Review Article

Recent Emergence and Re-Emergence of Crimean Congo Hemorrhagic Fever and Q Fever Zoonotic Diseases: Major yet Ignored Infectious Diseases Worldwide

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Abstract

The continuing emergence or re-emergence of vector-borne zoonotic Q fever (caused by *Coxiella burnetii*) and Crimean Congo hemorrhagic fever (CCHF, caused by *Orthonairovirus*) include indispensable extraordinary threat around the world. Low infectious dose and long-term environmental residence are major risks. Wildlife and domestic livestock act as hosts or reservoirs of the CCHF virus and ticks are carriers. The disease also poses a threat to public health services owing to its epidemic potential, high case fatality ratio (up to 40%) as well as difficulties in treatment, prevention, and control. Q fever is another zoonotic febrile disease mainly affecting workers involved in farming livestock. The causative agent of Q fever causes abortion in livestock. The pathogen is shed in large numbers in the waste of infected animals (amniotic fluids and placenta during parturition) and is transmitted by inhalation of contaminated aerosols. Vaccination is the most effective way of protecting against Q fever. The main way to prevent Q fever is to avoid contact with animals, especially while animals are giving birth, or consumption of unpasteurized milk and contaminated dairy products. Due to the increasing importation of livestock to meet the growing demand for dairy and meat products, new diseases are likely to be introduced. In our growing globalized world, where trade between countries increases, it is necessary to conduct more research on zoonotic diseases and to monitor any possible disease introduction to new areas. A continuing surveillance program and pathogen testing are important in tracking the emergence of new pathogens.

Keywords: Zoonoses, Crimean Congo hemorrhagic fever, Q fever, Risk factors

Introduction

Vector-borne zoonotic Crimean Congo hemorrhagic fever (CCHF), caused by Nairovirus, *Orthonairovirus* and *Bunyaviridae* family, and query (Q) fever *(Coxiella bornetii)* diseases have been outstandingly affecting the human as continuing threats (1). Indispensable factors such as high level of outbreaks, fatality rate and lack of efficient prevention or eradication pipelines have placed them in high priority category of the world health organization (WHO) R&D Blueprint (2-4). The Middle East countries such as Iran and Turkey have a high rate of CCHF, while there is no sufficient public knowledge or infrastructure

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equipment to encounter any future epidemic emergence. Occurring as acute febrile disease, firstly found in Crimea region in 1940, the CCHF is also globally called Karakhalak, Hungribta and Khunymuny (4, 5). CCHF is a single-stranded RNA and enveloped virus. The enveloped proteins include large (L, RNA-dependent polymerase), middle (M, nucleocapsid protein (NP) and small (S, participating in G1 and G2 glycoproteins) parts. According to recent studies, in addition, *C. burnetti* transmitted by *Dermacentor andersonii* ticks have a high potential for transmission.

Epidemiology

Although the CCHF is a transient benign infection lasting for up to a month, in livestock, it has a high mortality rate in humans following livestock blood or tissue contact, breathing, carcasses, livestock breeders, aerosols, the bite of a tick, and nosocomial route and occurs with acute fever and hemorrhagic syndrome. The *Hyalomma* spp *ixodidae* and other species of ticks *(Haemaphysalis, Amblyomma, Rhipicephalus, Dermacentor and Boophilus)* geographical distribution determine the CCHF prevalence and geographical disease pattern which take the virus from livestock and small vertebrates (6-8). The disease has a wide distribution in Africa, the Middle East and other areas in Asia (9, 10). Warm seasons (March to late September) are associated with higher tick's multiplication and maturity leading to a higher infection rate. The viral permanent transmission via transovarial and transstadial routes in various stages of the vector growth is considerable (7).

