11

Crimean-Congo Hemorrhagic Fever (CCHF)

Sadegh Chinikar, Ramin Mirahmadi, Maryam Moradi,
Seyed Mojtaba Ghiasi and Sahar Khakifirouz
Pasteur Institute of Iran
(Laboratory of Arboviruses and Viral Hemorrhagic Fevers)
(National Reference Laboratory)
Iran

1. Introduction

CCHF name derives from the separate regions in Asia and Africa where severe and often fatal human cases of hemorrhagic disease and fever were recognized in the 1940s and 1950s. Virus isolates from the two regions are antigenetically indistinguishable. The disease come to people's attention in the Crimea where about 200 military personnel become ill while helping peasants to harvest gain.

Russian scientists, led by Professor M.P.Chumakov, isolated the virus from human patients and from ticks. The same virus was isolated by C.Courtoise in 1956 from a 13 years old patient in the Belgium Congo. However, Crimean-Congo haemorrhagic Fever has a much longer history, with the first record in the early 12th century. Although ticks transmit CCHF virus to a wide variety of animal species, the severe disease only affects humans. Cattle, sheep and small mammals, such as hares may develop mild fever following infection. The disease in humans is comparatively rare but a cause for concern because of high mortality and transmission through contact with patients. Handling the virus requires the highest degree of laboratory containment (Knipe (2001), Chinikar(2009), Chinikar(2007)).

2. History of the virus in the world and in Iran

CCHF is a tick- borne Viral Zoonosis widely distributed in Africa, Asia and Eastern Europe within the ranges of ticks belonging to the genus *Hyalomma*. The virus is a member of the Nairovirus genus of the family Bunyaviridae. It causes mild fever and virema in cattle, sheep and small mammals such as hares.

Humans become infected by contact with infected blood or other tissues of livestock or human patients or from tick bite. Human infection is usually characterized by a febrile illness with headache, myalgia and petechial rash, frequently followed by a hemorrhagic state with necrotic hepatitis. The case fatality rate is approximately40%, but it can range from 20% to 80 %(Goodman (2005), Knipe (2001)).

A hemorrhagic disease with symptoms suggestive of CCHF infection was described in Eastern Europe and Asia as far back as the 12th century (Hoogstraal 1979). However, a disease given the name Crimean hemorrhagic fever was first described in people bitten by

ticks while harvesting crops and sleeping outdoors on the Crimean Peninsula in 1944. In the following year, it was demonstrated by inoculation of human subjects that the disease was caused by a filterable agent present in the blood of patients during the acute stage of illness and that the agent was also present in suspension prepared from ticks, suspected to be the vectors of the agent. The causative virus was finally isolated in a laboratory host, suckling mice, in 1967 (Chumakov 1974). In 1968 it was found that the agent of Crimean hemorrhagic fever was identical to a virus named Congo which had been isolated in 1956 from the blood of a febrile child in what was then the Belgian Congo (now democratic Republic of Congo), and since that time the two names have been used in combination (Casals 1980, Casals 1969, Chumakov 1970, Simpson 1976).

The first evidence that CCHF virus circulated in Iran was investigated by Chumakov et al in 1970, when 45% of a shipment of sheep sent from Tehran abattoir to Moscow tested positive for CCHF antigen. Although human infection with CCHF was suspected in areas close to the Azerbaijan border, the first confirmed human cases were not reported until 1974-1975. This lead to a large-scale serological study performed in collaboration with Yale University (New Haven, Connecticut, USA), focusing on the northern half of Iran. Using classic agar gel diffusion precipitation assays this study demonstrated high levels of seroconversion for humans (13%), cattle (38%) and sheep (18%).

In 1978, CCHF virus was isolated for the first time from engorged specimens of the ornithodoros tick Alveonasus lahorensis in the north eastern region of Iran. Since this early period to 1999 reports of CCHF were uncommon in Iran, the disease has however increased in prevalence since 2000 warranting new surveys and study (Chinikar2010, Chinikar2009 and Chinikar2007).

3. Etiology agent and biology

Crimean-Congo Hemorrhagic Fever virus is classified as a member of the genus Nairovirus, of the family Bunyaviridae (Knipe 2001).

The genus, consisting of 33 viruses, is divided into seven serogroups on the basis of antigenic relationships.

The CCHF serogroup contains CCHF virus, Hazara virus from Pakistan, and Khasan virus from the former USSR.

Apart from CCHF virus, the only members of the genus known to be pathogenic from humans are Nairobi sheep disease virus and Dugbe virus. Nairobi sheep disease virus of East Africa is believed to be identical to Ganjam virus of India and is a tick-borne pathogen of sheep and goats which sporadically causes benign illness in humans (Davies 1978).

Dugbe virus is a tick-borne virus commonly associated with mild infection of cattle and sheep in West Africa and infrequently causes benign human disease (Burt1996). The classification of the Nairoviruses was originally based on their antigenic relatedness; however, the groupings have subsequently been substantiated through demonstration of morphological and molecular affinities between the viruses (Calisher 1989).

Viewed using an electron microscope, CCHF virus appears spherical, approximately 100nm in diameter (buoyant density1.17 $g.ml^{-1}$) with a dense core (capsid) surrounded by a lipid

envelope, through which protrude spikes, 5-10 mm in length. The viral genome is segmented, comprising three circular, single strands of negative sense RNA.

All three genomic RNA segments have a unique 3'end sequence of 3'AGAG (A/U) UUCU.

The small (S) segment (approximately 1.7 Kb) has a single open reading frame encoding the nucleocapsid (N) protein.

In contrast, the medium (M) segment (approximately 5Kb) encodes a large polyprotein, which is processed into the two surface glycoproteins, G_1 and G_2, and several mon-structural proteins. The large (L) segment encodes a single L protein of approximately 460 KD_a, which is probably the viral polymerase (Fig. 1).

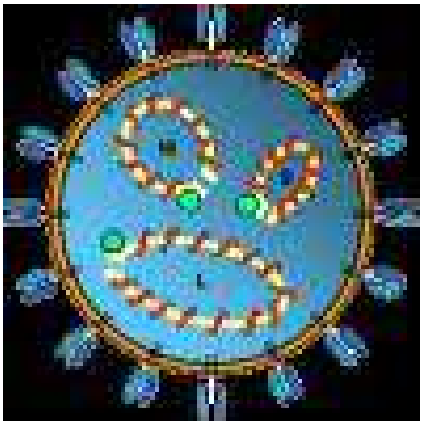


Fig. 1. CCHF virus Schema

Virus infection of cells is probably through receptor-mediated endocytosis, followed by fusion of the viral envelope with endosomal membranes. Monoclonal antibodies directed against G_1 (but not against the N protein) neutralized viral infectivity, suggesting an important role for G_1in the infection process. All stages of viral replication occur in the cell cytoplasm, although comparatively little is known of these events. As the viral RNA is negative-sense, the first step in replication is the transcription of the incoming genomic RNA into viral complementary RNA. The transcriptase has not yet been identified. Viral messengers RNA (mRNA) has host derived primer sequences, indicating a cap-snatching mRNA priming mechanism, as found with influenza viruses. Virion assembly occurs in the Golgi complex.

Nucleocapsids acquire their outer envelope by budding into the Golgi lumen. Virions are then transported to the cell membrane and released from the infected cell by exocytosis. (Richman 2002, Krasus2003, Isba2004, Van der Giessen 2004, Chhabra2003, M.W Service.2001, Goodman 2005, Fields2001).

