INTERNATIONAL JOURNAL OF ADVANCED BIOLOGICAL RESEARCH

www.scienceandnature.org

CRIMEAN-CONGO HEMORRHAGIC FEVER

Anushri Tiwari, Madhu Swamy, Yamini Verma and Amita Dubey
Department of Veterinary Pathology
College of Veterinary Science & Animal Husbandry, NDVSU, Jabalpur

ABSTRACT
India is fast becoming a hotspot for emerging illnesses. The last decade has seen infectious diseases and viral hemorrhagic fever of emerging and re-emerging forms in the country. Crimean-Congo hemorrhagic fever (CCHF) is a widespread disease caused by a tick-borne virus, *Nairovirus* of family *Bunyaviridae*. The disease was first characterized in Crimea in 1944 and then later in 1969 as the cause of illness in Congo, thus resulting in the current name of the disease. CCHFV has been isolated from domesticated and wild mammals including cattle, sheep, goats, water buffalo and hares. Nineteen samples of blood taken from cows and buffaloes were found positive for Crimean-Congo Hemorrhagic Fever (CCHF) antibodies in Jodhpur in September, 2019, where two people had already died of the disease. CCHF is a zoonotic disease and tick vectors are widespread, thus, numerous animals can be hosts. Major risk groups include farmers, veterinarians and abattoir workers in endemic areas and most of the affected cases deal with agriculture and/or domestic animal husbandry and slaughtering activities. Therefore, monitoring of virus circulation in zoonotic foci and education of high-risk groups are important. Animals become infected by the bite of infected ticks of the genus *Hyalomma* and play a crucial role in the life cycle of ticks as well as in the transmission and amplification of the virus and are, therefore in the focus of veterinary public health. The typical course of CCHF infection has four distinct phases- incubation period, pre-hemorrhagic phase, hemorrhagic phase and convalescent phase. The virus enters and gets released in endothelial cells causing endothelial damage, increased vascular permeability and impairment of the immune response. Infected mice revealed discolored liver and spleen, serosal petechia and intestinal hyperemia. The liver texture appeared brittle and white pulp in the spleen was diminished. There was massive hepatocyte necrosis and mild sinusoidal congestion. Human infection with CCHFV often results in severe hemorrhagic disease.

KEY WORDS: Crimean–Congo hemorrhagic fever; Tick-borne virus; Epidemiology, pathogenesis, diagnosis and treatment of CCHF

INTRODUCTION

India is fast becoming a hotspot for emerging illnesses. The last decade has seen infectious diseases such as Nipah, Avian influenza, pandemic influenza, Severe Acute Respiratory Syndrome (SARS) corona virus, chikungunya virus and viral hemorrhagic fever of emerging and re-emerging forms in the country. Few diseases have the capacity to stimulate the interest and instill concern both in the general population and the health-care community as do viral hemorrhagic fevers (VHFs). Some of the major hemorrhagic fever viruses like Lassa, Marburg, Ebola, agents of South American VHF, Hanta and Crimean-Congo, share a distinct characteristic that has important clinical and public health consequences, namely the potential for person-to-person transmission (Mardani and Keshtkar-Jahromi, 2007). Crimean-Congo hemorrhagic fever (CCHF) is a widespread disease caused by a tick-borne virus *Nairovirus* of the *Bunyaviridae* family which causes severe human hemorrhagic fever disease characterized by fever, weakness, myalgia and hemorrhagic signs (*Center for Disease Control* and Prevention, 2013).

HISTORICAL PERSPECTIVE

The virus was recognized from an outbreak of "Crimean hemorrhagic fever" in soldiers and peasants in the Crimean Peninsula in 1944 but was not isolated or characterized until 1967. "Congo hemorrhagic fever" virus, isolated from a patient in the former Zaire (now Democratic Republic of Congo) in 1956, was shown in 1969 to be the same virus. As a consequence, the names of both countries have been used in combination to describe the disease (Hoogstraal, 1979). Distribution of the virus reflects the broad distribution of *Hyalomma* ticks, the predominant vector of the virus (Swanepoel and Paweska, 2011).

DISCOVERY OF THE VIRUS

Crimean hemorrhagic fever (CHF) was first described as a clinical entity in 1944–1945 when about 200 Soviet military personnel were infected during an epidemic in war-torn Crimea (Chumakov,1947). After that, a viral etiology was suggested by reproducing a febrile syndrome in psychiatric patients undergoing pyrogenic therapy after inoculation with a filterable agent from the blood of CHF patients. In 1967, a breakthrough in CHF research came when Chumakov and his colleagues at the Institute of Poliomyelitis and Viral Encephalitides in Moscow first used newborn white mice for CHF virus isolation (Chumakov *et al*., 1968). The resulting Drosdov strain, isolated by this method from a patient (Drosdov) in Astrakhan, became the prototype strain for experimental work in Russia and elsewhere.

ETIOLOGY

The virus is now placed in genus Nairovirus, family Bunyaviridae, is an enveloped, negative sense trisegmented single stranded RNA virus. When viewed by negative stain electron microscopy, CCHF virions appear to be distinct from other viruses within the *Bunyaviridae* family, as they possess very small morphologic surface units with central holes arranged in no obvious order (Martin *et al*., 1985). Its genome is of three single-stranded RNA segments encapsidated by the nucleocapsid protein (CCHFV N) to form the ribonucleoprotein complex. This ribonucleoprotein complex is required during replication and transcription of the viral genomic RNA.

