Crimean-Congo Hemorrhagic Fever (CCHF) in Pakistan: The "Bell" is Ringing Silently

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ABSTRACT: Pakistan is being hit by communicable and noncommunicable diseases over time. Among these, tick-borne viral disease, Crimean-Congo hemorrhagic fever (CCHF) is one of the most fatal infections. Rapid climate change aroused by industrial, occupational, and agricultural activities to support ever-growing human population has been considered the single most causative basis for emergence or re-emergence of CCHF in Pakistan, where it has biannual peaks between the months of March–May and August–October. Many factors, including poor sanitation at farms, villages, and cities, unhygienic transportation and slaughter of animals at numerous sites within a city, inefficient tick-control programs, post-slaughter piles of animal remains other than meat, nomadic lifestyle, and lack of trained animal and human healthcare staff, are contributing to the spread of CCHF. Pakistan has confirmed cases of CCHF in almost every province: Sindh (Karachi), Punjab (Faisalabad, Multan, and Rawalpindi), Balochistan (Quetta) and Khyber Pakhtunkhwa (Peshawar). The root cause behind the spread of CCHF in Pakistan seems to be the absence of an effective disease surveillance system in the human as well as the animal populations. Most of the time, CCHF cases are not diagnosed, and if they are diagnosed they are not reported. If these cases are reported, there are not enough effective measures by the relevant provincial and district authorities. There is a need to educate the general public, farmers, and healthcare workers about the causes, transmission, and dangers of CCHF. An immediate plan for the implementation of a surveillance system, standard preventive measures, early detection, proper treatment, and timely response is urgently needed. Without such a plan, the accumulation of factors responsible for the sudden outbreak of CCHF may pose a serious threat to humans and animals in different geographical regions of the country.

KEY WORDS: Crimean-Congo hemorrhagic fever, epidemiology, surveillance, genotype, prevention

I. INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) is an emerging arboviral zoonotic disease caused by genus Nairovirus of Bunyaviridae family.[1] The name Crimean hemorrhagic fever was given after first outbreak in the Crimea in 1944–1945, when more than 200 cases of an acute, hemorrhagic, febrile illness occurred among soldiers. In 1969, this disease was documented as Crimean-Congo hemorrhagic fever because the same pathogen was responsible for an infection identified in 1956 in the Congo. The connection of these two place names resulted in the current name for the disease and the virus. The virus is transmitted to host either (animal or human) by direct contact with infected animal or by tick biting.[2] Different tick genera are capable of becoming infected with CCHF virus, but the *ixodid* tick of the genus *Hyalomma* is the primary vector.[3] All tick hosts, CCHF manifests only in humans. CCHF is characterized by severe multisystem syndrome associated with fever, myalgia (muscle ache), dizziness, neck pain and stiffness, backache, headache, sore eyes, photophobia (sensitivity to light), and hemorrhages. CCHF is the second most widespread arboviral disease after dengue fever; the virus is typically found in southern and eastern Europe, America, Central Asia, Africa, Southeast Asia, and the Middle East. In recent decades across the globe, CCHF has emerged in many countries including Albania (2001), Turkey (2002), and Georgia (2009). After long periods of absence, CCHF has re-emerged in

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various regions of the world such as southwestern Russia and Central Africa.[4]

II. GENOMIC ORGANIZATION

CCHF virus is an enveloped negative-sense single-stranded RNA virus with diameter ranging from 90 to 100 nm and a spherical shape.[5] The RNA has three genome segments referred as small (S), medium (M), and large (L), respectively. RNA-dependent RNA polymerase is expressed (L protein) by the large segment; the middle segment encodes mature glycoprotein; and the nucleoprotein encodes the small genomes segment (Fig. 1).[6]

III. EPIDEMIOLOGY

CCHF has been reported in Africa, Eastern Europe, the Middle East, and Central and Southern Asia. It has been confirmed in Abu Dhabi, Afghanistan, Albania, Bulgaria, China (Xinjiang Uygur Autonomous Region), Democratic Republic of Congo, Dubai, Greece, Iran, Iraq, Kazakhstan, Mauritania, Namibia, Oman, Pakistan, Russia, Saudi Arabia, Senegal, Serbia/Yugoslavia, South Africa, Tajikistan, Turkey, UAE, Turkey, and Uganda. The virus has been identified in Nigeria, Central African Republic, Kenya, Upper Volta, Madagascar and Ethiopia as well. The real number of CCHF-infected people likely higher than has been reported because the disease usually occurs in remote areas. CCHF usually appears in the spring and early summer in endemic countries in the Northern Hemisphere. *Ixodid* ticks are the natural reservoir of CCHFV, and they play a major role in the geographic circulation of CCHF. Climate change and changes in human occupation and agricultural activities are possible sources of the emergence or re-emergence of CCHF. Pakistan has confirmed cases of CCHF in the following areas: Sindh (Karachi), Punjab (Faisalabad, Multan, and Rawalpindi), Balochistan (Quetta) and Khyber Pakhtunkhwa (Peshawar).[7] In Pakistan, CCHF has biannual peaks between March–May and between August–October.[8]