Little information is available on the stability of the CCHF virus, but once enveloped, it is sensitive to lipid solvents (Karabatsos 1985), and it is known that its infectivity is destroyed by low concentration of formalin and B-propriolactone. The virus is labile in infected human tissues after host death (Hoogstraal 1979), but the examination of specimens from human

patients appears to show that infectivity is preserved for at least a few days at ambient temperature in separated serum.

Infectivity is destroyed by boiling in autoclaving, but the virus is stable at temperatures below-60°C.

CCHF virus replicates in a wide variety of primary cell and line cell cultures, including Vero, CER, and BHK21 cells, but not usually to high titer. The virus is poorly cytopathic, so that titers of infectivity are demonstrated by plaque production or immunoflorescence in infected cells (Hoogstraal1979, Calisher 1989 and Clerx 1981).

The virus has been isolated and titers have been determined most frequently by intracerebral inoculation of suckling mice (Hoogstraal 1979).

Because of its propensity for human-to-human transmission, its ability to cause infections in laboratory workers, and the severity of the disease in humans, CCHF is placed in biohazard class IV in countries which have relevant biosafety guidelines. This dictates that culture of the virus is permitted only in maximum-security biosafety level 4 (BSL-4) laboratories (Richman 2002, Knipe 2001).

4. Transmission and zoonotic hosts

Vectors are ixodid (hard) ticks. Although CCHF virus has been isolated from at least 31 different tick species and subspecies (including two argasid (soft) species), the primary vectors are *Hyalomma* species, particularly *H. marginatum marginatum, H. marginatum rufipes* (the African representative of the *H. marginatum* complex), and *H. anatolicum anatolicum*.

All three species are two-host ticks: immature stage (larvae and nymphs) feed on the same individual host before dropping off to mouth to the adult stage which then feeds on a second host.

Both immature stages and adults of *H. a. anatolicum* feed on domesticated mammals, whereas *H. m. marginatum* and *H. m. rufipes* immature stage and adults feed on dissimilar hosts: immature stages on birds, hares and hedgehogs, and adults on cattle and other large mammals. Adult ticks successfully attack humans, only being detected after a few days of feeding when they become enlarged with blood. Humans do not contribute to the transmission cycle. *Hares* (*Lepus* species), hedgehogs (*Erinaceus* and *Hemiechinus* species) and cattle are probably important amplifying hosts.

Feeding on viraemic animals may provide the source of infection for ticks. However, screening domestic and wild vertebrates for CCHF viremia has often failed to identify viraemic host species that maintain the viral enzootic cycle. Sheep and ground-feeding birds, such as Hornbills and Ostriches may act as non-viremic hosts. Otherwise birds play an important role in disseminating ticks, particularly those of the *H. marginatum* complex.

Virus can be transmitted sexually, from infected male to uninfected female ticks during mating (venereal transmission), and trans-ovarially, from infected females to their offspring. The epidemiologal significance of vertical (transovarial) transmission is unknown. However it could provide an important amplification mechanism if virus is transmitted from infected

to uninfected larvae co-feeding on the same host. Virus survival in infected ticks and the ability of *Hyalomma* species to survive at least 800 days without a blood meal indicate that ticks act as virus reservoirs (Fig. 2).

Direct transmission through contact with infected blood and body fluids and, possibly, crushed ticks are an important route of human infectious (M.W Service.2001, Goodman 2005, Knipe 2001). The virus causes in apparent infection or mild fever in livestock (Swanepoel 1985 S.Af.Med 68:638-641 – Swanepoel 1985.Af.Med 68:635-637).

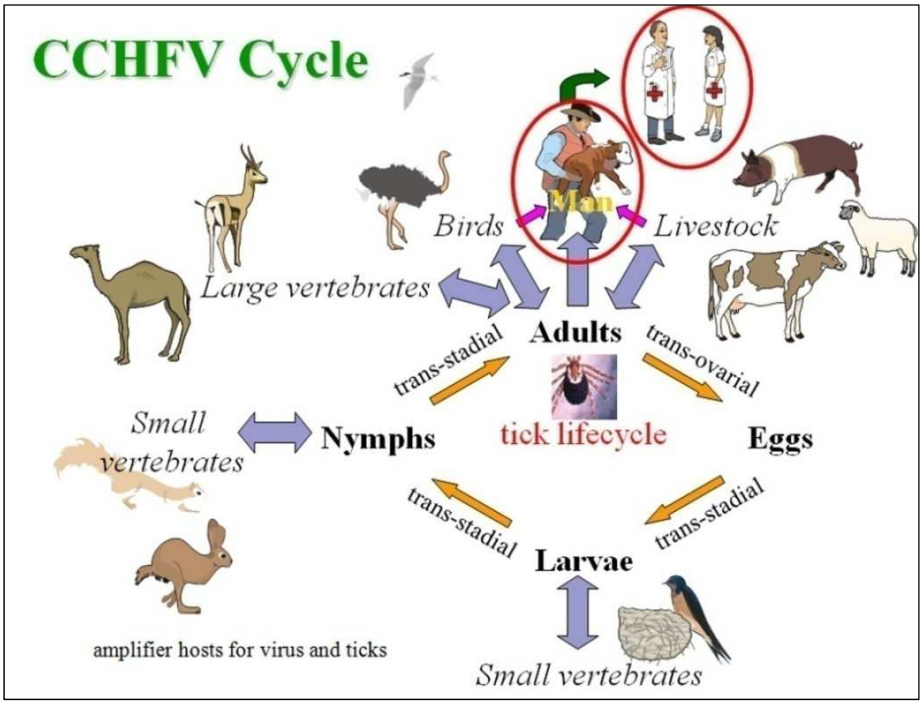


Fig. 2. CCHF virus Cycle in nature

Young ruminants, including calves and lambs, acquire maternal antibody from colostrums, but it has not been determined whether this is protective, and many animals seroconvert early in life after the occurrence of natural infection. Consequently, humans commonly become infected when they come into contact with the viremic blood of young animals in the course of performing procedures such as castrations, vaccination, insertion of ear tags, or slaughter of animals (Hoogstraal1979, Swanepoel1985). The evidence suggests that the infection in humans is acquire through contact of viremic blood with broken skin, and this is consistent with the observations that nosocomial infection in medical personnel usually results from accidental pricks with needles contaminated with the blood of patients or similar mishaps (Hoogstraal1979, Shepherd,A.J 1985).

In view of the serological evidence that infection of livestock occurs on a wide scale in areas infested by *Hyalomma* ticks, it is surprising that so few human infections are diagnosed. This

raises the possibility that many human infections are asymptomatic or mild and pass unnoticed, but the low prevalence of antibody generally detected in surveys and the sparse evidence of infection encountered among cohorts of cases of the disease suggest that a high proportion of CCHF infections does, in fact, come to medical attention (Fisher-Hock 1992).

Possible explanation for the low incidence of infection which occurs in humans include the fact that viremia in livestock is short lived and of low intensity compared to that in other zoonotic disease such as Rift Valley fever, which is more readily acquired from contact with infected tissues.

Furthermore, despite the fact that a high proportion of patients acquire infection from ticks, humans are not the preferred hosts of *Hyalomma* ticks and are infrequently bitten in comparison to livestock (M.W.Service 2001, Goodman2005 and Knipe 2001).

5. Epidemiology

5.1 CCHF in the world

The distribution of the disease coincides with that of the principal vectors of the virus, ticks of the genus *Hyalomma*. Cases of the naturally acquired human infection have been documented in the former Soviet Union, China ,Bulgaria, Yugoslavia, Albania, Kosovo, Pakistan, Iran, Iraq, United Arab Emirates, Saudi Arabia, Oman, Tanzania, Central African Republic, DRC(former Zaire) Uganda, Kenya, Mauritania, Burkina Faso, South Africa and Namibia.