Virions of members of the family *Bunyaviridae* contain three structural proteins: two envelope glycoproteins (G2 and G1 [more recently termed Gn and Gc, respectively] named in accordance with their relative proximity to the amino or carboxy terminus of the M segment encoded polyprotein) and a nucleocapsid protein (N), plus a large polypeptide (L) (approximately 200 kDa), which is the viron-associated RNA-dependent RNA polymerase. The viruses are approximately 80 to 120 nanometers in diameter and are enveloped viruses with negative sense, single stranded RNA genomes in three segments: L (large), M (medium) and S (small), each of which is contained in a separate nucleocapsid (N) within the virion, Gn and Gc glycoproteins, and the L polymerase respectively. The virus can be isolated from serum or plasma samples collected during the febrile or viraemic stage of infection or from liver of infected animals (OIE, 2018). The S segment codes for a nucleocapsid (N) protein, the M segment encodes a precursor for the 2 envelope glycoproteins Gn and Gc and the L segment codes for the RNA dependent RNA polymerase (Haferkamp *et al*., 2005).

CCHFV can survive for a short time in the environment, especially in some organic material. Infectious virus was found for up to 10 days and occasionally longer, in blood kept at 4°C (39°F). CCHFV was also reported to remain infectious in serum for at least a few days at unspecified ambient temperatures and to be stable "under wet conditions" for 15 days at 4°C, 11 days at 20°C (68°F) and 7 hours at 37°C (99°F). Dried virus was found to remain infectious for less than 24 hours (*Center for Food Security and Public Health, 2019).*

SPECIES AFFECTED

CCHFV has been isolated from domesticated and wild mammals including cattle, sheep, goats, water buffalo, hares, African hedgehogs and multimammate mice. Serological evidence of exposure has been reported in many additional species, such as horses, donkeys, camels, water buffalo, dogs, red foxes, wild dogs, Pallas cats, genets, a number of African ungulates, various rodents and bats (Chinikar *et al*., 2012).

Serological surveys in birds have mostly found no evidence for infection. However, ostriches are susceptible to CCHFV and are sometimes infected in nature. A low level of viremia was also reported in an experimentally infected guinea fowl and antibodies were found in one naturally infected magpie, although pooled sera from several groups of magpies were seronegative (Nalca and Whitehouse, 2007). A hornbill and a glossy starling developed antibodies to CCHFV and ticks fed on these birds apparently acquired the virus and transmitted it to rabbits (Turell, 2007). There is very little information about reptiles, but one tortoise from Tadzhikistan was seropositive (Spengler *et al*., 2016).

TRANSMISSION

Reservoir

Crimean-Congo hemorrhagic fever virus circulates in a silent enzootic tick-vertebrate-tick cycle, and there is no evidence that the virus causes any disease in animals. Tick is not only a vector but can also be a reservoir of the virus via vertical transmission (OIE, 2018). Ticks from the *Hyalomma* genus are the principal vectors of CCHF virus (Ozdarendeli *et al.*, 2008). Although the virus has also been isolated from other genera of Ixodid tick. *Hyalomma marginatum* is the main vector for CCHF in southern Europe; the virus has also been identified in *Hyalomma lusitanicum* in Spain (Estrada-Pena *et al.,* 2012).

Hares and hedgehogs act as amplifying hosts for the immature stages of the ticks. *Hyalomma marginatum* is usually activated by the increasing temperature in spring (beginning of April) and the immature stages are active in summer between May and September in the northern hemisphere (Ergonul and Whitehouse, 2007). Virus replicates in the host tick as it passes from the larva through adult stages (transstadial transmission) and it can also be transmitted from one generation to other (transovarial transmission).

Mode of transmission

Humans become infected through bites of infected ticks or by contact with infected blood or other livestock tissue. Nosocomial transmission may occur through direct contact with infected blood or body fluids, or through contaminated medical equipment or supply (Gurbuz *et al.,* 2009).

Risk groups

Major risk groups include farmers, veterinarians and abattoir workers in endemic areas and most of the affected cases deal with agriculture and/or domestic animal husbandry and slaughtering activities (Tekin *et al.,* 2010). Meat itself is not the source of infection because the virus is inactivated by post-slaughter acidification of the tissue, and CCHF virus does not survive cooking. Healthcare workers are the second most affected group while treating CCHF patients with severe bleeding and hemorrhage in a hospital setting, without strict barrier nursing procedures. Outdoor activities in endemic areas are a risk factor for tick exposure.

The natural cycle of CCHFV includes transovarial and trans-stadial transmission among ticks and a tick-vertebrate-tick cycle involving a variety of wild and domestic animals. Infection can also be transferred between infected and uninfected ticks during co-feeding on a host; also called 'non-viraemic transmission' phenomenon. *Hyalomma* ticks feed on a variety of domestic ruminants (sheep, goats, and cattle) and wild herbivores, hares, hedgehogs, and certain rodents. Although animal infections are generally subclinical, the

associated viraemia levels are sufficient to enable virus transmission to uninfected ticks (Swanepoel and Paweska, 2011). Although they do not appear to become viraemic, ground feeding birds may act as a vehicle for spread of CCHFV infected ticks. Results from serological surveys conducted in Africa and Eurasia indicate extensive circulation of the virus in livestock and wild vertebrates (Swanepoel and Burt, 2004).

EPIDEMIOLOGY

The CCHF virus causes severe viral hemorrhagic fever outbreaks with a case fatality rate of 10–40%. CCHF is endemic in Africa, the Balkans, the Middle East, Southern Europe, Eastern Europe (particularly in the former Soviet Union), throughout the Mediterranean, northwestern China, central Asia and the Indian subcontinent i.e. Asian countries and south of the 50th parallel north is the geographical limit of the principal tick vector (WHO, 2013). Seasonal variations have been described. In Iran, the high incidence was in August and September. In Pakistan, CCHF was more common between March and May and again, between August and October, depicting a biannual surge (Sheikh *et al*., 2005). Changes in climatic conditions have been suggested to be one of the factors that have facilitated reproduction of the tick population, and consequently the increased incidence of tick-borne infectious diseases (Gubler *et al*., 2001).

