Review

Highly infectious tick-borne viral diseases: Kyasanur forest disease and Crimean–Congo haemorrhagic fever in India

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ABSTRACT

Ticks are distributed worldwide and can harbour and transmit a range of pathogenic microorganisms that affect livestock and humans. Most tick-borne diseases are caused by tick-borne viruses. Two major tick-borne virus zoonotic diseases, Kyasanur forest disease (KFD) and Crimean–Congo haemorrhagic fever (CCHF), are notifiable in India and are associated with high mortality rates. KFD virus was first identified in 1957 in Karnataka state; the tick Haemaphysalis spinigera is the main vector. During 2012–2013, cases were reported from previously unaffected areas in Karnataka, and newer areas of Kerala and Tamil Nadu states. These reports may be the result of improved active surveillance or may reflect altered virus transmission because of environmental change. CCHF is distributed in Asia, Africa and some part of Europe; Hyalomma spp. ticks are the main vectors. The existence of CCHF in India was first confirmed in 2011 in Gujarat state. In 2013, a non-nosocomial CCHF outbreak in Amreli district, as well as positive tick, animal and human samples in various areas of Gujarat state, suggested that the virus is widespread in Gujarat state, India. The emergence of KFD and CCHF in various Indian states emphasizes the need for nationwide surveillance among animals and humans. There is a need for improved diagnostic facilities, more containment laboratories, better public awareness, and implementation of thorough tick control in affected areas during epidemics.

Key words: Crimean–Congo haemorrhagic fever, India, Kyasanur forest disease, tick-borne diseases, ticks

INTRODUCTION

Globally, ticks are the important arthropod vectors for transmission of numerous infectious agents and are responsible for causing human and animal diseases.1 Various wild and domestic animals are reservoir hosts for tick-borne pathogens of livestock, pets and humans.2 Ticks are obligatory blood-sucking ectoparasites that infest mammals, birds, reptiles and amphibians.3 Eighty per cent of the world’s tick fauna are hard ticks and the remaining 20% are soft ticks. However, only 10% of the total hard and soft tick species are known to be involved in disease transmission to domestic animals and humans.4,5 Ticks suck host blood during their lengthy attachment period (7–14 days); this may extend, depending on the association of the tick species and host.3 Tick-borne diseases are prevalent only in specific risk areas where favourable environmental conditions exist for individual tick species.3 Worldwide increases in the incidence of tick-borne diseases have been reported.5,6 Human tick-borne diseases have been recognized since the discovery of Lyme borreliosis, which is transmitted by Ixodid ticks.6 Rocky Mountain spotted fever, caused by Rickettsia rickettsia, is transmitted by Dermacentor spp. in the United States of America (USA). There are several other rickettsial infections like rickettioses and Boutonneuse fever (caused by Rickettsia conorii found in Europe), transmitted by Rhipicephalus sanguineus and other tick species. Generally, tick-borne viral diseases manifest three different clinical conditions: encephalitis, haemorrhagic fevers, and acute febrile illness. Among viral infections, European tick-borne encephalitis and the severe Russian spring–summer encephalitis are transmitted by Ixodid spp.7,8

Hyalomma anatolicum anatolicum and Haemaphysalis spinigera are the two important species of ticks present in India, which are responsible for causing the fatal tick-borne
viral diseases of Crimean–Congo hemorrhagic fever (CCHF) and Kyasanur forest disease (KFD), respectively.9,10 The tick species are widely distributed in different parts of world, including India (see Figure 1 and Table 1).11,12

The highly infectious nature of KFD and CCHF causes sporadic outbreaks among humans. These viruses have emerged in comparatively new areas, revealing gaps in many areas in understanding these diseases.13,14 This paper focuses particularly on the Indian scenario of KFD and CCHF infections, clinical and epidemiological features of the diseases, active surveillance programmes among humans and animals, the role of ticks as vectors, and current policies for management of their control and for raising public awareness.
Highly infectious tick-borne viral diseases in India

1. Kyasanur forest disease

The KFD virus (KFCV) is a member of the genus *Flavivirus* and family Flaviviridae. It was first recognized in 1957, when an illness occurred concomitantly in monkeys (*Semnopithecus entellus* and *Macaca radiata*) and in humans. The virus was initially suspected as a Russian spring–summer (RSS) complex of viruses, since isolates from monkeys and human showed relatedness to this virus.

**Mode of transmission of KFD virus**

KFDV is transmitted to the wild monkeys *Semnopithecus entellus* and *Macaca radiata*, through the bites of infected *H. spinigera* ticks. After infection, KFDV is transmitted to other ticks feeding on the infected animals. Infection causes severe febrile illness in some monkeys. When infected monkeys die, the ticks drop from their body, thereby generating hotspots of infectious ticks that further spread the virus (Table 1). The genus *Haemaphysalis* includes 177 species. The ticks are small (unfed adults <4.5 mm long), brownish or reddish, and eyeless, and have very short mouthparts. They are easy to differentiate from other genera by the characteristic lateral projection of a palpal article 2 beyond the margins of the basis capituli. All *Haemaphysalis* spp. are three-host ticks. *H. spinigera* is the main vector of KFD, which is endemic in Karnataka state, India. A large number of isolations have been obtained from ticks, and *H. spinigera* contributed about 95% of these isolations. This is the predominant tick species found in the forest. Among other susceptible species of *Haemaphysalis*, in addition to *H. spinigera*, are *H. turturis*, *H. papuana kinneari*, *H. minuta*, *H. cuspidata*, *H. bispinosa*, *H. kyasanurenensis*, *H. wellingtoni* and *H. aculeata*. Transmission of KFDV in the laboratory has been demonstrated in a number of *Haemaphysalis* and *Ixodes* species.