According to reports, CCHF remains a continuing high prevalent disease in Iran (8) such as in Zahedan, Southeast of the country (9). Recently, an outbreak also occurred in the northwest of Iran in 2021, where 10 confirmed cases were admitted to hospital (10). On the other hand, some studies have focused on detection of the pathogen in *Hyalomma* ticks isolated from livestock slaughterhouses in central and southern parts of Iran. A recent report by WHO has revealed that Iran and Turkey in the Middle East, Eastern Europe and Kazakhstan in Central Asia have the highest rate of CCHF disease (≥50 CCHF cases annually) (Figure 1). The disease has a lower rate in Europe mostly carried by ticks of *Hyalomma marginatum, H. lustanicum Rhipicephalus, H. marginatum* (Bulgaria and Albania), *R. bursa, and R. sanguineus species.*

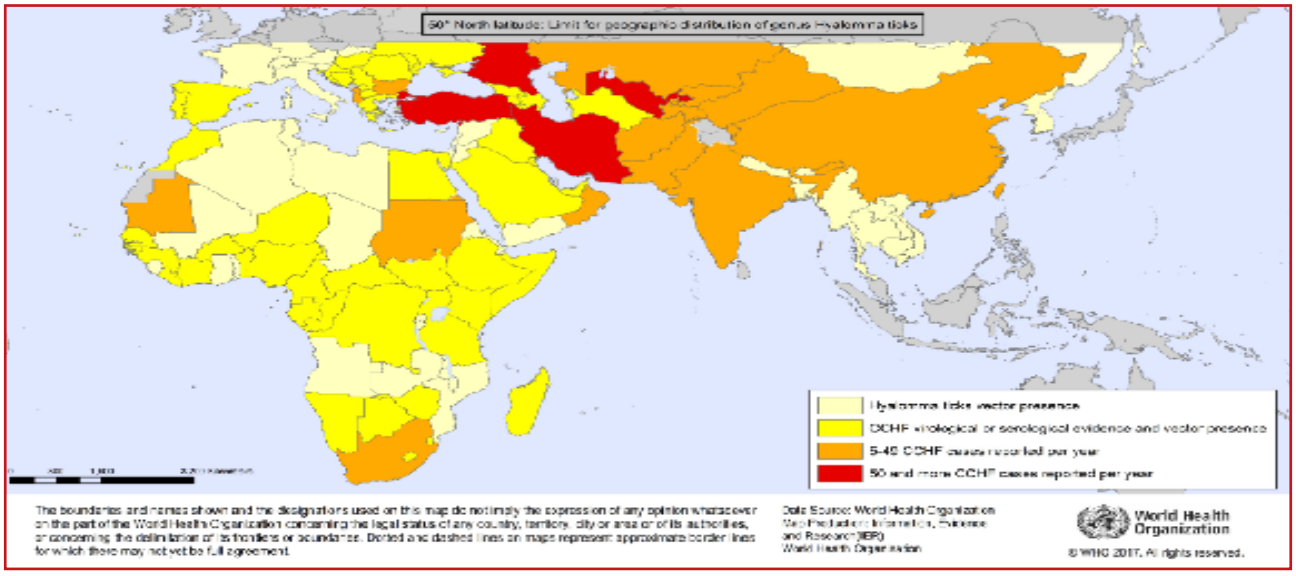

Figure1. The worldwide distribution of the CCHF highlights a tremendously high rate of infection in Iran and Turkey, Eastern Europe and Kazakhstan areas

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Risk factors

Individuals with a high risk of CCHF disease exposure and progress mostly include domestic animal breeders and veterinarians, physicians with contact with CCHF-infected patients, and nosocomial spread in healthcare workers (7). Direct contact with blood, breathing, aerosols, tissue debris and secretions of livestock or patients (person to person) leads to infection spread. During the epidemics of infection, the risk is outstandingly enhanced. In addition, places where the tick carriers can be survived and warm seasons should be considered as risk potentials (as aforementioned mainly *Hyalomma* ticks). The socioeconomic conditions are also an effecting cornerstone of the disease's spread and maintenance (11-16). As the contaminated ticks are permanent carriers, their existence is a paramount risk that should be solved (17-21). Decision makers play an indispensable role in the control of disease in rural areas. Climate change and human conflicts also affect the virus's thriving.