In addition, virus has been isolated from ticks or non-human mammals in Madagascar, Senegal, Nigeria, Central African Republic, Ethiopia, Afghanistan, Greece and Hungary (Swanepoel 1987).

The initial outbreaks of CCHF recognized on the Crimean Peninsula in 1944 and 1945 occurred under conditions of war when large members of soldiers and peasant farmers were exposed to tick bites while harvesting crops and sleeping outdoors (Hoogstraal 1979). Subsequent recognition of the presence of the disease in many countries in Eastern Europe and Asia similarly came about through the occurrence of highly visible epidemics or nosocomial outbreaks occasioned by human intervention , resulting in multiple exposure of people to infection. These include the institution of major land reclamation schemes or abrupt changes in animal husbandry practices in the former Soviet Union and Bulgaria in the 1950s and 1960s,nosocomial outbreaks of infection in Pakistan in 1976 and in Iraq and Dubai in1979, large - scale exposure of war refugees to outdoor conditions in Kosovo in 2000,Albania in 2001,and Pakistan in2001-2002, and multiple exposure of people to blood and ticks from the handling and slaughter of livestock imported from Africa and Asia to Saudi Arabia in1990,the United Arab Emirates in1994-1995 and Oman in1995.

The occurrence of these epidemics led to the perception that CCHF was an emerging disease. However, in many other countries in Eurasia and Africa the presence of the viruses was discovered because prospective laboratory investigations were undertaken, not because a specific clinical entity had been recognized, antibody surveys indicate that there is widespread circulation of virus in nature in many countries that have not yet recognized the occurrence of human disease (Hoogstraal 1979, Swanepoel 1987).

Hoogstraal pointed out that mechanisms for the dissemination of ticks and hence viruses, which include the movement of birds migrating annually on a north-south axis (Hoogstraal 1961, Hoogstral 1963) must have operated in Eurasia and Africa for millennia.

In addition, ticks can be dispersed between continents by movement of livestock. Although there is evidence that recent outbreaks of CCHF in the Arabian Peninsula resulted from trade of tick –infected livestock from Africa and Asia, long –established CCHF endemicity in the region cannot be excluded. Despite the potential for dispersal of the virus between the continents, it appears from phylogenetic analyses of CCHF isolates that the circulation of the virus is largely compartmentalized within the two land masses of Africa and Eurasia where the distribution of strains of the virus is probably related to the distribution and dispersal of the virus vectors.

The implication is not that there is continuing spread of CCHF from its present range, but that further investigation would reveal the presence of the virus and disease in the remaining countries of Africa, Eastern Europe and Asia, which lie within the distribution range of *Hyalomma* ticks.

Although the incidence of recognized cases of human infection is generally extremely low in countries where CCHF is endemic, it should be borne in mind in assessing the socioeconomic impact of the virus that the disease affects particular segments of the population, including those involved in the livestock industry and in health care. Hence, the occurrence of outbreaks can have dire consequences. For instance, the dedication of highly trained staff and expensive facilities and equipment to the intensive care of a single patient in isolation can prove to be very costly and disruptive of normal medical services .Bans imposed on the importation of slaughter livestock can seriously affect the economies of exporting countries (Goodman 2005).

5.2 CCHF in Iran

Although sporadic surveys of CCHF in livestock and humans have been undertaken since 1970, it was not however until the 1999 outbreak that CCHF was recognized as one of the country's major public health problems.

In consequence the laboratory of Arboviruses and Viral Hemorrhagic Fevers was established as a National Reference laboratory in the Pasteur Institute of Iran (a member of National Expert Committee on Viral Hemorrhagic Fevers).

The mission of the institution is to test all human, livestock and ticks suspected to be infected with CCHF viruses from all provinces of Iran with a rapid and charge free service. Thus since 2000, the percentage of CCHF infections throughout the country has been closely monitored. Twenty three (23) out of 30 provinces of Iran are endemic for CCHF virus and Sistan-Va-Baluchistan, Isfahan, Fars, Khuzestan are respectively the most heavily infected provinces.

In 2002 the CCHF virus genome was detected in 22.3% of ticks collected from Chaharmahal-va-Bakhtiari province, southwest of Iran.

In 2004, after a report of a human CCHF confirmed case in Hamadan province, western region of Iran, similar studies showed CCHF virus in 11.3% of ticks and nearly 30% of the livestock were IgG positive.

A study in 2003-2004 in Sistan-va-Balouchistan province, demonstrated that among 285 human volunteers, 6.3% were seropositive for CCHF infection. A seroepidemiological survey among livestock in Isfahan province between 2004 and 2005, showed seropositivity in almost 56% of the animals.

During the years 2003-2005, of 448livestock sera collected from Khorassan province, northeast part of Iran, 77.5% of 298 sheep samples and 46% of 150 goat samples were seropositive which implied a hyper enzootic region for CCHF. Other work that has focused on isolating and analyzing the CCHF viruses genome has led to the discovery of interesting phylogenetic relationship of the virus strains circulating in Iran. Thus Iran strains are very similar to the Matin strain of Pakistan (Chinikar 2010, Chinikar 2004).

In December 2008, a reemerging outbreak of CCHF occurred in the southern part of Iran. Five people were hospitalized with sudden fever and hemorrhaging, and CCHF was confirmed by RT-PCR and serological assays.

One of the cases had a fulminant course and died. Livestock was identified as the source of infection, all animals in the incriminated herd were serologically analyzed and more than half of them were positive for CCHF. Two routes of transmission played a role in this outbreak: contact with tissue and blood of infected livestock, and nosocomial transmission.

Phylogenetic analyses helped to identify the origin of this transmission. It is possible that a new strain occurred in the outbreak region, and future phylogenetic analyses are required to identify the precise origin of this genetic variant (Chinikar 2010).

In 2006, recombinant CCHF Virus antigen (nuclear protein) was produced by the Semliki Forest Virus expression system. The recombinant antigen is used in Elisa for serological diagnosis of CCHF, and is a useful advantage in that its production does not need biosafety level4 facilities.

In very recent years, new research projects focused on expression of a recombinant antigen to develop a subunit vaccine are being performed at the National Reference Laboratory as well as the previously mentioned projects (Chinikar 2005, Chinikar 2002, and Garsia 2006).

From June 2000 to 20 September2011, 2382 serum samples from CCHF probable patients have been collected from different provinces and transferred according to safety procedures to the laboratory of Arboviruses and Viral Hemorrhagic Fevers (National Reference Laboratory).

Among these 2382 probable cases, 853 were confirmed as positive CCHF either serologically and/or molecularly, and between these 853 confirmed cases 122 have died (Fig. 3.)

The data showed that the disease has been seen in a majority of Iranian regions, i.e. 23 out of 30 provinces of Iran (Fig. 4.).

More than one decade experience on CCHF in Iran has conducted to the following conclusions:

Sistan-va-Baluchistan is the most infected province (in the southeast of Iran, near the border of Pakistan and Afghanistan, where the disease is endemic) with 70% of positive cases.

The phylogenetic studies showed that the Iranian CCHF strain is very similar to the Matin (Pakistan) CCHF strain.