Humans become infected through the bite of infected unfed nymphs, which appear to be more anthropophilic than mature ticks. ticks have also been found to transmit this virus transstadially, thus also acting as a reservoir for the virus. In Karnataka state, the activity of nymphs is very high during November to May, correlating with a higher transmission rate of KFD at this time of year. Adult fed females lay eggs, which hatch to larvae under the leaves. They further infest small mammals and monkeys, as well as accidentally infesting humans, and feed on their hosts. Subsequently, they mature to nymphs, and the cycle is repeated. Nymphs and adults also transmit the disease to rodents and rabbits by bite, and this rodent–tick cycle continues for more than one life-cycle.

The finding that immature stages of *H. spinigera* infest a variety of hosts, such as birds, monkeys, rodents, cattle and buffaloes, and that these hosts are highly susceptible to the virus, suggests that this species of tick might indeed be an important vector for the disease.

Although human-to-human transmission is not known, more than 100 human cases have been reported in the past while working on the virus. During a recent outbreak of KFD (2013) in Bandipur Tiger Reserve forest, it was found that animal handlers became infected while handling sick monkeys. Owing to the large number of KFD laboratory-associated infections at the National Institute of Virology (NIV), Pune, work on this virus was stopped for 30 years; however NIV started working on the virus again after establishment of a Biosafety Level-3 (BSL-3) laboratory.

**Geographical distribution of KFD virus in India and its detection in newer areas**

After the discovery of KFD, it was mainly confined to three taluks (Sagar, Shikaripur and Sorab) of the Shimoga district of Karnataka, until 1972. Thereafter, foci were reported from four additional areas, namely Chikmagalur, Dakshina Kannada, Udipi and Uttar Kanada districts of Karnataka state. For a long time, KFD was thought to be endemic in the Shimoga district. However, serological evidence obtained in the past during different studies, suggests that KFD virus or related viruses are present in other areas of India, which include parts of the Saurashtra region in Gujarat state, forested regions west of Kolkata, West Bengal state, and the Andaman Islands.

Since 1957, the estimated incidence of KFD in India has been 400–500 cases per year. The epidemic period begins in November or December and peaks from January to April, then declines by May and June. Between 2003 and March 2012, there were 3263 reported human cases and, of these, 823 were laboratory confirmed. Large numbers of human infections were reported in 2003–2004, but a significant decline occurred in 2007 and again in 2010–2011. The frequency of cases can be correlated with the number of confirmed KFD-infected non-human primates. As far as spread in new areas is concerned, an outbreak of KFD was reported for the first time in December 2012 in Chamrajannagar district of Karnataka. In 2013, KFDV was detected in autopsy of dead monkeys in Nilgiris District, Tamil Nadu and in a human case from Wayanad district, Kerala (Figure 2). Already in 2014, an outbreak of KFD has been confirmed by screening human samples, monkey necropsy samples and tick pools from the Kannangi and Konandur areas in Thirthahalli taluk. Data indicate that, this year, the main focus activity was in Shimoga District (NIV unpublished data).

In earlier years, the geographic area affected was small and the number of cases was relatively low, while, with the increase in the number of foci, the incidence has increased. These facts suggest that constant changes in the ecobiology, including deforestation and new land-use practices for farming and timber harvesting, might have led to the spread of this disease to newer localities.

**Phylogenetic relation and ancestry of KFD virus in India**

The positive-sense RNA genome of the KFDV is about 11 kb in length and encodes a single polyprotein that is cleaved post-translationally into three structural (C, M and E) and seven non-structural (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5) proteins. Evolutionary studies undertaken earlier for KFDV, based on Bayesian molecular clock analysis (partial E and NS5 gene sequences of ~50 KFD viruses), revealed a rate of evolution of ~6.4 × 10^-4 substitutions/site/year with
the divergence of KFDV estimated to have occurred 62 years ago. However, a recent study based on analysis of full-length sequences (KFDV n = 3 and Alkhurma haemorrhagic fever virus n = 18 – a variant of KFDV in Saudi Arabia) revealed a slower rate of evolution (9.2 × 10⁻⁵ substitutions/site/year) and a much older ancestry of KFDV. Though the number of KFDV isolates that were available at the time of the study was limited, it appears that the analysis of full-length genomes might have provided a more accurate estimate of an older ancestry and also suggests that the evolution of tick-borne viruses was more gradual than that of rapidly evolving mosquito-borne viruses. Phylogeography studies for KFDV are also important to genetically characterize the recently circulating KFDVs and understand the dispersal pattern of the virus within Karnataka and to newer geographical areas.