Molecular epidemiology of African and Asian CCHF isolates by examining phylogenetic relationships were examined for 70 CCHFV isolates from southern, central, and western parts of Africa, the Middle East and Greece using sequence data determined for a region of the S segment of the genome. Analysis revealed up to 18% genetic differences (Burt and Swanepoel, 2005). Tree topology supports previous evidence for the existence of three groups of genetically related isolates; A, B, and C. Within group A, there are two clades: an African clade and a predominantly Asian clade comprising isolates from Pakistan, China, Iran, Russia, and Madagascar. Group B includes isolates from South and West Africa and Iran and group C includes a single isolate from Greece. Despite the potential of dispersal of the virus between Africa and Eurasia, it appears that circulation of the virus is largely compartmentalized within the two land masses, and the inference is that the geographic distribution of phylogenetic groups is related to the distribution and dispersal of tick vectors of the virus (Burt and Swanepoel, 2005).

Indian Scenario

With the presence of CCHF virus confirmed in adjoining Pakistan, China and Afghanistan – countries that India has had trade ties with for years – scientists have long suspected the presence of the virus here.

CCHF viral infection had not been reported in humans from India before, though previous seroprevalence studies have shown viral antibodies both in animals and humans. A total of 643 human sera were tested from all over India, out of which, nine samples from Kerala and Pondicherry were positive for anti-CCHF virus antibody. In the same study, 34 of 655 serum samples collected from sheep, horse, goat and domestic animals from all over India showed evidence of CCHF virus (Shanmugam *et al.*, 1976).

In 1977, a survey of Ixodid ticks was conducted to determine the Crimean hemorrhagic fever (CHF) virus activity in Jammu and Kashmir but CCHF virus was not isolated in any of the 138 pools comprising eight species under six genera of ticks (Kaul *et al*., 1990). However, a related species of the genus *Nairovirus* – Ganjam virus, that belongs to the Nairobi Sheep group transmitted locally by *Hemaphysalis* ticks was identified. This virus has veterinary importance in India and has been demonstrated in mosquitoes, man and sheep (Sudeep *et al*., 2009). The nosocomial outbreak of CCHF viral infection in Gujarat is the first notable report from India. The striking feature of this outbreak was high fatality and rapid spread among treating medical team, taking four lives including the treating medical team (Appannanavar and Mishra, 2011).

In Ahemdabad, Gujarat, a patient and her attending nurse were admitted to the hospital with history of high fever for last 3 days and vomiting. Haemoptysis, bleeding from the lips, haematuria, palatal petechiae, haematemesis and difficulty in breathing were observed and death due to multi-organ failure and disseminated intravascular coagulation. The patient was the first laboratory-confirmed case of CCHF from India (Mishra *et al*., 2011).

Studies conducted at the National Institute of Virology (NIV), Pune, had reported the presence of anti-CCHF IgG antibodies in domestic animals (Yadav *et al*., 2014) and in shepherds (Makwana *et al.*, 2015) from Sirohi district, Rajasthan State. A 30-year-old male was hospitalized in a private hospital in Jodhpur, Rajasthan State, who subsequently had developed thrombocytopenia and showed hemorrhagic manifestations and died in the hospital. Later on, four nursing staff from the same hospital also developed the similar symptoms (Yadav *et al*., 2016).

Nineteen samples of blood taken from cows and buffaloes were found positive for Crimean-Congo Hemorrhagic Fever (CCHF) antibodies in Jodhpur in September, 2019, where two person had already died of the disease. However, one tick out of 26 also tested positive for CCHF. Department of Animal Husbandry (DAH) had sent 30 samples of cattle (cows and buffaloes) and 10 samples of goat. From the test reports received from National Institute of Virology (NIV), Pune, it has been found that goats were not infected with CCHFV but it is cattle which have CCHF antibodies (Times News Network, 2019).

PATHOGENESIS

The interaction of the virus with host cells is most likely responsible for the pathogenesis of CCHF. The main contributors are endothelial cells (ECs) and immune cells. There are two theories underlying the CCHF pathogenesis: One is that the virus interacts with the ECs directly and the other that it interacts indirectly via immune cells with subsequent release of soluble mediators. Following steps are associated with the pathogenesis of the disease (Akinci *et al*., 2013).

1. VIRUS ENTRY AND RELEASE IN ENDOTHELIAL CELLS:

The virus probably overcomes its first barrier i.e. epithelium with the help of the tick bite. Viral attachment proteins are localized in the basolateral membrane. As the basolateral compartment of endothelial cells (ECs) is directed toward the blood vessels, viral release from the basolateral membrane into the bloodstream causes systemic dissemination.

2. DISSEMINATION OF THE VIRUS:

The tick bite promotes viral release into the vascular system and the virus then amplifies in tissue resident macrophages and dendritic cells (DCs) which may facilitate transmission of the virus to the local lymph nodes, spleen and finally to systemic circulation of the host. Primary replication occurs in blood which is subsequently followed by liver and spleen (Bente *et al*., 2010).

3. ENDOTHELIAL DAMAGE:

Activation of endothelial cells is critical for starting the inflammatory reactions involving leukocyte rolling, adhesion and transmigration into inflamed areas as well as organization of the immune response to infection and increase of vascular permeability (Connolly- Andersen *et al*., 2011). Release of proinflammatory cytokines like IL-1, IL- 6, IL-8, IL-10 and tumor necrosis factor-α (TNF-α) also contribute to the progression of the disease which have toxic effects on the endothelium leading to increased vascular permeability, vasodilatation, multiorgan failure and shock. Thus, the pathogenesis of CCHF and sepsis is similar.