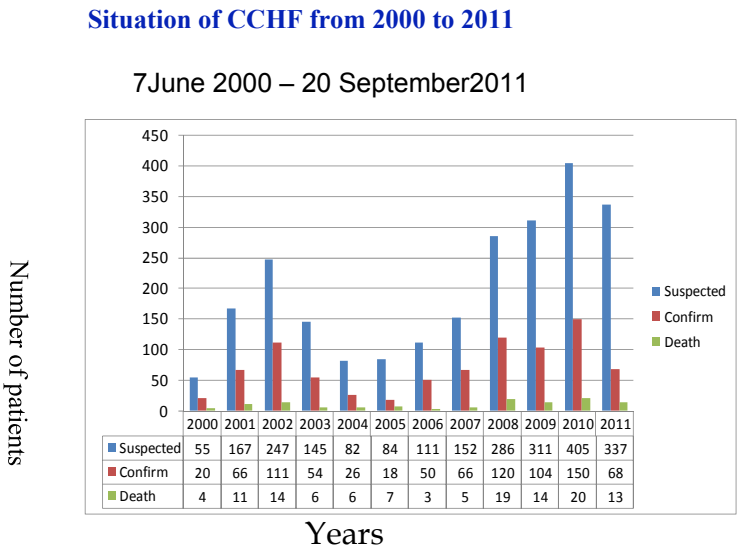


Fig. 3. Situation of CCHF from 2000 to September2011

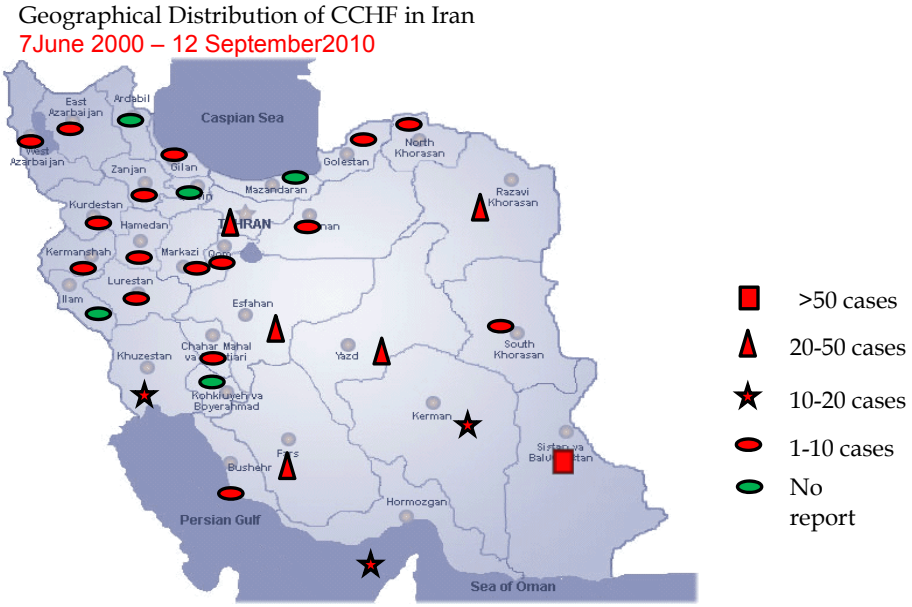


Fig. 4. Geographical Distribution of CCHF in Iran

The khorassan province and the Fars province are respectively the second and third infected province after Sistan-va-Baluchistan province (Fig. 5.).

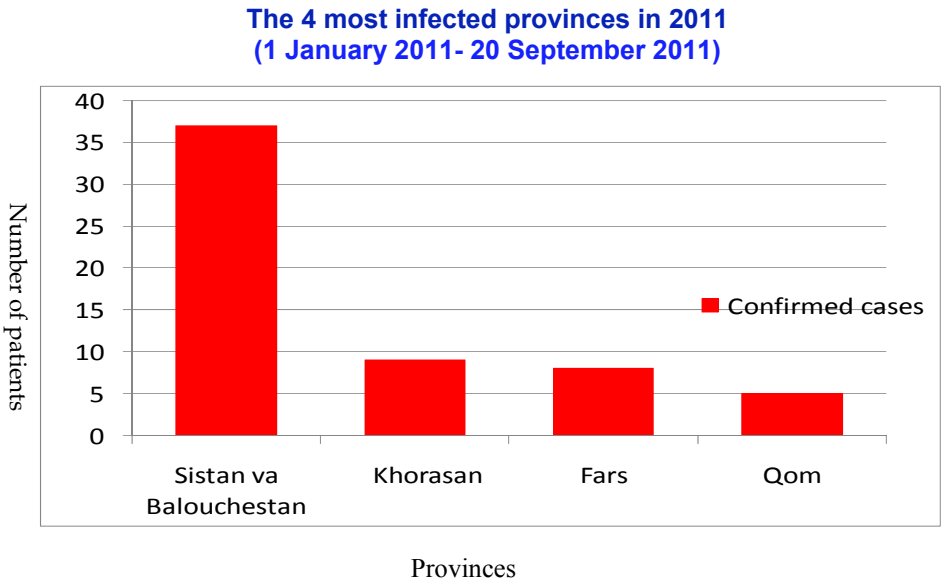


Fig. 5. The 4 most infected province in 2011

The epidemiological data showed that severity of the disease and also mortality rate of CCHF in different years and different provinces are different, so it seems more phylogenetic and pathogenesis studies should be done in this regard.

The majority of confirmed cases in Iran have profession like butchers, slaughterers, slaughter house workers, which have to deal with infected livestock blood or organs (Fig. 6.).

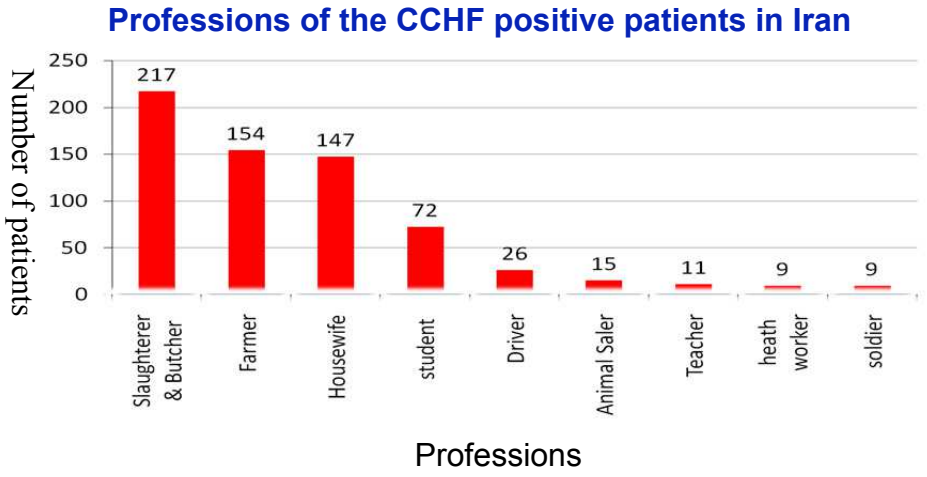
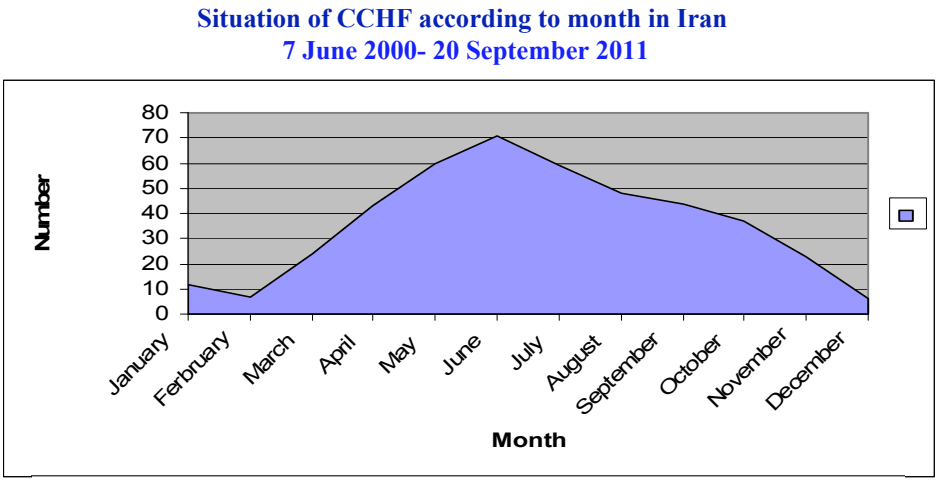


Fig. 6. Profession of the CCHF Positive patients in Iran

The majority of the positive cases in all the study years are seen in June and July (in warm months in which ticks population and their activity is high) (Fig. 7.).