**Clinical signs and symptoms**

In humans, the incubation period of KFD is estimated to be about 2 to 7 days after tick bites or exposure. The onset is sudden, with chills followed by severe frontal headache. Fever soon follows headache and rapidly rises to 104°F. This raised temperature is continuous and lasts for 5–12 days, or even longer. There is severe myalgia, which is reminiscent of dengue. Body pains are of high intensity at the nape of the neck, lumbar region and calf muscles. Diarrhoea and vomiting occur by the third or fourth day of illness. Bleeding from the nose, gums and intestines begins as early as the third day, but the majority of cases run a full course without any haemorrhagic symptoms. Gastrointestinal bleeding is evidenced by haematemesis or fresh blood in the stools. Some patients have persistent cough, with blood-tinged sputum and occasionally substantial haemoptysis. Physical examinations during the first few days of illness reveal an acutely ill, febrile patient with a severe degree of prostration. There is usually conjunctival suffusion and photophobia. The cervical lymph nodes are usually palpable, as are the axillary epitrochlear lymph nodes in some cases. A very constant feature is the appearance of papulovesicular lesions on the soft palate, but no skin eruption has been noted. The convalescent phase of the disease is prolonged. Often, the disease runs a biphasic course; the second phase occurs after a febrile period of 1 to 2 weeks. The fever lasts from 2 to 12 days (Table 2). It is initiated by headache and by this time abnormalities of the central nervous system are generally present. Neck stiffness, mental disturbance, coarse tremors, giddiness, and abnormality of reflexes are noted.
Clinically, KFD resembles Omsk hemorrhagic fever (OHF), which occurs in the Omsk Oblast in Siberia. The other tick-borne viral disease antigenically related to KFD and OHF is tick-borne encephalitis.29

**Case definition**

Owing to the variability in clinical illness associated with KFD infection, and the lack of data available on clinical diagnosis of KFD, it is essential to emphasize the importance of laboratory confirmation of the disease. The following case definitions are proposed:

**Case definition:** a patient of any age presenting with acute fever, headache and myalgia, and a history of exposure to ticks and/or a visit to a forest area and/or living in, a KFD-endemic area, particularly forest in Karnataka.33

**Suspected case:** a patient, within a radius of 5 km surrounding the villages reporting recent monkey deaths or laboratory-confirmed KFD cases, with sudden onset of high fever and one of headache or myalgia.33

**Probable case:** a clinically compatible illness that does not meet the SOPs for a confirmed definition, but with one of the following:

- epidemiological link to a documented exposure to a KFD-affected area (one or more of the following exposures within the 3 weeks before onset of symptoms);
- positive result on testing of clinical serum specimens using the immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA).

**Confirmed case:** a confirmed case of KFD is defined as a case that fulfils the criteria for a probable KFD case and, in addition, it should cover any of the following:

- exposure to secretions from a confirmed acute or convalescent case of viral haemorrhagic fever (VHF) within 10 days of that person’s onset of symptoms;
- isolation of KFDV in cell culture or in a mouse model, from blood or tissues;
- detection of KFDV-specific genetic sequence by reverse transcription-polymerase chain reaction (RT-PCR) or real-time RT-PCR from blood or tissues.

**Diagnosis**

In the KFD-endemic area of Karnataka state, India, the differential diagnosis should include consideration of influenza, typhoid and rickettsial group of fevers, for example, Q fever and mite-borne typhus in mild cases, and malaria and leptospirosis in moderate to severe cases.

**Hospital laboratory testing:** The following tests should be performed on blood samples from enrolled patients, according to standard hospital procedures:

- complete blood count (CBC): total leukocytic count (TLC)/differential leukocytic count (DLC), haemoglobin level, and platelet counts;
- liver function tests (aspartate aminotransferase (AST)/alanine aminotransferase (ALT), serum bilirubin, alkaline phosphatase);
- serum electrolytes, blood urea, serum creatinine;
- smear for malaria parasite or malaria rapid diagnostic test.

Standardized, detailed SOPs should be provided to each of the hospital wards and laboratories, to ensure proper collection of each of the specimens. Personal protective equipment should be used by the collecting technician or care provider in all cases. Blood samples should be collected from the hand or antecubital fossa by the treating physician, by venipuncture with an aseptic technique. For surveillance purposes, this should be done on two separate occasions — initially at the time of enrolment/admission, and then 10 days after initial specimen collection or at the time of discharge from the hospital (if less than 10 days). In cases of fatality, the second specimen should be collected at the time of death.
Diagnostic laboratory testing: For a long period, there were no studies done on KFD; hence, hemagglutination inhibition (HI), hemaglutination (HA), complement fixation and in vivo inoculation of patients’ sera into suckling mice were the tests of choice for diagnosis. During KFDV infection, the level of viraemia reaches up to $3 \times 10^6$ within 3–6 days and remains high for as long as 10–14 days of infection.\(^7\) Therefore, the period of higher viraemia coincides with the time at which patients usually report to hospital and collection of a blood sample for laboratory diagnosis. Therefore, an early and effective diagnosis strategy is essential.

After establishment of the first BSL-3 laboratory of India at NIV, Pune, real-time RT-PCR, RT-PCR and detection of IgM and IgG antibodies by ELISA were developed and standardized.\(^36\) KFDV can be isolated from the blood of patients (in acute phase 2–5 days), positive tick pools, or the blood or viscera of monkeys by inoculation into infant mice, or in vitro using Vero E6, BHK-21 or chick embryo cells. In all these systems, infant mice are found to be the most susceptible for virus isolation. KFD anti-IgM antibodies can be detected using ELISA during the acute phase (4 days onward) (see Table 2).\(^37\) The front-line test for KFD is real-time RT-PCR and RT-PCR from blood/serum of humans, blood and viscera of infected monkeys, or tissues of ticks.\(^36\) According to what is known, real-time RT-PCR can detect the virus in human samples after onset of febrile illness up to the 10th day (NIV data). Clear information about the KFD viraemia phase, the interrelationship of IgM and IgG antibodies, and the duration of persistence of these antibodies in naturally infected patients remains to be understood (Table 2). Suspected samples should be shipped according to international regulations for the shipment of infectious agents, following triple container criteria.\(^37\)

Treatment

No specific treatment for KFD is available; however, supportive therapy is important. This includes maintenance of hydration and the usual precautions for patients with bleeding disorders.