4. INCREASED VASCULAR PERMEABILITY:

The release of vasoactive mediators by activated ECs is likely to be responsible for increased vascular permeability. TNF-α possibly causes vascular leakage in CCHF. When ECs are exposed to TNF-α, vascular permeability due to destabilization of microtubules is observed with disruption of tight junctions followed by increased permeability and leakage (Petrache *et al*., 2003).

5. IMPAIRMENT IN THE IMMUNE RESPONSE:

CCHFV can impair the innate immune system and cause a delay in the adaptive immune response, which is critical for clearance of CCHFV (Saksida *et al*., 2010). The virus has many different ways to block the immune response like partial activation of macrophages and dendritic cells, leading to uncontrolled viral replication and the systemic spread of the virus throughout the body, hemophagocytosis, delayed induction of interferons, undetectable antibody response and depletion in the numbers of natural killer cells and lymphocytes. The systemic spread of virus to macrophages and dendritic cells leads to the release of mediators that modify vascular function and have procoagulant activity (Bray, 2005).

CLINICAL SIGNS:

CCHFV is thought to infect animals with few or no clinical signs (Nalca and Whitehouse, 2007). No illnesses have been attributed to this virus in naturally infected animals. Most experimentally infected livestock (cattle, sheep, goats, horses, donkeys) and wild species (e.g., hares, hedgehogs) also remained asymptomatic, although a transient mild fever was seen in some individuals and two calves were lethargic, with a reduced appetite, for a few days. One virus was isolated from a febrile cow during an outbreak of abortions, but whether other agents also occurred in the herd is unclear. Viremia levels and duration are relatively low and short and antibodies are detectable shortly after cessation of viremia.

Human beings are the only host of CCHFV in whom the disease manifestations are visible. In contrast to the inapparent infection in most other vertebrate hosts, human infection with CCHFV often results in severe hemorrhagic disease. The typical course of CCHF infection has four distinct phases - incubation period, pre-hemorrhagic phase, hemorrhagic phase and convalescent phase (WHO, 2018). The incubation period for CCHF virus is in the range of 3-7 days. The mean duration is largely influenced by the route of infection, viral load and source of infection-blood or tissue from livestock. The minimum viral load required for transmission of disease is 1-10 organisms (Franz *et al.*, 1997).

The disease begins with the pre-hemorrhagic phase, characterized by non-specific prodromal symptoms, during which it mimics other viral diseases. The major symptoms include high fever, myalgia, headache, nausea, abdominal pain and non-bloody diarrhea. This is accompanied by hypotension, relative bradycardia, tachypnea, conjunctivitis, pharyngitis and cutaneous flushing or rash (Swanepoel, 1994). The pre-hemorrhagic phase lasts for 4-5 days and in a majority of the patients, it progresses to hemorrhagic phase.

The hemorrhagic phase is generally short and has a rapid course with signs of progressive hemorrhage and diathesis. These include petechiae, conjunctival hemorrhage, epistaxis, hematemesis, hemoptysis and melena. Certain patients may also have hepatosplenomegaly (Ozkurt *et al*., 2006). The disease is fatal in 40-60% of the cases. In severe cases, death occurs as a result of multiorgan failure, disseminated intravascular coagulation and circulatory shock. Acute respiratory distress syndrome (ARDS) and diffuse alveolar hemorrhage, accompanied by systemic inflammatory reaction, have also been reported during hemorrhagic manifestations (Doganci *et al*., 2008). In the survivors, the convalescent period begins 10-20 days after the onset of illness. During this phase, patients may have feeble pulse, tachycardia, loss of hearing, memory loss and alopecia.

Clinical studies have demonstrated that thrombocytopenia, leucopenia and raised levels of liver transaminases are hallmarks of CCHFV infection and can be used to predict fatal outcome in 90% of patients (Joubert *et al*., 1985).

LESIONS:

Gross lesions: -

In study conducted by Negredo *et al.* (2017), gross examination of organs from CCHFV-infected STAT129 mice revealed discolored liver and spleen, serosal petechia and intestinal hyperemia. The liver texture appeared brittle and white pulp in the spleen was diminished (Bente *et al.*, 2010). In humans, gross examination revealed generalized visceral edema with substantial amounts of serohematic ascitic fluid and disseminated cutaneous and visceral hemorrhages. The liver was normal in both weight and size, with a brownish appearance and softened consistency (Negredo *et al*., 2017).

Histopathological lesions

Prominent histopathologic changes were observed in liver and spleen tissues in CCHFV-infected STAT129 mice in a study conducted by Bente *et al*., 2010. There was massive

hepatocyte necrosis, with sparing of narrow periportal and pericentral rims and mild sinusoidal congestion. No Kupffer-cell hyperplasia or inflammatory infiltrates were observed. The hepatocytes had a swollen appearance and widespread necrosis. In general, the hepatocytes contained cytoplasmic macro and microvesiculation. Although most mucosae were preserved, the appearance of the colon was striking owing to its complete epithelial denudation. The crypts were filled with basophilic mucoid material and walled by sloughed apoptotic cells, again without inflammatory infiltrates. Occasional microthrombi were observed. The bone marrow showed hemorrhages and a preserved megakaryocyte population with a normal morphologic appearance. The spleen showed slight lymphoid depletion and hemorrhage but no necrotic areas were observed by the workers.