IV. SURVEILLANCE

Pakistan has been faced with a multiple burden of communicable and no communicable diseases for many years. Among these, CCHF is one of the most fatal diseases. From 1976 to 2013, 14 deadly outbreaks and several deaths have been reported.[9] Overall, 3,426 infection cases were reported during 1998–2013 globally; of these, 230 were from Pakistan, with 92 deaths.[10] No proper surveillance system exists in Pakistan; the available data on CCHF cases during the past few years are very limited. It is likely that many cases have not been reported.[11] An extensive disease surveillance system is urgently needed within the country that is able to detect the occurrence of each targeted high-threat animal disease and any other unexpected serious exotic or emerg-

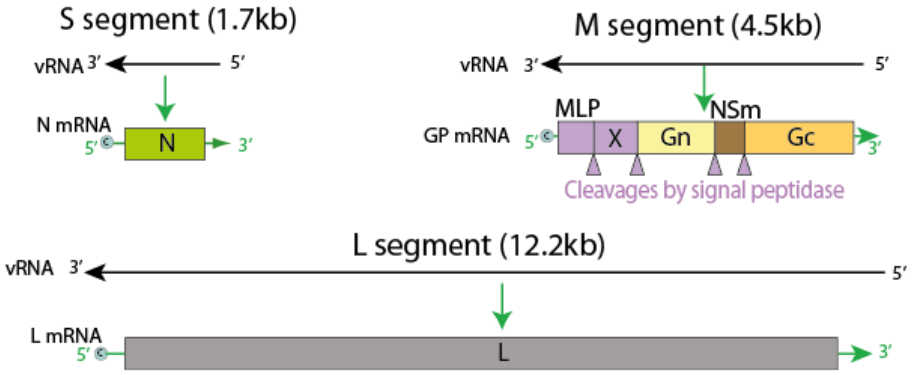

FIG. 1: Genome organization of Crimean-Congo hemorrhagic fever virus

ing diseases, at an early stage.[12] A comprehensive surveillance system should include:

- A wide geographical range and coverage of livestock populations (however, risk analyses may indicate the need for enhanced surveillance in one or more areas);
- A training program for veterinarians, veterinary paraprofessionals, and others involved in handling animals for detecting and reporting unusual animal health incidents;
- A legal obligation for private veterinarians in relation to the veterinary administration;
- A timely reporting system for events to the veterinary services;
- The ability to undertake effective disease investigation and reporting;
- Access to laboratories capable of diagnosing and differentiating relevant diseases;
- The ability to properly determine the significance of the results produced.[12]

V. CONGO GENOTYPE IN PAKISTAN

Seven major genetic clades have been identified via genetic analysis of the viral S and L-RNA segments and segregated on a geographical basis: Africa 1 (also classified as genotype 7), Africa 2 (genotype 5), Africa 3 (genotype 3), Asia 1 (genotype 1), Asia 2 (genotype 2), Europe 1 (genotype 4) and Europe 2 (genotype 6). Asia 1 and Asia 2 genotypes were identified in Pakistan during 1976–2002 and 2008, respectively. [13]

VI. CRIMEAN-CONGO HEMORRHAGIC FEVER OUTBREAKS IN PAKISTAN

CCHF is considered as an emerging disease across the Pakistan. In Pakistan, CCHF cases have generally been reported during the Islamic festival of Eid al-Adha, which is celebrated on days 10–13 of the last month of each lunar calendar year. This increase occurs because large flocks of sacrificial animals are brought into each city's animal mart from the rural areas of Pakistan.[14] CCHV was first isolated in 1960 from ticks in Lahore's forest Changa Manga. In 1976, Pakistan faced first outbreak when a patient from Rawalpindi with abdominal pain, melena, and hematemesis caused the death of a doctor and an operation theater attendant who were involved in the treatment of the infected person. In addition, 11 more patients had been infected with CCHV. A second outbreak occurred in December 1994 in Quetta, Baluchistan, which caused the death of the patient and the infection of two surgeons and a healthcare worker. In 2000, three cases were reported in Peshawar. In 2002, a case was reported from Azad Jammu Kasmir that caused the infection in two healthcare workers. The World Health Organization (WHO) reported 26 cases in 2010 by the National Focal Point Ministry of Pakistan. Overall, 69 cases were confirmed in 2014 and 25 CCHFV-positive cases were reported in Khyber Pakhtunkhwa in 2015; of these, 11 patients died.[1] In 2012, 62 were reported, and in 2013, 100 cases were reported in Pakistan. By mid-August 2016, 20 deaths from CCHF had been reported in Pakistan (Table 1).[15]