Guidelines for management of KFD cases

In recent years, state public health agencies in the affected districts have made efforts to ensure adequate staff and infrastructure at primary health-care (PHC) and secondary-level health facilities, to provide health care to critically ill patients, undertake training of staff, and minimize the frequency of transfer of trained staff. The role and responsibilities of health-care providers at different levels of health facilities are explained, and medical officers are trained to identify the suspected KFD cases. Government has made provision for well-equipped ambulances for transfer of critically ill patients from community to PHC or from PHC to secondary health-care facilities. For better organization of the health-care-delivery system, mapping of primary, secondary and tertiary care health facilities in high-risk areas has been made available. KFD cases are recorded annually, thereby monitoring the annual disease burden at all the health facilities, for better understanding and preparedness for KFD disease. Continuous information, education and communication activities with regard to early recognition of suspected KFD cases are carried out among newly recruited medical officers and other relevant populations. State government educates the villagers and tourists who visit the forest in Karnataka state about using repellent and gum boots and having prior vaccination. Whenever monkey deaths are reported, rapid action is taken to transmit information to health officers and veterinary staff for necropsy of monkeys, collection of specimens for diagnosis of monkey samples, and proper disposal of dead monkeys. If a monkey is found positive for KFDV, vaccination of human subjects should be carried out in those areas. Education has been provided in local languages every year. As soon as suspected cases are notified they are referred to NIV, Pune for investigation and confirmation.

Improper storage of vaccine and lack of maintenance of the cold chain result in ineffectiveness of the vaccine and could be another reason for the emergence of KFD despite routine vaccination. The trend of increasing numbers of patients infected with KFD in Karnataka state warrants development of a new vaccine – either recombinant, or a virus-like-particle-based vaccine – which will help in controlling the disease.\(^33\)

Prevention

The formalin-inactivated KFDV vaccine produced in chick embryo fibroblasts is currently in use in the endemic areas in Karnataka state of India.\(^38\) The vaccine was found to be immunogenic, potent, stable and safe. The production of inactivated vaccines carries the inherent risk of utilizing large quantities of potentially highly pathogenic viruses and the possibility of incomplete inactivation of viruses. In addition, vaccines based on inactivated viruses as antigens have shown a certain level of adverse reactions, especially in children, and this has to be carefully balanced with their efficacy and durability.\(^38\)

Control

Infected nymphs and larvae are shed in the forest, mainly by the monkeys, rats, shrews, porcupines, squirrels, and probably a few birds that form enzootic foci. Destruction of infected ticks would necessitate control of ticks throughout the entire forested area, but is not technically and economically feasible. This is the main reason why, once focus of this disease becomes established in any biotope, it cannot be eliminated easily. As association of human infections in the vicinity of dead monkeys has been shown, and the use of spray insecticides has been recommended in a 50-m radius around a dead monkey. However, although recommendations have been made for spraying of insecticides around the place of monkey death, it is technically difficult in certain inaccessible areas to transport the large volumes of water needed for the spray. Economic and logistical problems associated with regular insecticide spray in a large area makes implementation of a control programme difficult. Under these circumstances, the prevention of tick bites by the use of repellents should be considered.\(^29\)
2. Crimean–Congo haemorrhagic fever

The Crimean–Congo haemorrhagic fever virus (CCHFV) is also considered as an important zoonotic virus, owing to its wide distribution and ability to cause disease in humans, where it causes a high mortality rate. Secondly, it has the potential to cause nosocomial cases/outbreaks. CCHF was recognized for the first time in 1944, in the West Crimean region of the former Soviet Union, during a large outbreak, and the virus was subsequently isolated in 1956 from a human case.59-42 It is a member of the genus *Nairovirus* of the family Bunyaviridae. The average case-fatality rate is 30–50%; this varies between 5% and 80% in various outbreaks, as reported earlier.43

**Mode of transmission of CCHF virus**

Humans become infected through tick bites, by contact with a CCHF-infected patient during the acute phase of infection, or by contact with secretions, blood or tissues from viraemic livestock.44 Risk groups include individuals who are exposed to ticks (mainly farmers, shepherds and veterinarians), and persons who come in close contact with CCHF patients. Thus, in hospital settings, family or nosocomial outbreaks are observed. Observation of CCHF transmission in India during the year 2011 showed it was mainly nosocomial, and started with an index case history of tick bites and close contact with animals.44 During June 2012, another episode of nosocomial infections recorded from Ahmadabad city resulted in two fatalities.9 The history of exposure revealed that the treating physician had an accidental contact with the patient (the index case, resident of Bawla Taluka, Ahmadabad), who had similar symptoms of haemorrhagic fever and had died a week earlier. During the CCHF outbreak of 2013 in Karyana village, Amreli district, the main reason for transmission of virus was infected *Hyalomma* ticks infested on domestic animals. Once a human was infected, the disease was transferred to other close family relatives who either accompanied the infected individual to hospital, lived in the same house, attended the funeral of a person who had died due to CCHF, or came in contact with infected body fluids.9