LABORATORY DIAGNOSIS

Common laboratory findings reveal leucopenia and thrombocytopenia in patients with CCHF and indicate elevated levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), creatine kinase (CK) and lactate dehydrogenase (LDH) along with prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT) (Shayan *et al*., 2015). Also, increased myeloperoxidase expression in leukocytes leads to increased leukocyte lysis. Therefore, leucopenia in patients with CCHF may be attributed to lysis (Guven *et al*., 2013). Clinical studies have demonstrated that thrombocytopenia, leucopenia and raised levels of liver transaminases are hallmarks of CCHFV infection and can be used to predict fatal outcome in 90% of patients (Joubert *et al*., 1985).

As CCHF mimics a wide range of common illnesses caused by different etiological agents which are endemic in India, differential diagnosis should be done based on clinical, biochemical, haematological, bacteriological and virological findings. These include Kyasanur forest disease, hepatitis, *Neisseria meningitidis* infection, leptospirosis, borreliosis, typhoid, rickettsiosis, dengue and malaria. However, malaria diagnosis can be excluded in cases of suspected viral haemorrhagic fever (Zeller, 2007). Following methods can be used for identification of the causal agent:

Viral isolation

The most definitive way of diagnosis is the demonstration of virus or viral genome. The virus may be isolated from blood or tissue specimens in the first five days of illness and grown in cell culture (Shephered *et al.,* 1986). Viral isolation is done by using cell lines such as LLC-MK2, Vero, BHK-21, and SW-13.4 and can be achieved in 2-5 days. CCHF virus generally produces no or little cytopathic effect and can be identified by immune fluorescence assay with specific monoclonal antibodies. However, viral isolation is useful only in the early phase of infection when the viral load is high but suffers from poor sensitivity. Moreover, this can be done only if the Biosafety Level 4 containment facilities are available.

Molecular methods

Demonstration of viral genome is by far the most definitive form of diagnosis. Reverse-transcriptase PCR (RT PCR) is the method of choice for rapid laboratory diagnosis of CCHF virus infection. Another benefit to molecular diagnostic assays is their rapidity as compared to virus culture and a presumptive diagnosis can be made within 8 hours. The real-time PCR assay has various advantages like lower contamination rate, higher sensitivity and specificity and provides result in a few minutes. A one-step real-time RT-PCR assay for detecting CCHFV using primers to the nucleoprotein gene, using DNA-intercalating dye, SybrGreen I was used (Drosten *et al.*, 2003). Later, a real-time RT-PCR assay was developed using TaqMan-Minor Groove Binding Protein (MGB) probe, which had higher specificity and a shorter probe length (Whitehouse, 2004).

Serological assays

Serological tests are useful in the second week of illness. Serological tests formerly used for the detection of antibody to the virus, such as complement fixation, hemagglutination inhibition and reverse passive hemagglutination inhibition, lacked sensitivity and reproducibility, but indirect Immunofluorescence (IF) could detect IgG and IgM antibody responses by days 7-9 of illness in all survivors of the infection. Enzyme-Linked Immunosorbent Assay (ELISA) to detect specific IgM and IgG have largely replaced these conventional serodiagnostic tests. Specific IgM persists for up to 4 months post-infection while IgG remains detectable for at least 5 years. Recent or current infection is confirmed by demonstrating IgM, using IgM antibody capture (MAC)-ELISA in a single sample or a fourfold or greater increase in antibody titer in paired serum samples (Charrel *et al*., 2004). A recombinant nucleoprotein (rNP)- based IgG ELISA was developed for serological diagnosis of CCHF virus infections. This was shown to be a valuable tool for diagnosis and epidemiological investigations of CCHFV infections (Saijo *et al.*, 2002).

TREATMENT

Lack of significant clinical disease in livestock warrants no treatment considerations. Supportive therapy is the most essential part of case management in humans and includes the administration of thrombocytes, fresh frozen plasma and erythrocyte preparations. Replacement therapy with these blood products should be done after checking the patient's complete blood count, which should be done once or twice a day Fluid and electrolyte balance should also be monitored meticulously. Ribavirin is the recommended antiviral agent for infected patients, although its mechanism of action is not clear. In one in-vitro study, ribavirin was shown to inhibit viral activity and some CCHF viral strains appeared more sensitive than others (Watts *et al.,* 1989). In an experimental study done in mice, ribavirin treatment substantially reduced infant mouse mortality and extended the mean time to death (Tignor and Hanham, 1993).

CONTROL AND PREVENTION

Currently, 2 vaccines against CCHFV have been developed for use in humans. The first one is a formalin-inactivated vaccine, which was developed in Bulgaria from infected suckling mouse brain. The second is a DNA vaccine tested in mice. Neither vaccine has undergone official randomized clinical trials (Papa *et al.*, 2011).

Efficient ways to protect against CCHFV infection are tick control and limited exposure to infected livestock or humans. To minimize tick exposure, protective clothing and application of repellent is recommended (WHO, 2013). Insect repellants containing DEET (N, N-diethyl-m-toluamide) are most effective in warding off ticks. Clothing should be chosen to prevent tick attachment, especially covering legs and arms. Healthcare workers in endemic areas may be exposed to infected blood or tissue from patients with CCHF. Therefore, such workers should wear gloves, gowns and face masks to reduce the risk of exposure. Also, they must follow proper infection-control precautions to prevent occupational exposure (Bajpai and Nadkar, 2011). Illegal transportation of animals among countries may result in expansion of CCHFV; therefore, prevention of illegal transportation of animals may reduce the spread of CCHFV (Vorou, 2009).

