stages .

The result of this study showed disagreement with Dakhil, 2024 which recorded 62.2% and 37.8% in Thi-Qar and basrah province respectively at age 4-5 years while, recorded 31.3% and 44.5% in Thi qar and Basrah province respectively at age 3 months to 1 years in sheep.

A systematic review of 57 published articles on CCHF in all Arabic countries around the world has shown that there were only 321 CCHF cases reported within nine Arabic countries between 1978 and 2021 (Perveen and Khan, 2022).

In 2022, Alhilfi *et al.*,(2023) reported 219 CCHF cases within the first 6 months in Iraq, suggesting that the disease is still persisting in Iraq and the magnitude of the outbreak has increased by 4.3% compared with last year. While, Sabir *et al.*, (2023) studied of the epidemiological data of CCHF in Iraq within the first 6 months of 2023 were kindly provided by the Sulaymaniyah directory of the World Health Organization/Iraq that found of 229 (32%) positive CCHF cases were recorded in 722 suspected patients within the first 24 weeks of 2023.

In addition, 410 CCHF cases were recorded between 2000 and 2007 in Iran in 1029 suspected cases (Chinikar *et al.*, 2008). Moreover, only four CCHF cases were documented in 2022 in Pakistan (Tabassum *et al.*, 2023). Having more than 200 CCHF cases in Iraq in 2 subsequent years suggests a high prevalence of the disease in the country.

5.3. Hematological Parameters:

5.3.1. Complete Blood Picture:

Epistaxis, gingival bleeding, petechiae, large ecchymoses, hematemesis, melena, hematuria, vaginal bleeding, and bleeding from venipuncture sites may occur due to thrombocytopenia and prolonged PT and aPTT in the hemorrhagic phase of CCHF (Ergonul , 2006 ; Doganci *et al.*, 2008). Ocular findings with subconjunctival and retinal hemorrhage and without visual symptoms have also been reported in 73.7% of 19 patients (Engin et al., 2009). In severe cases with massive hemorrhage, intracranial bleeding, massive liver necrosis, and irreversible shock may develop, ultimately leading to death. The reported frequencies of hemorrhagic symptoms in fatal cases were 85% and 81.8% in the studies by Cevik et al., (2008).

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 hematological results of the present study revealed significant differences in WBC and RBC between positive and negative samples. However, no significant differences were observed in PLT, HGB and HCT. The absence of clinical signs in animals with positive samples (carriers) likely accounts for the lack of statistically significant differences in most of the hematological parameters analyzed. The decrease in RBC count, Hb and PCV% may be due to anemia which resulted from inhibition of erythropoietin hormone from the kidney which is important in production of RBCs. The reduction in the above parameters may result from the effect of Crimean- Congo hemorrhagic fever virus on bone marrow and this effect leads to inhibition in erythropoiesis process as well.

The statistically significant variations in hematological parameters between males and females might be explained by the impact of gender on the body's immune system or physiological reaction to infections or medical conditions. These discrepancies in immunological response and blood parameters like WBC count, RBC count, or platelet levels are probably due to variances in physiological constitution or immune activity between males and females.

The absence of significant differences in HGB levels and RBC between positive and negative female samples may be attributed to the probability that positive females were infection carriers who did not exhibit obvious clinical symptoms. Since this condition does not significantly affect the production or breakdown of red blood cells table.

The study showed no statistically significant differences between positive and negative pregnant ewes in WBC, RBC, and HGB. However, significant differences were observed in PLT and HCT. In non-pregnant ewes, significant differences were found in all hematological parameters between positive and negative samples, except for HCT, which did not show any significant differences table.

Also, the statistical analysis was appeared significant differences between positive and negative ewe in WBC, PLT, HGB and HCT parameter at less than one year's age, while no significant differences in RBC parameter between them. The age between 1 to 5 years was appeared no significant differences in RBC, PLT, HGB and HCT, while significant in WBC parameter between positive and negative samples.

Also, hematology tests were appeared significant differences between positive and negative samples at 5 to 9 years of age in WBC, RBC, PLT, and HCT while, no significant differences in HGB parameter between them, the differences of result because of many factors affected to hematology parameter such as parasite, malnutrition and other causes.

The absence of statistically significant differences in white blood cell (WBC) count, red blood cell (RBC) count, and hemoglobin (HGB) levels between positive and negative pregnant ewes suggests that the physiological changes associated with pregnancy may modulate the immune and hematological responses to infection. During pregnancy, the body undergoes various adaptations, including alterations in blood volume and composition, which may mask the effects of infection on these specific hematological parameters.