CCHFV circulates in nature in the enzootic “tick–vertebrate–tick” cycle.94 Ticks of the *Hyalomma* genus have been reported to be associated with the incidence of the disease and found to play a key role in transmission of CCHFV to mammals.46-50 The CCHF cases coincide with the life-cycle of *Hyalomma* ticks, and infection mainly occurs during the period when immature ticks are active. High tick activity is associated with warm winters and hot summers. The predominance of tick species as vectors of CCHFV differs geographically and includes *H. anatolicum* subspecies (*H. anatolicum anatolicum*), which are distributed throughout Eurasia, while in the northern half of Africa *H. marginatum* subspecies (*H. marginatum marginatum, H. marginatum rufipes, H. marginatum turanicum* and *H. marginatum isaei*) predominate. *Hyalomma* species of ticks are medium to large sized, with long hypostomes and eyes located in sockets. They are mainly found in semi-arid zones, and infest domestic and wild mammals as well as birds. Of the 25 known *Hyalomma* spp., 15 are important vectors of infectious agents of veterinary and public health importance.51 Among these, *Hyalomma anatolicum anatolicum* is important, and this species has wide distribution in India.52 Nymphs and unfed adults remain hidden in the dry and winter season in crevices in stone walls, stables and weedy or fallow fields. The life-cycle involves three hosts. This is medically important, since, during the developmental cycle, ticks infest a variety of hosts – smaller to larger mammals, birds or reptiles. They effectively withstand diverse habitats ranging from warm, arid and semi-arid, harsh lowland, and long dry seasons.53 *H. anatolicum anatolicum* is known to transmit virus to humans.53

CCHFV has a wide host range and can cause a transient viraemia in many wild, domesticated and laboratory mammals; antibodies against CCHFV have been detected in the sera of variety of animals.44,54-56 Viraemia does not develop in birds; however, migratory species could carry infected ticks and play a role in disseminating the virus over long distances.57

**Geographical distribution of CCHF virus in India**

CCHF is transmitted to humans and animals by the bite of *Ixodid* ticks, mainly those of the *Hyalomma* genus. Thus, the geographic distribution of CCHFV is closely related to the global distribution of *Hyalomma* spp. ticks. CCHFV has been reported in over 30 countries covering Africa, South-Eastern Europe, the Middle East and Western Asia.58-65

India has always been considered at high risk for CCHF, owing to its borders with affected countries such as China and Pakistan. Because of the long association of India with these adjoining countries and the possible trade of animals across the border, the risk of CCHFV being passed to the Indian subcontinent was recognized. The virus was first isolated from ticks in Pakistan in the 1960s and the first reported human case occurred in Rawalpindi in 1976.66,67 Since then, many sporadic outbreaks have occurred in Pakistan every year, resulting in high case fatality.68,69 In March 1998, an outbreak with 19 cases and 12 deaths (case-fatality rate 63.2%) was reported from Takhar Province in the northern part of Afghanistan.70 Since this episode, Afghanistan has seen many outbreaks of CCHFV in subsequent years.70 In Iran, CCHF was first isolated in 1978 and the disease re-emerged in 1999, with high case fatality.72,73 In China, CCHF was first isolated in 1965 from a human case and later, in 1984, from *H. asiaticum* ticks from the same region of Xinjiang province in north-western China, which is considered to be the most CCHF-affected area in the country.74,75

Until 2011, the existence of CCHFV was not known in India, apart from some serological evidence recorded in the past.76 Serological evidence of the presence of CCHF in India was reported by screening for HI antibodies in animal sera from Jammu and Kashmir, the western border districts, southern regions and Maharashtra state.76-77 Shanmugam et al. had reported the presence, in 1973, of CCHFV-specific antibody in nine human samples from Kerala and Pondicherry and in goats from South India.77 All these studies were based on serological findings only; no virus isolation could be achieved and hence no clear evidence of this virus could be obtained. During December 2010, just prior to the CCHF outbreak, blood samples were collected by NIV, Pune, to examine livestock from abattoirs in the northern adjoining state of Rajasthan.
and some more distant areas of Maharashtra and West Bengal states, for the presence of CCHFV-specific IgG antibodies. Serum samples of buffalo, goat and sheep from Sirohi district, in southern Rajasthan, were found to be positive for IgG antibodies against CCHFV.44

The presence of CCHF disease was confirmed for the first time in India during a nosocomial outbreak, in Ahmadabad district, Gujarat state.44 Samples from three suspected cases, 83 contacts, Hyalomma ticks and livestock were screened for CCHFV by real-time RT-PCR; of these, samples from two medical professionals and the husband of an index case were positive for CCHFV. About 17.0% of domestic animals from Kolat, Ahmadabad were positive for IgG antibodies, while only two cattle and a goat showed positivity by real-time RT-PCR. Surprisingly, in the adjoining village of Jivanpara, 43.0% of domestic animals (buffalo, cattle, sheep and goats) showed IgG antibodies but only one of the buffalo was positive for CCHFV. The H. anatolicum anatolicum ticks were positive by PCR and virus isolation. Retrospective screening of suspected human samples revealed that the virus was present in Gujarat state, during the year 2010, earlier than this outbreak (see Figure 2).44 After its confirmation in India, sporadic cases of CCHF were reported in 2011–2012.78 During the period of 23 June to 25 July 2013, a cluster of suspected VHF cases were reported in Karyana village, Amreli district and, simultaneously, sporadic cases were recorded from Surendra Nagar, Patan district and Kutch district, Gujarat state.9 Owing to high alertness over 2 months, a total of 198 human samples were processed by real-time RT-PCR and 19 samples were found to be positive for CCHFV. Human suspected cases from Amreli, Kutch, Patan, Rajkot and Surendranagar were found to be positive for CCHF viral RNA. IgG antibody positivity was recorded in animals from Karyana, Nilwada and Khambhala village from Amreli district and Kundal village, Ahmadabad district. Surveillance of anti-CCHF IgG in domestic animals showed a number of animals from 15 districts that were positive (see Figure 3) (NIV, unpublished data). This emphasizes the necessity of continuous monitoring and screening of syndrome-based cases for CCHF.