CONCLUSION

Crimean Congo hemorrhagic fever is a considerable public health threat which can have significant effect on abattoir workers and healthcare personnel, especially in resource-poor countries. The fact that a huge population of the livestock is in close contact with the human beings indicates the possibility of spread of this virus in different parts of India. In a developing country like India, only a few Biosafety level-4 laboratories are available and out of those only a few are capable to carry out viral diagnosis. This is one of the main limitations to deal with this infectious disease. It is also necessary to develop a network of health officials at root level to report the cases and co-ordinate with samples sharing, diagnosis and implementation of necessary actions in coordination with state governments for appropriate control of this disease. There is a need for active surveillance not only for existing pathogens in any geographic location but also for those that pose future threat. National inter-sectoral surveillance and response system and cross-border sharing of information and establishing special community-based laboratory surveillance programs for at risk population groups needs to be developed.

REFERENCES

Akinci, E., Bodur, H. and Leblebicioglu, H. (2013) Pathogenesis of Crimean-Congo hemorrhagic fever. *Vector- Borne and Zoonotic Diseases*, **13**(7): 429-437.

Appannanavar, S.B. and Mishra, B. (2011) An update on Crimean Congo hemorrhagic fever. *Journal of Global Infectious Diseases*, **3**(3): 285.

Bajpai, S. and Nadkar, M.Y. (2011) Crimean Congo hemorrhagic fever: requires vigilance and not panic. *Journal of Association of Physicians of India*, **59**(164): 7.

Bente, D.A., Alimonti, J.B., Shieh, W.J., Camus, G., U. Stroher, S. Zaki and Jones, S.M. (2010) Pathogenesis and immune response of Crimean-Congo hemorrhagic fever virus in a STAT-1 knockout mouse model. *Journal of Virology*, 84(21): 11089-11100.

Bray, M. (2005) Pathogenesis of viral hemorrhagic fever. *Current Opinion in Immunology*, **17**(4): 399-403.

Burt, F.J. and Swanepoel, R. (2005) Molecular epidemiology of African and Asian Crimean-Congo haemorrhagic fever isolates. *Epidemiology and Infection*, **133**(4): 659-666.

Center for Disease Control and Prevention (2013) Crimean-Congo hemorrhagic fever online https:// www. cdc. gov/ vhf/crimean-congo/ index.html

Center for Food Security and Public Health (2019) *online* http:// www. cfsph.iastate.edu/ Factsheets/ pdfs/ crimean congo_ hemorrhagic_fever.pdf

Charrel, R.N., H. Attoui, A.M. Butenko, J.C. Clegg, V. Deubel, T.V. Frolova, E.A. Gould, T.S. Gritsun, F.X. Heinz, M. Labuda, V.A. Lashkevich, V. Loktev, A. Lundkvist, D. V. Lvov, C.W. Mandl, M. Niedrig, A. Papa, V. S. Petrov, A. Plyusnin, S. Randolph, J. Suss, V. I. Zlobin and Lamballerie, X. (2004) Tick-borne virus diseases of human interest in Europe. *Clinical Microbiology and Infection*, **10**(12): 1040-1055.

Chinikar, S., R. Mirahmadi, M. Moradi, S. M. Ghiasi and S. Khakifirouz (2012) Crimean-Congo hemorrhagic fever (CCHF); in *Zoonosis*. Lorenzo-Morales, J. (ed.), pp 193-212, Janeza Trdine, Croatia.

Chumakov, M.P. (1947) A new virus disease-Crimean hemorrhagic fever. *News of Medicine*, **4**: 9-11.

Chumakov, M. P., A. M. Butenko, N. V. Shalunova, L. I. Martyanova, W. E. Smirnova, V. N. Bashkirtsev, T. I. Zavodova, S. G. Rubin, E. A. Tkachenko, V. Y. Karmy-Sheva, V. N. Reingold, G.V. Popov and A. P. Savinov (1968). New data on the virus causing Crimean hemorrhagic fever (CHF). *Voprosy Virusologii*, **13**: 377.

Connolly-Andersen, A. M., G. Moll, C. Andersson, S. Akerstrom, H. Karlberg, I. Douagi, and A. Mirazimi (2011). Crimean-Congo hemorrhagic fever virus activates endothelial cells. *Journal of Virology*, 85(15): 7766-7774.

Doganci, L., M. Ceyhan, N.F. Tasdeler, H. Sarikayalar and N. Tulek (2008) Crimean Congo hemorrhagic fever and diffuse alveolar haemorrhage. *Tropical Doctor*, 38(4): 252-254.

Drosten, C., B. M. Kummerer, H. Schmitz, and S. Gunther (2003) Molecular diagnostics of viral hemorrhagic fevers. *Antiviral Research*, **57**(1-2): 61-87.

Ergonul, O. and C. A. Whitehouse (2007) in *Crimean-Congo Hemorrhagic Fever: A Global Perspective*. Ergonul, O. and C. A. Whitehouse (eds.), pp 7, Springer, Dordrecht.

Estrada-Pena, A., Palomar, A.M., P., Santibanez, N., Sanchez, M.A. Habela, A. Portillo, Romero, L. and J.A. Oteo (2012) Crimean-Congo hemorrhagic fever virus in ticks, Southwestern Europe, 2010. *Emerging Infectious Diseases*, **18**(1): 179.

Franz, D. R., P.B. Jahrling, A.M. Friedlander, D.J., McClain, D.L. Hoover, W.R. Bryne, J.A. Pavlin, G.W. Christopher and E.M. Eitzen (1997) Clinical recognition and management of patients exposed to biological warfare agents. *Journal of the American Medical Association*, **278** (5): 399-411.

Gubler, D. J., P. Reiter, K. L. Ebi, W. Yap, R. Nasci and J. A. Patz (2001) Climate variability and change in the United States: potential impacts on vector-and rodent-borne diseases. *Environmental Health Perspectives*, **109** (2): 223-233.