Our data showed that the CCHF is gradually raised in March and reached into a peak in June and July months in all study years in Iran.

Fig. 7. Situation of CCHF according to month in Iran

The sex distribution of CCHF in Iran is a 3:1 ratio (male/female).

Age distribution: the majority of Iranian patients are between 20-40years (working age) (Fig. 8.).

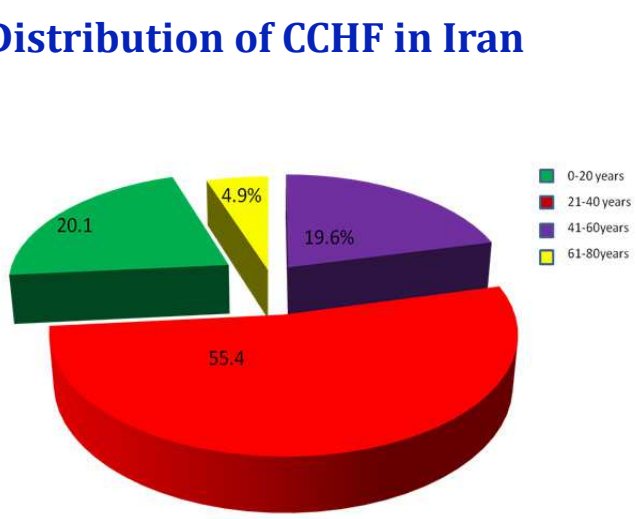


Fig. 8. Age Distribution of CCHF in Iran

6. Clinical manifestations

6.1 Symptoms

Disease severity appears similar wherever Crimean-Congo haemorrhagic fever occurs. Clinical signs of the disease follow an incubation period of 1-7 days but this may be longer when infection is by contagion rather than by tick bite. The disease is characterized by a sudden onset with severe headache, dizziness, neck pain and stiffness and photophobia. Fever with chills occurs at about the same times. Patients rapidly develop general myalgia and malaise, with intense leg and back pain. By the second to fourth day of illness, patients may have a flushed appearance. In severe cases, a petechial rash appears on the trunk and limbs by the third to sixth day of illness (Fig. 9.).

Fig. 9. CCHF Patient

Internal and external bleedings are common, although sometimes a tendency to haemorrhage is apparent only from the oozing of blood from injection or venepuncture sites. Severely ill patients may show hepatorenal and pulmonary failure from about day 5 onwards, and become progressively drowsy, stuporous and comatose. Deaths generally occur on the 5th to 14th day of illness. Recovery from CCHF begins on day 9 or 10 with the abatement of the rash and general improvement, although convalescence may continue for a month or longer (Richman 2002, Knipe 2001).

7. Clinical pathology and histopathology

Changes in the cellular and chemical composition of blood recorded during the first few days of illness in human patients include leukocytosis or leukopenia and elevated serum aspartate transaminase, alanine transaminase, gamma-glutamyl transferase, lactic dehydrogenase, alkhaline phosphatase, and creatine kinase levels, while bilirubin, creatinine and urea levels increase and serum protein levels decline during the second week (Swanepoel 1989). Thrombocytopenia, elevation of the prothrombin ratio, increased

thrombin time, and elevated levels of fibrin degradation products, as well as depression of fibrinogen and hemoglobin values, are evident during the first few days of illness.

Complete autopsies are seldom performed on patients who die of CCHF, and examination of tissue is often confined to liver samples taken with biopsy needles. Lesions in the liver vary from disseminated foci of necrosis, to massive necrosis involving over 75% of hepatocytes and a variable degree of hemorrhage (Swanepoel 1985).

Inflammatory cell infiltrates in necrotic areas are absent or mild and unrelated to the extent of hepatocellular damage.

Limited observations of splenic tissue show lymphoid depletion, focal necrosis, and scattered lymphoblasts in periarterial sheaths. In addition, diffuse alveolar damage, intra-alveolar hemorrhage, hyaline membrane formation, and a mononuclear interstitial pneumonitis have been observed in the lungs, and congestion and slight interstitial edema have been noted in the heart. Lesions in other organs include congestion, hemorrhage, and focal necrosis in the central nervous system, kidneys, and adrenal glands, and general depletion of lymphoid tissue. None of the histopathologic features is pathognomonic, and similar features can be seen in other viral, rickettsial, and bacterial infections, as well as toxic exposures. Hence, a definitive diagnosis can be established only by immunohistochemical or virological tests (Richman 2002, Goodman 2005, and Knipe 2001).

7.1 Pathogenesis

The Pathogenesis of the disease is incompletely understood (Shepherd, A.J 1989), but by analogy with other arthropod-borne virus infections it can be surmised that CCHF virus undergoes some replication at the site of inoculation and that there is hematogeneous and lymph-borne spread of infection to organs such as the liver, which are major sites of replication. Localization of CCHF virus in tissues by immunohistochemistry has shown that mononuclear phagocytes and endothelial cells are also major targets of virus infection (Burt 1997). A similar tropism is exhibited by many lethal hemorrhagic fever viruses. The mononuclear phagocyte system may constitute a mechanism for viral clearance in some patients, but in others replication of virus in these cells may enhance viremia. Infection of mononuclear phagocytes and depletion of lymphoid cells may protect the virus from phagocytosis and immune inactivation and enhance the spread of virus. In addition it may play a role in the pathogenesis of CCHF through the release of physiologically active substances, including cytokines, tumor necrosis factor and other inflammatory mediators and procoagulants.

The occurrence of disseminated intravascular coagulation (DIC) appears to be an early and central event in the pathogenesis of the disease. The hepatocytes are a major target of the virus, and the occurrence of minimal inflammatory infiltration suggests that hepatocellular necrosis may be mediated by a direct viral cytopathic effect. Hepathocellular necrosis leads to further release of tumor necrosis factor and other procoagulation into the circulation, and ultimately to impairment of the synthesis of coagulation factors to replace those which are consumed in DIC. Wide spread infection of endothelium with degenerative change rather than necrosis is associated with capillary dysfunction, which contributes to the occurrence of a hemorrhagic diathesis and the generation of a petechial rash (Richman 2001, Krasus 2003, Goodman 2005, Knipe 2001).

8. Diagnosis

8.1 Generalities

A diagnosis of CCHF should be suspected when severe influenza-like illness with sudden onset and short incubation period, usually less than 1 week, occurs in persons exposed to tick bites or fresh blood and other tissues of livestock or human patients. The disease is easier to recognize once a rash appears and there are hemorrhagic signs such as epistaxis, hematemesis and melena.

Etiologic investigation of suspected CCHF infections should be performed in a BSL-4 laboratory (Krasus 2003, Isba 2004, Van der Giessan2004 and Chhabra 2003).

Confirmation of the diagnosis in the acute phase of illness consists of detection of viral nucleic acid by reverse transcriptase PCR (RT-PCR), demonstration of viral antigen by enzyme-linked immunoassay (ELISA) of serum samples, or isolation of the virus (Burt 1994 and Burt 1998).