Figure 3: Pictorial presentation of different outbreaks and spread of CCHF in Gujarat State.
Ancestry of CCHF virus in India

Sequence-based molecular characterization of the Indian CCHFV has shown that the virus possesses the functional motifs known to occur in the S, M and L gene segment products, as in other CCHF viruses. The complete genome was found to be 19.2 kb in length. The CCHFV strains cluster into 6–7 distinct groups; West-Africa in group I, Central Africa in group II, South-Africa and West Africa in group III, Middle-East and Asia in group IV, Europe in group V and Greece in group VI. Group IV may split into two distinct groups, Asia 1 and Asia 2.

The S segment of the six Indian CCHFVs showed 99.8% nucleotide identity. Notably, both tick isolates shared 100% nucleotide identity with one of the Indian human isolates of 2011. Phylogenetic analysis based on the S segment demonstrated that the Indian CCHFV isolates formed a distinct cluster in the Asian–Middle East group IV of CCHF viruses. The S segment was closest to a Tajikistan strain TADJ/HU8966 of 1990 (98.5% nucleotide identity) and was of South-Asia 2 type, while the M segment was of type M2. Both M and L segments were closest to an Afghanistan strain Afg09-2990 of 2009 (93% and 98% nucleotide identity) respectively. Complete genome analysis of Indian CCHFV isolates not only revealed high genetic diversity but also showed recombination and reassortment, which resulted in more complicated evolutionary routes of the virus than mutation-based selective forces. The molecular clock of Indian isolates has revealed the ancestry of these viruses is not very recent and dates back to about 33 years, on the basis of the S segment, whereas it is about 15 years based on the M segment.

Clinical signs and symptoms

The initial nonspecific symptoms of CCHF can mimic other common infections that occur in India, which may lead to misdiagnosis. The delay of proper treatment and precautionary measures may result in outbreaks, including nosocomial outbreaks of this high-risk group of viruses. In India, this disease needs to be differentiated from other infections such as dengue, leptospirosis, rickettisiosis, brucellosis, Q fever and other haemorrhagic fevers. Patient history is very informative, especially when tick bite or travel to currently known endemic areas in Gujarat is evident.

The clinical signs and symptoms are observed between 1 and 3 days (maximum 9 days) after a tick bite; however, when infection is contracted from direct contact with viraemic livestock or CCHF patients, these signs and symptoms may be seen from 5 to 6 days later (maximum 13 days). They include abrupt high fever, severe headache, malaise, nausea, vomiting, diarrhoea and sore throat. Typically, the disease follows a four-phase course: incubation, pre-haemorrhagic and haemorrhagic phases, and convalescence. Laboratory investigations during the first 5 days of the CCHF disease mostly show leukopenia, thrombocytopenia, elevated liver enzymes and prolonged blood coagulation times. In most cases, platelets $20 \times 10^9$/L or less, AST 700 U/L or more, ALT 900 U/L or more, partial thromboplastin time 60 s or more, and fibrinogen 110 mg/dL or less are suggestive of a fatal outcome. High viral load is also associated with a fatal outcome. In severe cases, approximately 5 to 7 days after the onset of the disease, haemorrhagic manifestations are observed – mainly petechiae, epistaxis, haematomas and vaginal bleeding. Death usually occurs between 5 and 7 day of illness, while survivors show progressive improvement (see Table 2). The disease is milder in children and in secondary or tertiary cases. Haemophagocytosis is also one of the consistent features in many cases; however, increased serum ferritin levels caused by haemophagocytosis is also one of the consistent features related to the severity of disease. The average period of incubation is around a week. The first phase includes a few days of fever, tiredness, headache and muscle pain, followed by a long asymptomatic period. After that phase, the first signs that the central nervous system has been compromised start appearing, including meningitis, encephalitis and myelitis, which can lead to neurological sequelae and, in a few cases, even death. Information on the time of appearance of symptom of VHF has varied, ranging between 1 and 21 days after exposure to the virus. Symptoms also depend on the viral species involved and may include fever, tiredness, dizziness, muscle pain, weakness and exhaustion. In more serious cases, there is bleeding under the skin or in internal organs, or bleeding out of the mouth, eyes, ears and vagina. Patients with serious illness can show signs of shock or coma, involving neurological symptoms like delirium and convulsions.

After a tick bite, the incubation period is of short duration (3–7 days). The pre-haemorrhagic period is characterized by sudden onset of fever, headache, myalgia, dizziness and further symptoms of diarrhoea, nausea and vomiting. Hyperaemia of the face, neck and chest; congested sclera; and conjunctivitis are also noted. The haemorrhagic period is short, rapidly progresses and typically begins at the third to fifth day of the illness. The haemorrhagic signs vary from petechiae to the appearance of large haematomas on the mucous membranes and skin. Bleeding, commonly from the nose, gastrointestinal system, urinary tract, respiratory tract and other sites including the vagina; gingival bleeding; cerebral haemorrhage; and bleeding from unexpected sites has been reported. The convalescence period begins 10–20 days after the onset of disease. It is characterized by labile pulse, tachycardia, temporary complete loss of hair, polyneuritis, difficulty in breathing, poor vision, loss of hearing and loss of memory.