Pathogenesis

CCHF employs various virulence factors to invade the macrophages, monocytes and endothelial cells and cause systemic infection which affects the liver, endothelium and also nervous system. Alongside virulent and fulminant status of CCHF infection, the virus renders proinflammatory cytokines induction and cytokine storm (IL-1, IL-6, TNF-α, and IL-10), exacerbating the disease comorbidity. These cytokines cause severe clinical symptoms which pose a high mortality rate (up to 50%) (22-25). It is worth mentioning that CCHF escapes the response type I interferon via a variety of mechanisms (23, 24).

The disease duration is divided into four stages which include latent or incubation (lasting 1-3 days up to 9 days), pre-hemorrhagic, upper mucous membranes and skin hemorrhage, and a recovery phase. Clinical symptoms include fever, chills, muscle aches, and pains. Internal hemorrhage eventually leads to shock, pulmonary edema, and death (25-29).

Viral agent identification

In under-developed areas, CCHF is misdiagnosed with malaria. Timely identification is crucial for the survival of a patient or the control of the disease. A series of diagnostic tools are available to identify the CCHF arbovirus agent such as antigen-antibody assays, serum neutralization, culture or isolation and molecular techniques such as quantitative real-time polymerase chain reaction (RT-qPCR). High sensitive and specific tools and gene sequencing facilities are promising for rapid and accurate detection of the agent (30, 31).

Treatment

In addition to a rapid identification strategy, timely antiviral eradication approach is an unmet requirement. Noticeably, supportive care uses adjusting water and electrolyte balance and overcoming dysuria. Antiviral drugs such as ribavirin (targeting the transcription of viral mRNA) (5, 32, 33) and favipiravir (inhibition of viral replication) 48 hours after infection are effective (25, 26).

Control Measures

Prevention or hinder of exposure to the agent includes paramount primary control of its spread. Those high-risk individuals are recommended to avoid or harness contact with livestock secretions or respiration, ticks and suspected patients. Examples of preventive strategies include gloves coverage and protected contact with naked skin or tissues or blood of diseased animals which can be accurately efficient (3, 27). The application of Acaricide compounds in endemic areas can also mitigate a load of arthropods as the extraordinary part of the transmission. These preventive strategies can be also appropriately efficient regarding medical personnel in contact with CCHF suspected patients. Transport of livestock or related products or human travel from endemic areas should be performed with awareness of societies and physicians to prevent neighborhood or human-to-human transmission and spread of efficient and timely treatment (28).

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Therefore, confined contact and prevention of tick bite include the cornerstone of CCHF control. Owing to the lack of preventive vaccines in humans and animals, rapid detection and eradication of viral agent is essential (29) (13). The use of reference laboratories for the diagnosis, improvement of health systems, increase in public knowledge, early-preparedness strategy fulfillment, vector prevention and eradication, protection equipment availability and multisectoral coordination can be also helpful to success in aims (9-11,13,21,22).

Q Fever

Q fever (caused by an obligate intracellular bacterium, *C. burnetii*) is another challenging zoonotic bacterial disease that requires accurate diagnosis. This highly contagious and pathogenic bacterium and because of risk factors similar to the CCHF, most control strategies are similar. A recent study in Southeast Iran showed a common infection of Brucella spp and *C. burnetii* in milk (data not published). Owing to the higher thermostability of *C. burnetii,* accurate milk boiling is necessary. The disease usually spread through secretions of infected animals and inhalation of aerosol contaminated with the placenta (30, 31). Despite having similar morphology to Rickettsia spp, *C. burnetii* is yet to be entirely known in terms of environmental stability and virulence, host-bacterium interactions or clinical characters. *C. burnetii* has a low infectious (10–15 CFU/mL) and the incubation period depends on the infectious dose. Because of this, Q fever prevention strategies should incorporate human, animal, and environmental domains (32-34).