VII. DIAGNOSIS

Polymerase chain reaction (PCR), virus isolation in cell culture, and IgM detection by enzyme-linked immunosorbent assay (ELISA) are the diagnostic techniques mainly used for detection of CCHFV. Blood, plasma, serum, or other body fluids, biopsy material, and liver samples are used for laboratory confirmation of CCHF. As a potential bioterrorism agent, testing with CCHFV should be performed at biosafety level 4 (BSL-4) facilities.

A. Virus Isolation Cell

Mammalian cell cultures can be used for CCHFV isolation. During days 1–5 postinoculation, vero cells yield isolates. CCHFV is feebly cytopathic; therefore, its infectivity is titrated by the expression of immunofluorescence in infected cells. For virus isolation, the SW-13 cell line has also been used extensively, generating plaques within 4 days postinoculation. Recognition of a CCHFV isolate

TABLE 1: Cases and deaths from Crimean-Congo hemorrhagic fever in Pakistan during 2010–2016

Year	Cases	Deaths	Reference
2012	62	18	3
2013	100	20	3
2014	≤ 80	≤ 20	14
2015	25	11	14
2016	≥ 27	20	15

has to be confirmed using immunofluorescence or molecular techniques.

B. Nucleic Acid Detection Techniques

Molecular-based diagnostic assays, such as RT-PCR, serve as the front-line tool in the diagnosis of CCHF as well as other viral hemorrhagic fevers. Molecular diagnostic assays are more rapid methods than virus cultures; diagnostic results can frequently be reported within a few hours after receiving a specimen. RT-PCR is a sensitive method for diagnosis, but designing primers or probes that allow detection of all circulating strains of the virus is challenging due to the genetic diversity of CCHFV. A real-time RT-PCR is extremely sensitive; it is capable of identify as few as 1,164 viral RNA copies per milliliter of plasma. In 2007, Wolfel et al.[16] developed a real-time PCR assay that targets the S-segment of the CCHFV genome and perceives strains from different geographical regions. The method employs an *in vitro* transcribed RNA copy of the full S-segment as a quantitative RNA standard. The assay is supported by a pair of primers and three probes. Viral RNA, which is used as template in the assay, is extracted from a patient's sample using any standard viral RNA extraction method or commercially available kit.

C. Serological Tests

Serological tests include virus neutralization assay, ELISA, immunofluorescence (IFA), and immunochromatographic test devices. Virus neutralization is highly specific for diagnosis, but *Nairvirus* genus induces a weaker neutralizing antibody response than do members of other genera of the family *Bunyaviridae.* Therefore, it is rarely used for CCHFV diagnosis. Recently, a small number of CCHFV commercial kits for IgM or IgG by ELISA or IFA have been designed for the human diagnostic market. However, it is feasible to adapt these commercial ELISAs and IFAs for serological testing in animals. In addition, some in-house ELISAs have become available for the detection of CCHFV-specific antibodies in animals. IgM, IgG, and total antibodies can be detected IgM-capture ELISA, an IgG-sandwich, or indirect ELISA and competitive ELISA, respectively. The advantage of competitive ELISA is the capability to investigate different animal species because they are host-species independent.[17]

VIII. TREATMENT

A. General Supportive Measures

Asymptotic or nonspecific febrile sickness with CCHFV does not require hospitalization or specific therapy. Intravenous fluids largely supportive for those patients who develop hypotension, hemorrhage, and organ perfusion, with careful monitoring to prevent the development of pulmonary edema. Fresh-frozen plasma and platelets are needed for coagulation abnormalities, whereas the considerable hemorrhaging will require blood transfusion. A combination of high-dose methylprednisolone, intravenous immunoglobulin, and fresh-frozen plasma was recently reported in Turkey to be beneficial, but the authors did not include a control group.[6]