Case definition: Defining a case is an important aspect of a surveillance system. The case definition of the disease will be more accurate when it is combined with laboratory confirmation with clinical manifestations.

The following are the case definitions for CCHF infection:

Suspected case: a patient with abrupt onset of high fever $>38.5^\circ$C and one of the following symptoms: severe headache, myalgia, nausea, vomiting, and/or diarrhoea and a history of tick bite within 14 days prior to the onset of symptoms; or history of contact with tissues, blood, or other biological fluids from a possibly infected animal (e.g. abattoir workers, livestock owners, veterinarians) within 14 days prior the onset of symptoms; or health-care workers in health-care facilities, with a history of exposure to a suspected, probable, or laboratory-confirmed CCHF case, within 14 days prior to the onset of symptoms.
**Probable case**: a probable CCHF case is defined as a suspected CCHF case fulfilling the following additional criteria: thrombocytopenia <50,000 cells/mL and two of the following haemorrhagic manifestations: haematomata at an injection site, petechiae, purpuric rash, rhinorrhagia, haematemeses, haemoptysis, gastrointestinal haemorrhage, gingival haemorrhage, or any other haemorrhagic manifestation in the absence of any known precipitating factor for haemorrhagic manifestation.

**Confirmed case**: a confirmed CCHF case is defined as a case that fulfills the criteria for probable CCHF and, in addition, is laboratory confirmed with one of the following assays: detection by ELISA or immunofluorescence assay of specific IgM antibodies against CCHFV, or a 4-fold increase in specific IgG antibodies against CCHFV in two specimens collected in the acute and convalescence phases, or detection by RT-PCR of CCHF viral RNA in a clinical specimen, confirmed by sequencing of the PCR product or CCHFV isolation.

**Diagnosis**

Haemorrhagic fevers are contracted through contact with the blood of infected animals, and the bite of infected ticks (CCHF and KFD) and mosquitoes (dengue fever). Some of these fevers can be transmitted from person to person. Laboratory diagnosis of the disease is established by molecular methods, while IgM and IgG antibodies become detectable by indirect immunofluorescence assay or ELISA after the fifth day (see Table 2).

However, there are reports that in severe cases no antibody response is observed. Real-time RT-PCR for rapid diagnosis of CCHFV infections is the test of choice in the acute phase, and for ticks. The isolation of CCHFV requires a high-containment BSL-4 laboratory, while viral RNA detection combined with serology for laboratory diagnosis in BSL-3- or BSL-2-compliant laboratories can be carried out following good microbiological practices with standard protocol. The Gujarat Government has planned to upgrade laboratories to provide CCHF diagnosis, with proper biosafety precautions and handling of these samples, after training and acquisition of all the required biosafety equipment.

**Treatment**

So far, there is no specific treatment for CCHF. A vaccine based on formalin-inactivated suckling mouse brain, which is not yet approved by the Food and Drug Administration of the USA (FDA), has been used in Bulgaria and the former Soviet Union. Since no specific treatment is available, supportive treatment includes careful fluid and electrolyte balance, monitoring and replacement with platelets, fresh frozen plasma and erythrocyte preparations. The effect of ribavirin is still controversial; the drug has not been approved for the treatment of CCHF by the FDA, but is, at present, the only antiviral agent with promising effect, if administered before the fifth day of the disease. It was observed that CCHF cases in India supported the use of ribavirin. Ribavirin is contraindicated in patients with chronic anaemia and haemoglobin levels below 8 g/dL, and in patients with severe renal impairment (creatinine clearance <30 mL/min). The drug may accumulate in patients with impaired renal function. If ribavirin is administered, patients should be carefully monitored during therapy for signs and symptoms of toxicity, such as anaemia. Patients with hypotension or haemodynamic instability should be managed following standard guidelines for the treatment of shock, which include resuscitation, fluid supplements (crystalloids/colloids) and inotropic support. In suspected secondary bacterial infection, patients should be treated according to standard guidelines/practice for community-acquired/nosocomial infections. CCHF–venin, an immunoglobulin preparation particularly from the geographical areas where this disease is endemic, may be useful by the intravenous route for treatment of patients with severe CCHF.

**Guidelines for management of CCHF cases**

**In the hospital setting**

The following precautions are recommended:

- isolate the patient in a room that is separate from other patients in the hospital;
- medical staff handling the patient should wear gloves and a gown, to avoid direct contact with the patient;
- after handling the patient, medical staff should thoroughly wash their hands, as well as any other parts of their body that came into contact with the patient, using soap and water;
- clinical procedures that are likely to cause spraying of bodily fluids should be avoided, or only performed by medical staff wearing a face shield, or a mask and eye goggles;
- bleach can be used for disinfection. A 1:100 dilution of bleach should be used to clean surfaces, medical equipment, and bedding and clothes. A 1:10 dilution of bleach should be used to clean up bodily fluids. Alternatively, 5% Lysol may be used.

**In the family/community setting**

- Family members and friends who had direct contact with the patient should be monitored for 14 days, for onset of a febrile illness.