Causative agent

This bacterium *C. burnetii* can be transmitted through ticks *(Dermacentor andersonii)*, urine, feces, milk, placenta, and amniotic fluid of animals. However, when these products become dry, the bacteria would persist in inactive form for a long time and can spread as part of the peripheral dust in the air. *C. burnetii* is resistant to environmental stresses such as high temperatures, osmotic pressure, and ultraviolet light (31). The pathogen is capable of changing the phase as passes two antigenic stages. Small cell varieties (SCVs) forms are resistant to heat, pressure, and mechanical agents, and survive well in the environment. Additionally, large cellular varieties (LCVs) proliferate only in host monocytes or macrophages (32). *C. burnetii* delays the phagosomes-lysosome fusion after penetrating the host phagosomes and is likely to convert from SCV to LCV mode through this mechanism. SCVs and LCVs are distinguished using electron microscopy (31). Owing to intracellular habitat, the bacterium does not proliferate *in vitro* conditions and is directly derived from patients or animals being in phase I of the infection. In order to isolate the bacterium, multiple passages through embryonic eggs or chicken embryos (in Phase II) should be carried out at 35°C and the maximum growth and development rate is close to the fetal death time. The time for bacterial isolation in chicken embryo is approximately 12 hours. Tissue culture is also possible using endothelial cells or embryonic fibroblasts of chickens or mouse cells. The bacterium in Phase I of the infection with a capsular polysaccharide and high pathogenicity is isolated from patients or infected animals, which is used for production of the vaccine. Bacteria in phase II are less pathogenic and the pathogen loses the capsule due to frequent growth in embryonic eggs.

Epidemiological studies in Iran and other countries

In a study among130 camel blood samples, 14 samples were positive for the existence of *C. burnetii.* During another study, 11 of 100 samples from cow milk were positive, while sera anti-phase II antibodies in slaughterhouse animals have outlined a higher (68% IgG positive) rate of infection (33). Another work revealed that 33.9% of sheep and 22.4% of goats in the southern regions of Iran had antibodies against *C. burnetii* (34). Furthermore, of 190 sera samples using

ELISA, 14.4% were *C. burnetii*-positive (33,34). Recently, an epidemiological survey from northeast Iran showed that seroprevalence of antibodies against *C. burnetii* was 17.2% among butchers, slaughterhouse workers, farmers, and veterinarians. Interestingly, 12 individuals also had Q fever and brucellosis co-infection, with a prevalence of 6.4% (35). One study also tried to detect and determine the frequency of *C. burnetii* in milk samples of dairy animals in three provinces (Tehran, Hamadan, and Mazandaran) using RT-qPCR. Out of 162 samples, 23 (14.2%) were positive for the pathogen (36).

A recent systematic review showed that *C. burnetii* was detected in 4.8% of tested ticks in Europe with a significantly higher prevalence observed in Southern European countries (36). In a study conducted in Italy 2016, 5738 sheep and goat sera were collected and analyzed using ELISA test for the presence of specific IgG antibodies to *C. burnetiid*. They found that 15.9% of the samples were positive for specific antibodies. Bond and co-workers (2016) found that prevalence of *C. burnetii* in non-pregnant goats was 15%, and the morbidity of the infection was 49.5% as evaluated by PCR technique (37). Another investigation from Portugal (2017) recorded seven patients with hepatitis, and evaluated the epidemiologic history of Q fever. Identification of Q fever agent was confirmed in 5 cases using PCR test, and the results were positive for both serologic and PCR tests (PCR), though serologic tests were negative at the beginning of the infection (38, 39). In France, Gache et al, observed much higher positive frequencies of the disease. Indeed, around 36% of cows, 55% of sheep, and 61% of goats were positive for Q fever. Moreover, the abortion rate associated with *C. burnetii* in 2695 cattle, 658 sheep, and 105 goats was examined using PCR techniques (40). In a study carried out by Van Roeden et al (2018), 439 febrile people were evaluated. Of these patients, 166 cases had a chronic fever, with 14% acute aneurysms, 13% of heart problems and 10% of non-cardiac abscesses. The mortality rate for acute febrile seizures was 38% (41, 42, 43).

Pathogenesis

The low pathogenicity dose of *C. burnetii* (1-10 CFU/ml) and long-term persistence in the environment have made *C. burnetii* one of the most pathogenic organisms around the world (44). Q fever is appeared in two forms, acute and chronic forms, but noticeably asymptomatic condition is very common. The Q-fever also shows various clinical symptoms (45). Since the infection has variable, non-specific symptoms and the fever does not always occur, it is usually difficult to diagnose the disease. The mortality rate is 1-2%. Endocarditis is one of the main causes of death, occurring in 1% of cases.