B. Ribavirin

Ribavirin, a guanosine analogue is a licensed drug for the treatment of respiratory syncytial virus

infections and hepatitis C. It has been used to treat CCHF patients for more than two decades.[6] Viruses of Bunyaviridae family are commonly susceptible to ribavirin.[5] The drug was effective against CCHF infection with apparent benefit. According to its WHO Fact Sheet, it is effective in both oral and intravenous formulations. The inhibition of CCHFV replication has been verified in a minigenome system, in virus-infected cells, in newborn mice, and in STAT-1 KO mice. The drug was given as postexposure prophylaxis in a nosocomial outbreak in South Africa for the first time in 1985. In 1995, three CCHF patients in Pakistan were treated with ribavirin and appeared to respond rapidly to therapy. The authors concluded that "a randomized controlled trial in the context of good quality supportive care is justified." No report has described any serious adverse effects of ribavirin therapy. Other antiviral therapies include type I interferon (IFN), which also inhibits CCHFV replication *in vitro*.[18]

C. Antibody Therapy

Anti-CCHF immune globulin, prepared from the plasma of disease survivors, was suggested as therapy by Chumakov et al.[23] at the time of the 1944–1945 Crimean outbreak, but later assessments in the Soviet Union found little indication of advantage. However, immune globulin therapy was initiated in Bulgaria, where it continues in use. Intramuscular and intravenous anti-CCHF immunoglobulin showed rapid improvement in CCHF infected patients in 1990, but its random clinical trials for efficacy was not carried out. Hyperimmune globulin therapy has been associated with the clinical improvement of patients in South Africa and Turkey.[6]

D. Vaccine

The US National Institute of Allergy and Infectious Diseases registered CCHFV as a category C priority pathogen. No antiviral compounds or vaccines are effective against it because of the severity of disease and ease of transmission. The UK Advisory Committee on Dangerous Pathogens classified the CCHFV as a hazard group 4 pathogen. A lack of suitable animal models has severely hindered the research progress for CCHFV.[19] The Soviet Union developed a formalin-inactivated mouse-brain CCHF vaccine that was approved for use in 1970. Repeated vaccination in several thousand recipients showed low-level neutralizing antibody response in serum; on the other hand, its protective efficacy was not evaluated.[18] A similar unlicensed chloroform-inactivated suckling-mouse-brain CCHFV vaccine has been used in eastern Europe, but its neutralizing antibody titers were low, even in people who had received 4 doses. This vaccine is unlikely to gain widespread international regulatory approval due to its crude formulation, and no controlled studies on protective efficacy have been reported (Karen R. Buttigieg, March 2014). Current vaccine advances for CCHF comprise a DNA-based vaccine expressing the CCHFV M segment, which stimulate neutralizing antibodies in ~50% of vaccinated mice, but the efficacy could not be demonstrated due to the lack of a challenge model. One more vaccine candidate via transgenic tobacco leaves expressing G_N and G_C was fed to mice and induced IgG and IgA. However, antibodies were neither tested for neutralization titers nor tested for protection. No investigation for cellular immune responses for these studies has been conducted. Currently, a candidate vaccine is being developed based on recombinant MVA expressing the CCHFV glycoproteins to assess the induction of cellular and humoral immunity and to evaluate efficacy in a challenge model that represents human disease. Indeed, MVA vaccines are currently in clinical trials up to phase III for diseases including tuberculosis, malaria, HIV-AIDS, cancer, influenza, and hepatitis.[19]

IX. PREVENTION AND CONTROL

Prevention and control of CCHFV can be attained by avoiding or minimizing contact with the virus. The following control measures can help prevent and control the spread of this disease.

A. Controlling CCHF in Animals and Ticks

The control and prevention of CCHF in the animal host and tick vector is very complicated because the disease in animals is asymptomatic and ticks are prevalent in endemic regions. Animals transport from CCHF-infested areas should be treated at "entry points" through direct use of insecticides (acaricides) to bodies of domestic animals. For tick control, insecticide treatment is helpful 12–15 days before slaughtering animals.[18] Currently, no vaccines are available for animal protection.