Gurbuz, Y., I. Sencan, B. Ozturk and E. Tutuncu (2009) A case of nosocomial transmission of Crimean–Congo hemorrhagic fever from patient to patient. *International Journal of Infectious Diseases*, **13**(3): 105-107.

Guven, F. M. K., H. Aydin, G. Yildiz, A. Engin, V. K. Celik, D. Bakir, and K. Deveci (2013) The importance of myeloperoxidase enzyme activity in the pathogenesis of Crimean–Congo haemorrhagic fever. *Journal of Medical Microbiology*, 62(3): 441-445.

Haferkamp, S., L. Fernando, T. F. Schwarz, H. Feldmann and R. Flick (2005) Intracellular localization of Crimean-Congo hemorrhagic fever (CCHF) virus glycoproteins. *Virology Journal*, **2**(1): 42.

Hoogstraal, H. (1979) The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe and Africa. *Journal of Medical Entomology*, **15**(4): 307-417.

Joubert, J. R., King, J. B., Rossouw, D. J. and Cooper, R. (1985) A nosocomial outbreak of Crimean-Congo haemorrhagic fever at Tygerberg Hospital. Part III. Clinical pathology and pathogenesis. *South African Medical Journal*, **68**(10): 722-728.

Kaul, H. N., Shetty, P. S., Ghalsasi, G.R. and Dhanda, V. (1990) Survey of ticks (Acarina: *Ixodidae*) for Crimean haemorrhagic fever virus activity in Jammu & Kashmir state, India. *The Indian Journal of Medical Research*, **91**: 5-8.

Makwana, D., P. D. Yadav, A. Kelaiya and D.T. Mourya (2015). First confirmed case of Crimean-Congo haemorrhagic fever from Sirohi district in Rajasthan State, India. *The Indian Journal of Medical Research*, **142**(4): 489.

Mardani, M. and M. Keshtkar-Jahromi (2007) Crimean-Congo hemorrhagic fever. *Archives of Iranian* Medicine, **10** (2): 204-14.

Martin, M. L., H. Lindsey-Regnery, Sasso, D. R., McCormick J.B. and Palmer, E. (1985). Distinction between Bunyaviridae genera by surface structure and comparison with Hantaan virus using negative stain electron microscopy. *Archives of Virology*, **86(**1-2): 17-28.

Mishra, A. C., M. Mehta, D. T. Mourya, and S. Gandhi (2011) Crimean-Congo haemorrhagic fever in India. *The Lancet*, **378**(9788): 372.

Nalca, A. and C. A. Whitehouse (2007). Crimean-Congo hemorrhagic fever virus infection among animals; in *Crimean-Congo Hemorrhagic Fever*. **Ergonul**, O. and C.A. Whitehouse (eds.), pp 155-165, Springer, Dordrecht.

Negredo, A., F. de la Calle-Prieto, E. Palencia-Herrejon, M. Mora-Rillo, J. Astray-Mochales, M. P. Sanchez-Seco, E. Bermejo Lopez, J. Menarguez, A. Fernandez-Cruz, B. Sanchez-Artola, E. Keough-Delgado, E. Ramirez de Arellano, F. Lasala, J. Milla, J. L. Fraile, M. Ordobas Gavin, A. Martinez de la Gandara, L. Lopez Perez, D. Diaz-Diaz, M. A. Lopez-Garcia, P. Delgado-Jimenez, A. Martin-Quiros, E. Trigo, J. C. Figueira, J. Manzanares, E. Rodriguez-Baena, L. Garcia-Comas, O. Rodriguez-Fraga, N. Garcia-Arenzana, M. V. Fernandez-Diaz, V.M. Cornejo, P. Emmerich, J. Schmidt-Chanasit and A. R. Arribas (2017) Autochthonous Crimean-Congo Hemorrhagic Fever in Spain. *New England Journal of Medicine*, **377**(2): 154-161.

OIE (2018) Crimean-Congo hemorrhagic fever online https://www.oie.int/fileadmin/Home/eng/Health_standards/tahm/3.01.05_CCHF.pdf

Ozdarendeli, A., K. Aydin, S. Tonbak, M. Aktas, K. Altay, I. Koksal, Y. Bolat, N. Dumanli and A. Kalkan (2008) Genetic analysis of the M RNA segment of Crimean-Congo hemorrhagic fever virus strains in Turkey. *Archives of Virology*, **153**(1): 37-44.

Ozkurt, Z., I. Kiki, S. Erol, F. Erdem, N. Yilmaz, M. Parlak, M. Gundogdu and M.A. Tasyaran (2006) Crimean–Congo hemorrhagic fever in Eastern Turkey: clinical features, risk factors and efficacy of ribavirin therapy. *Journal of Infection*, 52(3): 207-215.

Papa, A., E. Papadimitriou and I. Christova (2011) The Bulgarian vaccine Crimean-Congo haemorrhagic fever virus strain. *Scandinavian Journal of Infectious Diseases*, **43**(3): 225-229.

Petrache, I., A. Birukova, S. I. Ramirez, J. G. Garcia and A. D. Verin (2003) The role of the microtubules in tumor necrosis factor-α–induced endothelial cell permeability. *American Journal of Respiratory Cell and Molecular Biology*, **28**(5): 574-581.

Saijo, M., T. Qing, M. Niikura, A. Maeda, T. Ikegami, C. Prehaud, I. Kurane and S. Morikawa (2002) Recombinant nucleoprotein-based enzyme-linked immunosorbent assay for detection of immunoglobulin G antibodies to Crimean-Congo hemorrhagic fever virus. *Journal of Clinical Microbiology*, **40**(5): 1587-1591.