In contrast, the significant differences observed in platelet (PLT) count and hematocrit (HCT) in pregnant ewes indicate that these parameters are more sensitive to changes associated with infection, possibly due to their roles in

coagulation and fluid balance during pregnancy. Elevated PLT counts may reflect a response to inflammatory processes, while changes in HCT could indicate variations in plasma volume or red blood cell mass.

For non-pregnant ewes, the presence of significant differences in all hematological parameters, except for HCT, between positive and negative samples suggests a more pronounced impact of infection on hematological profiles in this group. This could indicate that non-pregnant ewes exhibit a more typical immune response to infection, with alterations in WBC, RBC, and HGB levels reflecting the body's reaction to the disease. The lack of significant differences in HCT among non-pregnant ewes may imply that factors other than infection, such as hydration status or physiological stress, could be influencing this parameter.

The results of the present study are in line with those obtained by (Rajendran and Hafeez, 2003; Kumar *et al.*, 2010; Barznji *et al.*, 2014) they found that the hematological in sheep infested with *Hyalomma* species were lower in RBC, PCV, HGB, 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). Hemoglobin levels are debatable in the diagnosis of early significant bleeding (Hongzhao *et al.*, (2023). The decreased in hemoglobin levels, may be be result from the beginning of anemia caused by bleeding. Techetgna *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).

5.3.2. Biochemical Study:

The levels of aspartate aminotransferase (AST/GOT) and alanine aminotransferase (ALT) in serum can help diagnose body tissues especially the heart and the liver are injured or not. The present study was research and find relation between carrier ewe to virus and the level of enzyme which secreted from kidney and liver. An elevation in the serum creatinine and urea may be an indication of renal damage, Urea level can be increased by many other factors such as dehydration, antidiuretic drugs and diet, while creatinine is more specific to the kidney, since kidney damage is the only significant factor that increases the serum creatinine level (Nwanjo *et al.*, 2005). Experimentally infected animals, such as calves, horses, guinea pigs, hamsters, and rabbits, develop little or no viremia and high levels of neutralizing antibodies but do not display clinical signs of disease (Shepherd *et al.*, 1989; Nalca and Whitehouse, 2007). In line with our findings, various studies have pointed to the increase in ESR levels in Crimean fever patients (Bozkurt and Esen, 2021; Oygar *et al.*, 2023).

One of the important target organs of the CCHF virus is the liver and hepatocyte infection leads to decreased albumin synthesis and abnormal liver function tests and raised level of liver injury biomarker enzymes such as Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT) Nasirian, r(2020).

Liver infection is characterized by massive hepatocellular necrosis. these elevated biomarkers of liver injury have been recognized as prominent prognostic factors for poorer outcomes in Crimean-Congo hemorrhagic fever and their raised level is often associated with the severity of the diseases,

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.

Also, the present study was connected between pregnant and non-pregnant animals with kidney and liver enzyme of positive and negative carrier ewe, the statistical analysis was appeared significant differences between pregnant and non-pregnant in positive and negative samples at urea , ALP and creatine, ALT in

pregnant, while this study do not find significant differences between them in creatine in non-pregnant , AST in pregnant and ALT in non-pregnant table (4-11), this differences between them may be because of pregnant effect to metabolic and enzyme activity.

Age of the animals is play an important role in the spread of some viruses because of some viruses have special receptors at a certain age, therefore, this study was interested and connected between age of animals and CCHF virus, the statical analysis was appeared significant differences between positive and negative samples in urea UA, ALT and ALP while no significant differences in creatine and AST at less than one years of age. While it was appeared significant differences between them in urea, creatine, ALT and ALP while no significant differences in ALT and ALP at one to five years of age.

The age between five to nine years was appeared significant differences between positive and negative samples in creatinine, UA, AST, ALT and ALP except urea parameter do not find significant differences between them at this age.

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 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).

Chapter Six

Conclusion and Recommendation

6. Conclusions and Recommendations

6.1: Conclusions:

- 1- Sheep may play an important role as a reservoir in the epidemiology of CCHFV.
- 2- CCHFV is highly endemic in the different region of Thi – QAR province
- 3- Seropositivity of CCHFV in sheep is higher in male sheep than female.
- 4- Pregnancy is not risk factor for CCHFV infection in sheep.
- 5- Sheep aged 5 to 9 years have higher CCHFV infection rate.
- 6- Regarding physiological variations (hematological and biochemical parameters), this study revealed the carrier state of sheep with CCHFV have no effects on these parameters.