B. Controlling Animal Movements

Local authorities should restrict uncontrolled movement of livestock animal between endemic countries by applying official regulations and penalties. This approach will be useful in preventing the spread of new antigenic (or genetic) variants of the virus between countries. Animals and related document controls should be checked by veterinary and treasury officers synchronously during the transport of animals at check points. Only acricide-treated animals should be imported and exported, and competent communication between the countries can prevent uncontrolled animal movement.[20]

C. Reducing the Risk of Infection in People

The key to protecting people from infection with CCHF is raising awareness and education among high-risk groups and those in endemic regions. Occupations such as butchers, veterinarians, and shepherds are included in the high-risk category; therefore, concerned people should take every possible measure to avoid exposure to virus-infected ticks or virus-contaminated animal blood and other tissues. The use of personal protective equipment (e.g., gloves, gowns, plastic boots, etc.) are effective control measures against exposure to fresh blood and other tissues.[21] Standard infection control precautions should be taken by healthcare workers when caring for suspected or confirmed CCHF patients or when handling specimens from them. These include basic hand hygiene, use of personal protective equipment, safe injection practices, and safe burial practices. Only trained and qualified staff should handle samples from suspected CCHF people in properly equipped laboratories.[3]

D. Reducing the Risk of Tick-to-Human Transmission

The risk of tick-to-human transmission can be reduced by avoiding travel to tick-prevalent areas and by undertaking special safety measures in the most active tick season. People should look for medical advice after visiting tick-infested areas and inform healthcare workers about travel history in areas where tickborne diseases are common, especially if there is unexplained illness with fever. People in rural areas, where exposure to ticks is high, become infected easily when bitten by infected ticks. Tick bites on naked skin can be minimized by using commercially available insect repellents, including diethyl toluamide. The use of permethrin-treated clothes is also beneficial against tick bites.[21] Do not use bare hands to remove tick if it attaches to skin. The tick should be detached using fine-tipped tweezers as soon as possible, and the bite area and hands should be thoroughly washed with soap and water. An antiseptic should then be applied to the bite site.[20]

E. Reducing the Risk of Animal-to-Human Transmission

Animals play an important role in the life cycle of ticks and, consequently, in the transmission and amplification of the virus.[20] Animals importing from endemic areas must be treated with pesticides two weeks before slaughter to inhibit possible tick infestation. Gloves and personal protective equipment must be worn by people handling animals in endemic areas. During slaughter, butchering, and gathering procedures in slaughterhouses or at home, gloves and other protective clothing should be worn while handling animals or their tissues in endemic areas.[3] Use gloves for handling the hides of animals. Rather than discarding the waste and blood of animals into

streams and watercourses, methods such as rendering, landfill, composting and anaerobic digestion should be adopted.[20]

F. Reducing the Risk of Human-to-Human Transmission

Healthcare personnel are at risk from occupational infection during treatment of CCHF-infected patients, so they should avoid close physical contact with the infected person. Hands should be washed properly and regularly after each visit to an ill person.[3] In developed countries, healthcare workers should wear efficient air respirators for protection; but unfortunately, this practice is not practicable in a country such as Pakistan.[3,20] Face shields, safety goggles, and surgical masks should be used as preventive measure against CCHF. Isolation of the patient and barrier nursing is also recommended.

G. Prevention of CCHF During Eid al-Adha

Eid al-Adha is a religious festival celebrated every year with pomp and show. Prevention and control of CCHF infection during Eid al-Adha is difficult due to uncontrolled movement and trade of animals within and between the countries. Movement of animals from disease-endemic areas should be strictly controlled. Strong surveillance programs must be constructed for tick-prevalent areas. Checking animals for CCHFV infection should be conducted at each entry point. Only acricide-treated animals should be distributed to animal markets. These regulations are very difficult to apply before Eid al-Adha due to the high demand for animals. Spread of the virus between infected animals and human is a potential risk during Eid al-Adha.[22] Hence, on this occasion, regulations must be followed to reduce possible disease outbreak and to protect public health. Commonly, during Eid al-Adha, nonprofessional butchers freelance, going from house to house to sacrifice the animals. Slaughter should be conducted only in a restricted zone, rather than on the road or at home. Rendering, landfill, composting, and anaerobic digestion should be adopted for disposal of waste and unused internal organs. Preventing the access of pests and pets into slaughtering area as well as disposal areas will improve environmental hygiene. Only trained butchers and people should be involved in the sacrificing practice and handling of animal tissues. Disinfectant, soap, detergents, and personal protective equipment must be used as preventive measures. Physical contact with suspicious material such as blood, skin, and tissues should be prevented. Animal skin should be handled carefully because large numbers of ticks attach to the hide. All these precautions play important roles in preventing infection between ticks, livestock, animals, and humans. Training should be provided to public and animal health professionals and policy makers. Education and information on prevention of CCHF should also be given those involved in the slaughter and handling of animals. Awareness of disease control should be provided through advertisements on social media platforms, short movies on television, ads on television, radio, billboards, magazines, and newspapers before and during Eid al-Adha.[20]

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