In samples collected later, the diagnosis is confirmed by demonstration of an immune response. RT-PCR using conventional thermocycling or real-time PCR constitutes a rapid and sensitive technique for diagnosing CCHF infection during the early stage of infection before an antibody response is demonstrable or in fatal cases where an antibody response is frequently not demonstrable (Burt 1998). Virus may be isolated in cell cultures, commonly of vero cells or by intracerebral inoculation of 1- day- old mice. The virus is detected and identified in cell cultures by performing an immunofluorescence (IF) test.

Isolation of the virus in cell cultures can be achieved in 1 to 5 days, compared to 5 to 8 days in mice, but mouse inoculation is more sensitive for isolating virus that is present at low concentrations. Nairoviruses in general, including CCHF, induce a weak neutralizing antibody response, and serum samples frequently contain nonspecific inhibitors of virus infectivity. Hence, neutralization test have found limited application for demonstrating antibody response. In contrast, indirect IF has proved to be a rapid and sensitive technique for detecting an immune response to CCHF virus. The Elisa is also a sensitive technique, and both Elisa and IF can distinguish between immunoglobulin G (IgG) and IgM antibodies.

Both IgG and IgM antibodies become demonstrable by IF from about day 5 of illness onwards and are present in the sera of all survivors of the disease by day 9 at the latest. The IgM antibody activity declines to undetectable levels by the fourth month after infection, and IgG titers may begin to decline gradually at this stage but remain demonstrable for at least 5 years. Recent or current infection is confirmed by demonstrating seroconversion, a fourfold or greater increase in antibody activity in paired serum samples, or IgM activity in a single specimen.

Patients who succumb rarely develop a demonstrable antibody response, and the diagnosis is confirmed by isolation of virus or detection of viral nucleic acid in serum samples, liver samples taken after death, or demonstration of CCHF antigen by immunohistochemical techniques with paraffin embedded liver sections. Virus antigen may sometimes be demonstrated in liver impression smears by IF or in serum or liver homogenate by Elisa.

Observation of necrotic lesions compatible with CCHF in sections of liver provides presumptive evidence in support of the diagnosis. (Richman 2002, Chhabra 2003, Goodman 2005, Knipe 2001)

8.2 Differential diagnosis

The vast majority of suspected cases of CCHF prove to be severe infections with more common agents, including bacterial septicemias, malaria, viral hepatitis, rickettsioses and complications of human immunodeficieng virus AIDS. In arriving at a diagnosis, it is important to take into account an accurate history of possible exposure to infection, signs and symptoms of illness and clinical pathology findings (Richman 2002, Knipe 2001).

In Africa, CCHF should be distinguished from other febrile diseases associated with ticks (Burt 1996) and particularly from tick borne typhus which has an incubation period of 7 to 10 days and a more insidious onset than CCHF. Tick-borne typhus is associated with a petechial rash and is capable of causing a fatal disease in humans with hemorrhagic manifestations similar to CCHF, but it is amenable to treatment with appropriate antibiotics. Other tick-borne diseases occurring in Africa which could be considered include Q fever and relapsing fever borreliosis. In addition, there are a number of tick-borne viruses in Africa apart from CCHF, which have been associated with human disease such as Dughe and Nairobi sheep disease viruses (M.W. Service 2001).

Rift-Valley fever can also be acquired from contact with the tissues of livestock in Africa, but it usually occurs in the context of massive epidemics involving abortion and death of sheep and cattle at irregular intervals in years when heavy rains favor the breeding of the mosquito vectors of the virus.

Particular consideration should be given to the other viral hemorrhagic fevers of Africa. In brief, they include Marburg disease and Ebola fever, caused by members of the family Filoviridae, and Lassa fever, caused by a virus of the family Arenaviridae. Marburg and Ebola viruses cause sporadic outbreaks of highly lethal disease in tropical Africa, often in association with similar disease in non-human primates, but the source of these viruses in nature remains unknown. Lassa fever virus causes chronic renal infection of rodents in West Africa and transmission to humans occurs through contamination of food and house dust with rodent urine.

Another group of rodent-associated viruses which belongs to the Hantavirus genus of the family Bunyaviridae are found in Europe, Asia and the Americas.

Diseases caused by the Hantaviruses of Europe and Asia are known collectively as hemorrhagic fever with renal syndrome, and these could conceivably be confused with CCHF on occasion. The Hantaviruses of north and South America are associated with the so-called Hantaviruses pulmonary syndrome, which is less likely to be confused with CCHF. There is inconclusive evidence for the presence of Hantaviruses in Africa.

Yellow fever and dengue virus (of which there are four serotypes) are mosquito-borne flaviviruses capable of causing fatal hemorrhagic disease in humans within defined geographic ranges.

Chikungunya virus is a mosquito-borne alphavirus which has been associated with hemorrhagic disease in Asia, although in Africa it is reported as a benign febrile illness with severe joint pain. Although not found in Africa, Omsk hemorrhagic fever and Kyasanur forest disease (tick-borne flavivirus infections) might also be considered in the differential diagnosis in their respective ranges.

Distinguishing between the various possible causes of suspected viral hemorrhagic fever is a specialized task, normally undertaken in laboratories dedicated to the purpose (Goodman 2005).

9. Common laboratory diagnostic methods

9.1 Serological assay

Serological methods have been developed to diagnose CCHF using either inactivated virus or extract from infected suckling mouse brain. Since CCHF is highly pathogenic for humans and the available therapeutical means are limited to the use of ribavirin when administered early upon onset of syndromes, it must be handled in BSL4 containment, rendering difficult the production of native antigen. Due to these limitations, recombinant antigens were produced, replacing native antigen in Elisa and other antigen dependent assays. As the nucleoprotein of CCHF virus is recognized as the predominant antigen inducing a high immune response in most Bunyavirus infection, the recombinant nucleoprotein of the CCHF virus has been produced through Semliki Forest virus and baculovirous expression systems and used to detect IgM and IgG in human and animal serum.

9.1.1 IgG Elisa (Swanepoel, R 1987)

The wells were coated overnight at 4°C with the mouse hyper immune ascetic fluid diluted at1:1000 in 0.05% Tween 20-PBS containing 5% skim milk as a saturating reagent. This solution was used to dilute antigen and sera. The native or the recombinant antigen at a 1:100 dilution was added for 1h 37°C. Peroxydase labeled antihuman or anti- animal immunoglobulin was added at 1:1000 for 1h at 37°C.After 10min of incubation with the TMB substrate (KDL, Gaithersburg MD,USA),the OD was read at 450and 620nm (Chinikar 2005, Chinikar 2002, Chinikar 2010).

9.1.2 IgM Elisa

The Elisa plates are coated with the goat IgG fraction to human IgM (anti μ chain) diluted in PBS 1x and incubate over night at4°C.After addition of diluted recombinant or native antigen, diluted immune-ascite is then added .After a definite incubation , peroxydase – labeled anti – mouse immunoglobulin is added and incubated in 37°C. The plates then are washed three times with PBST containing 0.5% Tween. Finally, hydrogen peroxyde and TMB (3, 3', 5, 5' tetra methyl benzedrine) are added and after a short incubation, the enzymatic reaction is stopped by the addition of 4N sulfuric acid. Then, the plates are read by one Elisa reader at 450nm (Chinikar 2005, Chinikar 2002, and Chinikar 2010).