6.2. Recommendations

- 1- Further Sero-epidemiological studies of CCHFV should be made in wild, other domestic animals and birds in Iraq.
- 2- Conduction of serological and molecular study of CCHFV in human in endemic regions of Iraq, particularly in Thi-qar province.
- 3- Further studies should be made to determine the effect of CCHFV infection on B 12 vitamin, folic acid, iron, lipid profile and oxidative stress in human and animals.
- 4- Conduction of comparative molecular and phylogenetic studies to determine Iraqi CCHFV strains in human, ticks and animals.
- 5- Preventing tick bites, wearing personal protective equipment, and controlling CCHFV in animals by applying acaricides in animal farms.
- 6- Before being killed, animals should be kept in a quarantine for 14 days to guarantee they are free of ticks. This prevented human infection for anyone who came into touch with the carcasses and reduced the chance that the animal would get infected after its killing.

Chapter Seven

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الخلاصة

فايروس حمى القرم والكونغو النزفية مرضا مميتا ينتقل بواسطة القراد وله اعراض سريرية حادة لدى البشر ولكنه لا يسبب اعراضا في الحيوانات البرية والاليفة، يعتبر المرض مستوطن في مناطق كثيرة من العالم وتعتبر احدى المؤشرات الرئيسية على انتشار المرض وتوطنه في منطقة ما هو وجود اجسام مضادة خاصة لفيروس حمى القرم في مجموعات الحيوانات وان العوامل التي تساعد في انتشار الفيروس بين الأنواع المختلفة غير معروفة. أظهرت الدراسات المصلية ان الأغنام مصدر مهم يساعد في انتشار وباء فيروس الحمى النزفية في الطبيعة ومع ذلك هناك حاجة لمزيد من الدراسات حول هذا الموضوع في العراق.

تم اخذ عينات الدم من ٢٠٠ راس من الأغنام مختلفة الجنس والاعمار والحالة الفسيولوجية، بينما تم استخدام ١٨٦ عينة للاختبار الاليزا حسب الطاقة الاستيعابية للكت المستخدم للفحص، وتم كذلك اختيار ١٠٠ عينة مصل للاختبارات الفسيولوجية بعد ظهور نتائج فحص الاليزا للمقارنة بين العينات الموجبة والسالبة.

أظهرت نتائج الدراسة الحالية ارتفاع معنوي في الأغنام الحاملة للفايروس مقارنة بغير الحاملة حيث أظهرت الدراسة ان نسبة الأغنام الحاملة للفايروس بلغت ٧٨,٥٣ % بينما الأغنام غير الحاملة للفايروس أظهرت نسبة 21.46 % في محافظة ذي قار والتي شملت مناطق العيكة والرفاعي والكرمة والتي سجلت نسبة إصابة حوالي 85 % و ٩٠ % و ٩٠ % على التوالي مقارنة بمناطق أخرى من محافظة ذي قار الفضلية والطار والمنار والتي أظهرت فيها مستويات منخفضة من الإصابة والتي سجلت حوالي ٧٥ % و ٥٠ % و ٦٠,٧1 % على التوالي. كذلك ركزت الدراسة الحالية وربطت الحالات الفسيولوجية المختلفة والعمر والجنس مع الحيوانات المصابة، حيث أظهرت الدراسة ارتفاع في نسبة الإصابة لدى الذكور مقارنة بالإناث حيث بلغت الإصابة 83.92 و 74.61 على التوالي، كذلك أظهرت الدراسة ارتفاع في نسبة الإصابة في الأغنام غير الحاملة مقارنة بالأغنام الحوامل، كما أظهرت الدراسة الحالية ارتفاع مستوى الإصابة في الاعمار من ٥ الى ٩ سنوات حيث بلغت 82. ٣٥ % مقارنة مع الاعمار الأخرى والتي سجلت ٧١,١١ و ٧٨,٥٠ على التوالي.

إضافة الى ذلك اظهر التحليل الاحصائي وجود اختلاف معنوي بين الاناث والذكور في تحاليل الدم بين العينات الموجبة والسالبة بينما وجد اختلاف معنوي بين الاناث الحاملة للفايروس وغير الحاملة في كريات الدم الحمراء وهيملوبين الدم.

أظهرت نتائج التحليل الإحصائي وجود فرق معنوي بين الأغنام الحوامل الموجبة والسالبة في فحص كريات الدم البيضاء وكريات الدم الحمراء وهيملوغلوبين بينما لم يكن هناك فروق معنوية في الصفائح الدموية وهيملاتوكريت. أما الأغنام غير الحوامل فقد أظهرت الدراسة وجود فروق معنوية بين العينات الموجبة والسالبة في جميع فحوصات الدم باستثناء هيملوغلوبين لم تكن فروق معنوية بين العينات الموجبة والسالبة.