The most important route of infection is the inhalation of contaminated dust, while the oral route is considered of secondary importance. Once inhaled or ingested, the extracellular form of *Coxiella burnetii* (or SCV after small-cell variant) attaches itself to a cell membrane and is internalized into the host cells. Then, phagolysosomes are formed after the fusion of phagosomes with cellular acidic lysosomes. The multiple intracellular phagolysosomes eventually fuse together leading to the formation of a large unique vacuole. *C. burnetii* has adapted to the phagolysosomes of eukaryotic cells and is capable of multiplying in acidic vacuoles (46, 47). In fact, acidity is necessary for its metabolism, including nutrients assimilation and synthesis of nucleic acids and amino acids. Multiplication of *C. burnetii* can be stopped by raising the phagolysosomal pH using lysosomotropic agents such as chloroquine.

Three proteins contribute to the intracellular survival of the pathogen: a superoxide dismutase, a catalase, and a macrophage infectivity potentiator (Cbmip). *In vitro* studies on persistently infected cells with phase I and phase II bacteria reported a similar mitotic rate in infected and noninfected cells (23). Moreover, the researchers frequently observed asymmetric cellular divisions in infected cells and suggested that this phenomenon could allow maintenance of persistent infection.

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The intracellular cycle of *C. burnetii* leads to the formation of two development stages of the bacterium known as "small-cell variant" (SCV) and "large-cell variant" (LCV). LCVs can differentiate into spore-like bacteria by binary asymmetrical division. The endogenous spore-like forms undergo further development and metabolic changes until finally reaching the SCV form. Finally, cell lysis, or possibly exocytosis, releases the resistant bacteria into the extracellular media (48-50).

Infection forms

The acute form of the infection is similar to the flu with some degree of pneumonia and hepatitis. Symptoms may occur 3 to 30 days after exposure to the bacteria, which include fever and severe headache often along with sweating, muscle aches, joint pain, loss of appetite, fatigue, and severe weight loss. Skin rashes occur in 5-20% of cases. Hepatobiliary is associated with jaundice in rare cases, but hepatomegaly enlargement and elevated levels of liver enzymes are common. The acute form of the infection is usually self-limited and in some cases may lead to death (31, 32, 38, 45). In people with underlying illnesses, heart valve impairment, blood vessel abnormalities, immune deficiency, and acute renal failure, the acute form of the infection can be fatal. In chronic conditions, usually, there is no fever. The chronic condition of the infection can develop a month or even one year after the acute form of the infection. Due to the delay between the development of the infection and its diagnosis at this stage, the mortality rate increases in chronic form. The most common chronic symptoms include endocarditis and osteomyelitis appeared usually in patients with underlying illnesses. Chronic fatigue syndrome and cardiovascular infection are the long-term complications of this phase of the infection (40-43). Patients with a negative culture of endocarditis should be considered as Positive-Q fever. Without antibiotic therapy, endocarditis is usually fatal. Most articles published in the context of Q fever in children are case reports. Because the fever is usually not diagnosed in children, the general belief is that fever is rare among children. Clinical manifestations of Q-fever in children are similar to those in adults, and fever is self-limited, but in rare cases, it may be associated with death. Osteomyelitis and endocarditis occur in chronic conditions, and fever may recur again. Fever has been reported in all countries except New Zealand, and the last epidemic was in the Netherlands in 2008, where almost 2300 people were affected by the disease. Overall, clinical symptoms of the Q fever are non-specific and are not helpful in the diagnosis. An examination of the history of contact with cattle, sheep, and goats can be helpful. Children suspected to have a fever should be checked for heart valve infection due to the fact that they may be predisposed to endocarditis (40-43).

Diagnostic approaches

Current approaches for diagnosing Q fever rely upon serological methods, PCR techniques, and histological findings.

1) Serology: Indirect immunofluorescence (IF) is a reference method for serological diagnosis. Enzyme immunoassay (EIA) and complement fixation (CF) are routine methods used in serology. The CF test is more long-lasting and has less specificity than the IF test. Serological diagnostic tests for Q-fever have cross-reactivity with *Legionella and Leptospira* infections (40, 41). Khalili and colleagues used ELISA to test the phase I antibodies in patients with suspected Q fever in Iran, and 24% of patients had phase I antibodies and 36% had phase II antibodies.