9.2 Molecular assay

Viral RNA is extracted from 140μl of serum or phenol extracted tick suspensions using QIAamp RNA mini kit according to the instructions of the manufactures (QIAgen GmbH, Hilden, Germany). The extracted viral RNA is analyzed by gel – based and Real -Time RT – PCR using a one -step RT-PCR kit(QIAgen GmbH , Hilden ,Germany) and specific primers F2 5´TGGACACCTTCACAAACTC 3´and R3 5´GACAATTCCCTACACC 3´,which amplify a 536 bp fragment of the S segment of CCHF virus genome. The PCR reaction is done in 50μl total

volume and 30min at 50°C, 15min at 95°C, and 40cycles including 30s at95°C, 30s at 50°C, 45s at 72°C, and finally10 min in 72°C as a final extension. (Chinikar 2010, Chinikar 2004)

10. Prevention, control and treatment

Nosocomial infections have been associated with needle stick injuries or contact of broken skin with infected blood, tissues, and body fluids of patients.

Aerosol transmission is not considered a primary mode of transmission, in situations where infection with CCHF virus is suspected, patients should be isolated and subjected to barrier nursing techniques until the diagnosis is confirmed or excluded, to protect health care workers from potential exposure to infection.

In brief, the patient should be isolated in a room with an adjoining anteroom, if possible, for storage of supplies required for barrier nursing and patient care. Health care workers should wear protective clothing such as disposable gowns, gloves, masks, goggles and overshoes, which are discarded on leaving the isolation room via the anteroom.

All items removed from the isolation ward should be safety disposed of or suitably disinfected. Blood samples should be wrapped in absorbent material such as paper towels and placed in secondary leak-proof containers, such as rigid metal or plastic screw-cap containers or sealed plastic bags for safe transport to the laboratory.

Clinical laboratory tests should be kept to a maximum security and performed by experienced staff wearing protective clothing, and automated analyzers must be decontaminated after use, commonly with dilute chlorine disinfectants, CCHF virus is classified as a biohazard class IV pathogen, hence, specific diagnostic tests and culture of the virus are undertaken only in BSL-4 laboratories in countries which have relevant biosafety regulations.

Acaricide treatment of livestock and controlling the numbers of hares are effective in reducing the populations of infected ticks and hence the risk of infection. However, tick control is impractical in many regions of the world where *Hyalomma* ticks are most prevalent.

Clothing impregnated with pyrethroid acaricides can give some protection against tick bites. Wearing gloves and limiting exposure of naked skin to fresh blood and other tissues of animals are practical control measures that should be undertaken by veterinarians, slaughter workers and others involved with potentially infected livestock, and by medical staff treating patients.

Treatment of the disease consists essentially of supportive and replacement therapy with blood products .Immune plasma has been used, but the efficacy of this treatment is not clear, since there has been no systematic investigation with a uniform product of known virus-neutralizing activity.

Promising results were obtained in limited trials with the chemotherapeutic drug Ribavirin, but the disease is often recognized only at a late stage, ideally, treatment should commence before day 5of illness.

Owing to the occurrence of vomiting and hemorrhagic gastroenteritis, oral ribavirin is not very useful in severely ill patients who need treatment most, and the intravenous form of

the drug is often difficult to obtain, since it is produced on a limited scale owing to the lack of demand, and it is not available in Iran.

Oral ribavirin can be used prophylactically in instances of known exposure to infection, such as in needle stick injuries with the blood of patients with a confirmed diagnosis.

Inactivated vaccines prepared from infected mouse brain were used for the protection of humans in Eastern Europe and the former Soviet Union in the past, but no commercial vaccines are currently available (Richman 2002, M.W.Service 2001, Goodman 2005).

11. References

Burt F.J., P.A.Leman, J.F.Smith, R.Swanepoel. (1998). The use of a reverse transcription – polymerase chain reaction for the ection of viral nucleic acid in the diagnosis of Crimean-Congo haemorrhagic fever. *Virol. Methods* 70:129-137.

Burt, F.J., Swanepoel, R., Shieh, W.J., Smith, J.F., Leman P.A., Geer, P.W., Coffield, L.M., Rollin, P.E., Ksiazek T.G. Peters, C.J., Zaki, S.R. (1997). Immunohistochemical and in situ localization of Crimean-Congo Hemorrhagic fever (CCHF) virus in human tissues and implications for CCHF pathogensis. *Arch. Pathol. Lab. Med*. 121: 839-846.

Burt,F.J, Spencer, D.C., leman, P.A. Patterson, B. Swanepoel, R.(1996) Investigation of tick-borne viruses as pathogens of humans in South Africa and evidence of Dugbe virus infection in patients with prolonged thrombocytopenia. *Epidemiol. Infect*. 116: 353-361.

Burt, F.J., Leman, P.A., Abbott, J.C., Swanepoel, R., (1994) "Serodiagnosis of Crimean-Congo haemorrhagic fever". Epidemiol. Infect. 113: 551-562.

Calisher, C.H & karabatsos N. (1989). "Arbovirus serogroups: definition and geographic distribution, p 19-57. In T.P. Monath *(ed.)*". The *Arboviruses: Epidemiology*, vol1. CRC Press, Inc, Boca Raton, Flc.

Casals, J and Tignor G.H(1980) "The Nairivirus genus" *Intrvirology* 14: 144-147.

Casalas, J. (1969) "Antigenic similarity between the virus causing Crimean Haemorrhagic fever and Congo virus". Proc.*Soc.Exp.Biol.HED* 131: 233-236.

Chhabra, Daljeet & Arora, Sushrut (2003) "*Atext book on General and Microzoonoses*"(first editon),JAYPEE BROTHERS, ISBN 81-8061-043-8, New Delhi.

Chinikar.S, Ghiasi.S.M,Hewson.R, Moradi.M, Haeri.A (2010) "Crimen-Congo hemorrhagic fever in Iran and neighboring countries" *Journal of Clinical Virology47* (2010) 110-114.

Chinikar.S, Ghiasi.S, Moradi.M,et al . (2010) "Phylogenetic analysis in a recent controlled outbreak of Crimean_ Congo haemorrhagic fever in the south of Iran" . *Eurosureveill, 2010*;15 (47):pii=19720. December 2008.

Chinikar.S, Ghiasi.S, et al. (2009) An overview of Crimean_ Congo haemorrhagic fever in Iran. *Iranian journal of Microbiology* (spring 2009) 7-12

Chinikar.S, Mirahmadi R., Mazaheri V., Nebeth P., Saron MF., Salehi P. (2005) "A serological survey in suspected human patients of Crimean-Congo haemorrhagic fever in Ira by determination of IgM specific ELISA method during2000-2004". Arch. *Iranian Med* 2005: 8: 52-55.

Chinikar.S., Persson Stine-Mari, Johansson Marie, Bladh Linda, Goya Mehdi, Houshmand Badakhshan, Mirazim Ali, Plyusnin Alexander,et al.(2004) "Genetic Analysis of Crimean- Congo Hemorrhagic Fever Virus in Iran".*Journal of Medical Virology 73*:404–411

Chinikar S., Fayaz A., Mirahmadi R., Mazaheri V., Mathiot C., Saron M.F.(2002) "The specific serological investigation of suspected human and domestic animals to Crimean-Congo haemorrhagic fever in various parts of Iran using Elisatechniques". *Hakin J* 2002: 4: 294-300.

Chinikar.S (2007). Crimean_ Congo haemorrhagic fever "*A Global Perspective Book*" (Published by springer,Onder Ergonul ChrisA.Whitehouse Editors) (89-99) ISBN 978-1-4020-6106-6 (e-book)

Chumakov, M.P(1974) "*Contribution to 30years of investigation of Crimean-Congo haemorrhagic fever*"Abad.Hed.Naul SSR 22:5-18.

Chumakov, M.P, Smirnova, S.E and tkachenho, E.A. (1970) "*Relationship between strains of Crimen haemorrhagic fever and Congo viruses*" Acta Virol. 14:82-85.

Clerx J.P. Casals, J. Bioshop, D.H.(1981) "*structural characteristics of nairoviruses (genus Nairovirus, Bunyaviridae*". J.Gen.Viral. 55:165-178.