كما أظهر التحليل الإحصائي وجود فروق معنوية بين الأغنام الموجبة والسالبة في معايير كريات الدم البيضاء و الصفائح الدموية و الهيملوغلوبين و هيملاتوكريت عند عمر أقل من سنة ، في حين لا توجد فروق معنوية في كريات الدم الحمراء ، بينما أظهر معدل العمر من ١ - ٥ سنوات اختلافات معنوية في كريات الدم الحمراء و الصفائح الدموية و الهيملوغلوبين و هيملاتوكريت ، بينما أظهر اختلاف معنوي في كريات الدم البيضاء بين العينات الموجبة والسالبة ن كذلك أظهرت اختبارات الدم وجود اختلاف معنوي بين العينات الموجبة والسالبة عند عمر ٥ الى ٩ سنوات في كريات الدم البيضاء و الحمراء و الصفائح الدموية و هيملاتوكريت بينما لا توجد اختلافات معنوية في الهيملوغلوبين بين العينات الموجبة والسالبة.

تأثر الكلية والكبد بفيروس الحمى النزفية من CCHFV لذلك الدراسة الحالية ربطت بين انزيمات الكبد والكلية والحيوانات الحاملة وغير الحاملة للفايروس، التحليل الإحصائي أظهر اختلافات معنوية بينهما في اليوريا وناقل امين الاسباريت وناقل امين الالانين بينما لا توجد اختلافات معنوية بين العينات الحاملة وغير الحاملة للفايروس في قياس الكرياتينين وحامض اليوريك والفوسفاتيز القلوية. كذلك أظهر التحليل الإحصائي اختلافات معنوية بين الذكور والاناث في العينات الموجبة والسالبة في اختبار اليوريا والكرياتينين وناقل امين الالانين والفوسفاتيز القلوية بينما هذه الدراسة لم تجد اختلافات معنوية بين العينات الموجبة والسالبة في اختبار حامض اليوريك وناقل امين الاسبارين ماعدا حامض اليوريك أظهر اختلاف معنوي في الاناث الحاملة وغير الحاملة للفايروس.

إضافة الى ذلك ربطت الدراسة الحالية بين الأغنام الحوامل وغير الحوامل والتي تحمل نتيجة موجبة وسالبة لفايروس الحمى النزفية وانزيمات الكبد والكلية وقد أظهر التحليل الإحصائي اختلافات معنوية بين الأغنام

الحوامل وغير الحوامل الحاملة للفايروس وغير الحاملة للفايروس في اختبار اليوريا والفوسفاتيز القلوية والكرياتينين وناقل امين الالانين في الأغنام الحوامل بينما لا توجد اختلافات معنوية بينهما في اختبار الكرياتينين في الأغنام غير الحاملة وناقل امين الاسباريت في الأغنام الحوامل وناقل امين الالانين في الأغنام غير

الحاملة. عمر الأغنام يلعب دور مهم في انتشار الفايروسات بسبب وجود مستقبلات خاصة في بعض الاعمار أكثر من الأخرى لذلك ربطت الدراسة بين العمر وانتشار الفايروس حيث أظهر التحليل الاحصائي اختلافات معنوية بين العينات الموجبة والسالبة في اختبار اليوريا وناقل امين الالنين والفوسفاتيز القلوية في عمر من ١ الى ٥ سنوات، العمر من ٥ الى ٩ سنوات اظهر اختلاف معنوي في فحص الكرياتنين وناقل امين الاسباريت وناقل امين الالنين والفوسفاتيز القلوية ماعدا اليوريا لم يظهر اختلاف معنوي في عمر من ٥ الى ٩. تشير نتائج البحث ان الأغنام تلعب دور مهم في خزن فايروس حمى القرم والمساهمة في انتشار وبائية هذا الفايروس.



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دراسة مصلية وفسلجية لحمى القرم – الكونغو النزفية في الأغنام في محافظة ذي قار

رسالة مقدمة إلى
مجلس كلية الطب البيطري - جامعة ذي قار
كجزء من متطلبات نيل درجة الماجستير في علوم الطب البيطري في
الاحياء المجهرية

من قبل

حنين حسين طالب

بكالوريوس طب وجراحة بيطرية عامة (٢٠١٩)

بإشراف:

أ.م. د. حكمت كاظم طاهر

١٤٤٥ هـ

أ.م. د. نبيل مهدي عبد

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