2) PCR technique: If the PCR test gives a positive result before antibody detection, it can be used as a rapid diagnostic method. The PCR test is highly valid and accurate in tissue samples taken from the heart valves because of the large number of bacteria (42, 43).

3) Histology and other laboratory methods: Histological findings are mostly non-specific.

Immunostaining is beneficial for fresh tissue samples and those that have been fixed using formalin (40, 53) In the acute phase of the infection, the count of white blood cells is usually normal; thrombocytopenia is observed in 25% of cases with an increase in the level of liver enzymes (49-52).

Treatment and Control

The first-line treatment for Q fever is doxycycline, and doxycycline combined with hydroxychloroquine is recommended for chronic Q fever. The duration of treatment varies, depending on the type of illness (acute or chronic). In chronic conditions, doxycycline is used for a period of 18 months to several years (to prevent the infection recurrence). In the case of endocarditis, doxycycline consumption needs to be accompanied by quinolone for 2 to 4 years. It should be noted that even in the acute form of the infection, there is a possibility of healing of the untreated patient, and in the case of incomplete healing, the patients should receive doxycycline for 2-3 weeks. Even after complete treatment, it is necessary to follow up with the patient.

In the case of a Q fever outbreak, sanitary and prophylactic measures ought to be applied at the herd and human levels, in order to restrict disease spread. Human and animal infections must be diagnosed as early as possible and treated immediately to avoid the development of chronic infections and secondary complications. For instance, when Q fever has been detected on a dairy farm in France, milk from the aborted females should be thrown away. In fact, the sale, transformation, and treatment of such milk are strictly prohibited. Guidelines published by the Health Protection Agency in the UK advise that 2% formaldehyde, 1% Lysol, 5% hydrogen peroxide, 70% ethanol, or 5% chloroform should be used for decontaminating surfaces, and spills should be cleaned up immediately with hypochlorite, 5% peroxide, or phenol-based solutions (41, 44, 45). Infections can be avoided by preventing direct contact with amniotic fluid and animal secretions, vaccinating livestock, and applying quarantine rules for imported livestock (51). In Cyprus, the prevalence of Q fever among sheep and goats was reduced by destroying infected aborted material, isolating infected dams, and disinfecting the premises.

Vaccination with formalin-inactive whole-cell bacteria has been performed and proved effective in humans and animals. Q-Vax® is a phase I whole-cell vaccine, and its licensed use is limited to Australia. However, inactive whole-cell vaccines present several effects. Some of the most common side effects of vaccines include injection site reaction (pain, heat, swelling and redness), flu-like symptoms, headache and fever. Recombinant vaccines have been developed in experimental conditions and have great potential for the future (41). Live and cellular vaccines for the infection are being studied and some of them have been approved. A full-cellular vaccine has been licensed in Australia (Q-Vax) and also vaccines produced from bacterial residues and deactivated using phenol-chloroform (CMR) have been approved in the United States (53).

Conclusion

CCHF and Q fever zoonotic infections are global threats with an increasing trend. Most CCHF cases have concentrated on the Middle East and North Africa, Central Asia and Eastern Europe. Thereby, health policymakers and healthcare providers are recommended to fulfill public knowledge and control strategies to hinder its spread. Avoidance to contact with livestock and ticks, and their control alongside the awareness of wildlife movements and multinational policies include determining implementations with this regard. It is worth mentioning that the international surveillance conducts are needed to be extended and supported to better predict, investigate and control these difficult-to-combat infectious diseases. In order to predict and monitor the CCHF and Q fever, regional rather than countrywide scale cooperation is mandatory. Immigration or travel also remains an important factor influencing the dissemination of these agents. The use of reference laboratories for the diagnosis, improving

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health systems, increasing public knowledge, early-preparedness strategy fulfillment, vector prevention and eradication, protection equipment availability and multisectoral coordination can be also helpful to succeed its aims.

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Conflict of interest

None.

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