Davies, F.G, casals J, Jesset D.M., and Ochieng, P.(1978). "*The serological relationships of Nairobi sheep disease virus*.J.Camp.Pathol. 88:519-523.

Fisher-Hock S.P, Mc Cormick, J.B., Swanepoel, R., Van Middle loop, A. Harvey , S. Kustner H.G.(1992) "Risk of human infections with Crimean-Congo haemorrhagic fever virus in a South African rural community". *An.J. Trop. Med. Hyg*. 47:337-345

Garsia.S, Chinikar.S, Coudrier.D, et al: "Evaluation of a Crimean-Congo Hemorrhagic fever virus recombinant antigen expressed by Semliki Forest suicide virus for IgM and IgG antibody detection in human and animal sera collected in Iran". *Journal of Clinical Virology 35* (2006) 154-159.

Goodman, L.Jesse.(T.Dennis David, and Shine E.Sonen)(Ed). (2005) "*Tick-Borne Disease of Humans* " ASM Press, ISBN1-55581- 238-4, Washington,DC.

Hoogstraal, H. (1979). "The epidemiology of tick-borne Crimean-Congo hemorrhagic fever in Asia, Europe and Africa". *J.Med.Entomol*.15: 307-417.

Hoogstral, H. Kaiser, M.N., Traylor, M.A., Guindy, E., Gaber, S. (1963). "Ticks (Ixodidae) on bird's migratig from Europe and Asia to Africa" 1959-61. *Bull.W.H.O*. 28: 235-262.

Hoogstraal, H., Kaiser, MN., Traylor, M.A., Gaber , S. and Guindy, E. (1961). "Ticks (Ixodidae) on bird's migration from Africa to Euroupe and Asia". *Bull.W.H.O*.24: 197-212

Isba Rachel (2004)"*Rapid infectious Disease and Tropical Medicine*" (First edition) Blackwell, ISBN 1-4051-1325-1, Massachusetts,USA.

Karabatsos, N.(1985) "*International Catalogue of Arboviruses (including certain other viruses of vertebrates*" (3rd ed) American Society of tropical Medicine and Hygiene, San Antonio, Tex.

Knipe, David M. Howely, Peter M. (Eds) (2001) Bunyaviridae, In: *Fields Virology* ,LIPPINCOTT WILLIAMS & WILKINS, ISBN 0-7817-1832-5, USA .

Krasus, Harmut., Weber, Albert., Appel, Max., Enders, Burkhards., D.Isenberg, Henry., Gerd Schifer, Hans. Slenczka, Werner., Von Graevenitz,Alexander., Zahner, Horst. (2003) *ZOONOSES Infectious Disease Transmissible from Animals to Humans* (Third edition)ASM Press,ISBN: 155812368,Washington DC.

M.W. Service.(Ed) (2001) "*The Encyclopedia of Arthropod_transmitted Infectious of Man and Domesticated Animals* "CABI Publishing, ISBN 0851994733New York,USA

Richman, Douglas D .(WHITLey ,Richard J. and Hayden, Frederick G.)(Ed) (2002) *Clinical Virology*. ASM Press, ISBN 1 55581- 226-0, Washington,D.c.

Shepherd, A.J., Swanepoel, R., Leman, P.A. (1989). "Antibody response in Crimean-Congo haemorrhagic fever". *Rev. Infect. Dis*. 11: 806-811

Shepherd,A.J, Swanepoel, R. Shepherd, S.P. leman, P.A, Blachbun , N.K. and Hallett, A.F.(1985) "A nosocomial outbreak of Crimean-Congo haemorrhagic fever at Tygenberg Hospital". *S.Af.Med.J*. 68: 733-736.

Simpson, D.K, Knight, M.C, G.Courtois, Weinbren , M.P and Kibuhanusoke, J.W. (1976.) Congo virus a hitherto undescribed virus occurring in Africa.*E.Af.Med.J* 44:87-92.

Swanepoel, R., Gill, D.E., Shepherd, A.J., Leman, P.A., Mynhardt, J.H., Harvey, S. (1989). "The clinical pathology of Crimean- Congo haemorrhagic fever". *Rev. Infect. Dis*. 11:794-800.

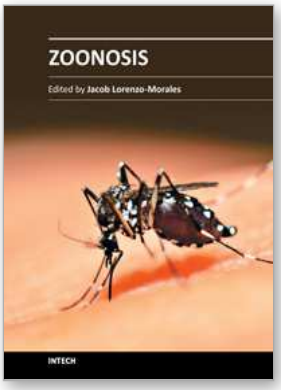
Swanepoel, R., shepherd, A.J. leman, P.A. shepherd, S.P, Mc Gillivray, G.M. Erasmus ,M.J. Seark, L.A. Gill ,D.E.(1987). "Epidemiology and clinical features of Crimean-Congo haemorrhagic fever in southern Africa" .*Am.J.Trop.Med.Hyg*:36:120-132.

Swanepoel, R., shepherd, A.J, leman, P.A. shepherd , S.P. Miller, G.B. (1985) "A common-source outbreak of Crimean- Congo haemorrhagic fever on a diary farm". *S.Af.Med*.J. 68:635-637.

Swanepoel, R. , shepherd A.J. leman,P.A . Shepherd, S.P. (1985). "Investigation foolowing initial recognition of Crimean-Congo haemorrhagic fever in South Africa". *S.Af.Med*.J.68:638-641.

Van der Giessen, J.W.B., Isken L.D., Tiemersma E.W. (2004) *Zoonoses in Europe* :*A risk to public Health* (First Edition National Institute for public health and the environment, Bilthoven, The Netherlands.

Watts, D.M. Ksiazek, T.G. Linthicum ,K.J. Hoogstraal, H. (1989). "Crimean-Congo haemorrhagic fever". In Month, T.P. (ed), the *Arboviruse: Epidemiology and Ecology*, (p 177-222) vol2, CRCPress, Inc. Boca Raton, Fle.



Zoonosis

Edited by Dr. Jacob Lorenzo-Morales

ISBN 978-953-51-0479-7
Hard cover, 436 pages
Publisher InTech
Published online 04, April, 2012
Published in print edition April, 2012

Zoonotic diseases are mainly caused by bacterial, viral or parasitic agents although "unconventional agents" such as prions could also be involved in causing zoonotic diseases. Many of the zoonotic diseases are a public health concern but also affect the production of food of animal origin thus they could cause problems in international trade of animal-origin goods. A major factor contributing to the emergence of new zoonotic pathogens in human populations is increased contact between humans and animals. This book provides an insight on zoonosis and both authors and the editor hope that the work compiled in it would help to raise awareness and interest in this field. It should also help researchers, clinicians and other readers in their research and clinical usage.

How to reference

In order to correctly reference this scholarly work, feel free to copy and paste the following:

Sadegh Chinikar, Ramin Mirahmadi, Maryam Moradi, Seyed Mojtaba Ghiasi and Sahar Khakifirouz (2012). Crimean-Congo Hemorrhagic Fever (CCHF), Zoonosis, Dr. Jacob Lorenzo-Morales (Ed.), ISBN: 978-953-51-0479-7, InTech, Available from: http://www.intechopen.com/books/zoonosis/crimean-congo-hemorhagic-fever-

INTECH
open science | open minds

InTech Europe
University Campus STeP Ri
Slavka Krautzeka 83/A
51000 Rijeka, Croatia
Phone: +385 (51) 770 447
Fax: +385 (51) 686 166
www.intechopen.com

InTech China
Unit 405, Office Block, Hotel Equatorial Shanghai
No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
Fax: +86-21-62489821