Saksida, A., D. Duh, B. Wraber, I. Dedushaj, S. Ahmeti and T. Avsic-Zupanc (2010) Interacting roles of immune mechanisms and viral load in the pathogenesis of

Crimean-Congo hemorrhagic fever. *Clinical and Vaccine Immunology*, **17**(7): 1086-1093.

Shanmugam, J., S. E. Smirnova and M. P. Chumakov (1976) Presence of antibody to arboviruses of the Crimean Haemorrhagic Fever-Congo (CHF-Congo) group in human beings and domestic animals in India. *Indian Journal of Medical Research*, **64**(10): 1403-1413.

Shayan, S., M. Bokaean, M.R. Shahrivar and S. Chinikar (2015) Crimean-Congo hemorrhagic fever. *Laboratory Medicine*, **46**(3): 180-189.

Sheikh, A.S., A.A. Sheikh, N. S. Sheikh, M. Asif, F. Afridi and M.T. Malik (2005) Bi-annual surge of Crimean-Congo haemorrhagic fever (CCHF): a five-year experience. *International Journal of Infectious Diseases*, **9** (1): 37-42.

Shepherd, A. J., R. Swanepoel, P. A. Leman and S. P. Shepherd (1986) Comparison of methods for isolation and titration of Crimean-Congo hemorrhagic fever virus. *Journal of Clinical Microbiology*, **24**(4): 654-656.

Spengler, J.R., E. Bergeron and Rollin, P.E. (2016) Seroepidemiological studies of Crimean-Congo hemorrhagic fever virus in domestic and wild animals. *Public Library of Science Neglected Tropical Diseases*, **10**: 4210.

Sudeep, A. B., Jadi, R. S. and Mishra, A.C. (2009) Ganjam virus. *Indian Journal of Medical Research*, **130** (5): 514.

Swanepoel, R. (1994) Crimean-Congo haemorrhagic fever; in *Handbook of Zoonoses: Viral Zoonoses*, 2nd Edn., Beran, G. W. (Ed.), pp 149-161, CRC Press, USA.

Swanepoel, R. and F. J. Burt (2004) Crimean–Congo haemorrhagic fever; in *Infectious diseases of livestock with special reference to South Africa*, 2nd Edn., Coetzer, J.A.W. and Tustin, R.C. (eds.), pp 1077–1085, Oxford University Press, South Africa.

Swanepoel, R. and J. T. Paweska (2011) Crimean-Congo hemorrhagic fever; in *Oxford Textbook of Zoonosis: Biology, Clinical Practise and Public Health Control*, 2nd Edn. Palmer, S. R., L. Soulsby, P. R. Torgerson and D. W. G. Brown (eds.), pp 287–293, Oxford University Press, UK.

Tekin, S., S. Barut, A. Bursali, G. Aydogan, O. Yuce, F. Demir and B. Yildirim (2010) Seroprevalence of Crimean-Congo haemorrhagic fever (CCHF) in risk groups in Tokat Province of Turkey. *African Journal of Microbiology Research*, **4**(3): 214-217.

Tignor, G. H. and C. A. Hanham (1993) Ribavirin efficacy in an in vivo model of Crimean-Congo hemorrhagic fever virus (CCHF) infection. *Antiviral Research*, **22**(4): 309-325.

Times News Network (2019) 19 samples of cattle test positive for Congo fever. *Times of India*, 18, September 2019, pp 2.

Turell, M. J. (2007) Role of ticks in the transmission of Crimean-Congo hemorrhagic fever virus; in *Crimean-Congo Hemorrhagic Fever*. Ergonul**,** O. and C. A. Whitehouse (eds.), pp 143-154, Springer, Dordrecht.

Vorou, R. M. (2009) Crimean-Congo hemorrhagic fever in southeastern Europe. *International Journal of Infectious Diseases*, **13**(6): 659-662.

Watts, D. M., M. A. Ussery, D. Nash, and C. J. Peters (1989) Inhibition of Crimean-Congo hemorrhagic fever viral infectivity yields in vitro by ribavirin. *The American Journal of Tropical Medicine and Hygiene*, **41**(5): 581-585.

Whitehouse, C.A. (2004) Crimean–Congo hemorrhagic fever. *Antiviral Research*, **64**(3): 145-160.

WHO (2013) Crimean-Congo haemorrhagic fever online https://www.who.int/news-room/factsheets/detail/crimean-congo-haemorrhagic-fever

WHO (2018) Introduction to Crimean-Congo Haemorrhagic Fever - Managing infectious hazards online https:// www.who.int/ emergencies/ diseases/crimean-congo- haemorrhagic-fever/introduction.pdff

Yadav, P. D., C. G. Raut, D. Y. Patil, T. D. Majumdar and D. T. Mourya (2014) Crimean-Congo hemorrhagic fever: current scenario in India. *Proceedings of the National Academy of Sciences, India Section B: Biological Sciences*, **84**(1): 9-18.

Yadav, P. D., D. Y. Patil, A. M. Shete, P. Kokate, P. Goyal, S. Jadhav, S. Sinha, D. Zawar, S. Sharma, A. Kapil, D. K. Sharma, K. Upadhyay and D. T. Mourya (2016) Nosocomial infection of CCHF among health care workers in Rajasthan, India. *BMC Infectious Diseases*, **16**(1): 624.

Zeller, H. (2007) Laboratory Diagnosis of Crimean-Congo Hemorrhagic Fever; in *Crimean-Congo Hemorrhagic Fever: A Global Perspective*. Ergonul, O. and C.A. Whitehouse (eds.), pp 233-43, Springer, Dordrecht.