**Dead body disposal**

- Rubber gloves or double surgical gloves should be used for handling the dead body. The persons handling the dead body in hospitals should also wear a mask and use personal protective equipment.
- The dead body should be sprayed with 1:10 liquid bleach. It should then be wrapped with a winding sheet, which is then sprayed with bleach solution.
- The wrapped and bleached body should be placed in a plastic bag, which is then sealed with adhesive tape before transport.
- The ambulance/transport vehicle should also be disinfected after use.
Prevention

The main means of CCHF outbreak control, namely breaking the transmission chains by avoiding/minimizing exposure to the virus-infected material, is recommended for prevention. Barrier nursing techniques are essential while treating confirmed and suspected cases of CCHF. Close contacts of the infected patient should be followed up with daily temperature recording and monitoring of symptoms for at least 15 days after the putative exposure. Health-care workers should take all the necessary precautionary measures to prevent occupational exposure.

Control

After confirmation of the CCHF outbreak in Gujarat state, a highly efficient network of hospital reporting of admissions of suspected cases, and help in monitoring the contacts and family members was activated. Animal diseases health officials and national vector-borne diseases (NVBCD) officials are on alert for sampling of ticks and domestic animal samples from the area of any suspected case in Gujarat state. With the diagnostic support of NIV, Pune in the last 3 years, every suspected case has been referred to NIV for a quick diagnosis, to avoid the spread of infection.9,44,57,78 An awareness programme is conducted in different government and private hospitals, for reporting and sending samples to NIV, Pune for confirmation. The strategic actions taken by the state government included active human, animal and entomological surveillance. Whenever any deceased CCHF-positive patient is reported, immediate surveillance is conducted for members of the community, for unusual fever symptoms, as well as IgG antibody screening of domestic animals and viral RNA detection in infested ticks. Isolation and treatment of cases following universal precautions is carried out, with contact tracing and monitoring of contacts, spraying cattle in the affected area with anti-tick agents, spraying human dwelling with residual sprays, and communicating the risk to the public. Owing to proper precautions and quick preventive measures, avoidance of nosocomial infection in humans is optimized.13,14,98

The individuals in outbreak areas who are vulnerable to tick bites, or exposed to infected animals or animal tissues, or health-care and laboratory workers are considered to be at risk of contracting CCHF. Thus, control measures should be mainly focused on tick control in outbreak areas and on personal protective measures for persons caring for CCHF patients.97–101

- if animals are slaughtered, proper disposal of carcasses should be performed;
- the left-over feed should be sprayed with 3% bleach and should be disposed of, to ensure that other animals do not feed on it;
- these procedures should be followed for at least 2 weeks and blood samples should be drawn and confirmed to be free of viraemia

Tick bites are best prevented by people avoiding tick-infested areas or by wearing long trousers that are tucked into boots. Tick bites can be prevented by application of a topical repellent to exposed skin and treatment of clothing with insecticide, which gives nearly 100% protection. Dipping is the primary method of tick control for livestock and has been found to be highly effective for controlling several tick-borne diseases. Spraying is another method used to apply the chemical acaricides that kill ticks.99–101 However, resistance of ticks to acaricides poses an increasing risk to livestock.

DISCUSSION

According to a recent study from the Zoological Society of London, United Kingdom, along with researchers from Georgia and New York, USA, India is considered a “hot spot” for emerging infectious disease, on a global map.102 In recent years, vector-borne diseases have emerged as a serious public health problem in countries of the South-East Asia, including India. Many emerging zoonoses have spread globally at the human–animal interface.103 Risk factors for emergence reside in multiple sectors. India has extremes of climatological and geographical conditions: temperatures that vary from extremely low to high, temperate regions and desert, thick evergreen forest, and areas of high rainfall. Increased population, urbanization, international travel, change in agricultural practices, environmental factors, change in lifestyle, deforestation, close contact of animals, and a porous international border make this country a high-risk area for outbreaks of emerging and new diseases.102

Vector-borne zoonoses now occur in epidemic form on an almost annual basis, causing considerable morbidity and mortality.104 All these have impact not only on public health but also on the livelihood and economy of affected countries. A network of laboratories, trained laboratory staff, more high-containment diagnostic laboratories, surveillance programmes, modern equipment and trained medical professionals are required in order that the country is prepared to deal with this kind of emergency situation. KFD was originally assumed to be restricted only to Karnataka state, but there is now evidence of its spread; similarly, CCHF is not restricted to one district but human positivity has now been recorded in seven districts.9 The recent sero-survey study by NIV, Pune, has revealed that domestic animals are positive for anti-IgG antibody in at least 15 districts of Gujarat state (NIV unpublished data). Though the main endemic foci are in Gujarat state, CCHF has been suspected in other parts of the country, based on earlier serology data.76,77 Strengthening of public health system networking for
CONCLUSION

KFD and CCHF are both of high importance for public health in India, as cases are observed almost every year in Karnataka and Gujarat states, respectively. It is important that the health system should be able to distinguish these tick-borne haemorrhagic diseases from other diseases, which have diverse and often overlapping, clinical presentations. In general, as the incidence of tick-borne diseases increases in any area, surveillance in other/adjoining geographic areas should also be expanded. Keeping in view the current status, the Indian Council of Medical Research (ICMR) has taken the initiative to survey to determine the probability of the existence of KFD in states adjoining Karnataka. Similarly, a joint initiative has been taken up by ICMR and the Indian Council of Agricultural Research (ICAR) to conduct a survey of IgG antibodies against CCHF in domestic animals in different states of India. In view of the “One health concept” put forward by WHO, it is proposed to establish a joint ICMR and ICAR committee on zoonoses, to create facilities for an ICMR–ICAR centre of excellence on zoonoses, which will be upgraded to a National Institute of Zoonoses. This institute will be dedicated to research and development in the areas of zoonotic diseases and will significantly contribute towards early diagnosis and control of re-emerging and newly emerging zoonotic diseases.

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