Kyasanur Forest Disease: a Status Update

Jeny Kalluvila John1, Jobin Jose Kattoor2, Anoopraj Rajappan Nair4, Aswathi Plantharayil Bharathan3, Rekha Valsala4, Gurupriya Vijayasaraswathy Sadanandan

1Department of Veterinary Pathology, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P; 2Department of Virology, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P; 3Central Avian Research Institute, Izatnagar, Bareilly, U.P; 4Department of Bacteriology and Mycology, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P; 5Department of Animal Biochemistry, Indian Veterinary Research Institute, Izatnagar, Bareilly, U.P
*Corresponding author: jenykj@gmail.com


INTRODUCTION

Kyasanur Forest Disease (KFD) is an emerging zoonotic viral tick borne disease affecting mainly monkeys. Every year lots of human cases are reporting with a morbidity rate of around 2–10% in South India (Gould and Solomon, 2008). It was first noticed when cases of monkey mortality occurred in a forest area of Shimoga district, India, followed by acute, febrile haemorrhagic disease in humans nearby during 1937 (Work et al., 1959). Around 400–500 cases of KFD are reporting from India every year (Work et al., 1957; Pavri, 1989). As a tick borne infection, it has a seasonal occurrence from January to June. Monkeys and humans are the only known host species that build up clinical disease with KFD virus. KFD virus circulates through small mammals such as porcupines, squirrels, rodents, shrews and ground birds and also in tick species in the endemic areas (Pattnaik, 2006). Kyasanur forest disease is endemic in Karnataka, Tamil Nadu and Kerala (CDC, 2013). As prophylactic measure formalin inactivated tissue culture vaccine is used in the diseased areas. In spite of vaccination, every year new cases are reporting from these areas. The possible factor for emergence of new cases can be due to low coverage of the vaccine (Kasabi et al., 2013) or due to lack of proper control of tick in endemic areas. The present review attempts to summarize on the various aspects of disease, its etiology, transmission, clinical features, epidemiology, diagnosis and various control strategies.

ETIOLOGY

The etiological agent of KFD is Kyasanur Forest Disease virus (KFDV), a RNA virus of the genus Flavivirus, family Flaviviridae (Lin et al., 2003; Thiel et al., 2005). The virus is positive sense with single stranded RNA virus. The KFDV is spherical (40–65nm in size), enveloped virus with an icosahedral nucleocapsid. The genome of Flavivirus has 3 structural proteins (Capsid, prM, and Envelope) and 8 non-structural proteins (NS1, NS2A, NS2B, NS3, NS4A, NS4B, NS5 and NS5B). The genomic RNA is similar to cellular mRNA except for not having poly–adenylated tail. The replication of Flaviviruses takes place in the cytoplasm of infected cells utilizing host cell’s polymerase (Kofler et al., 2006; Villordo, and Gamarnik, 2009).

Based on mode of transmission, the flaviviruses can be grouped into two: the vector borne viruses and the other...
with no known vector (Kuno et al., 1998). The vector borne viruses can be subdivided into a mosquito borne viruses and as tick borne viruses (Gaunt et al., 2001).

KFDV variants have been isolated from Saudi Arabia (Carletti et al., 2010; Memish et al., 2011) and China (Zhang et al., 1989; Zaki, 1997; Wang et al., 2009). During 1995 and 2001, a novel Flavivirus was isolated from haemorrhagic fever patients in Jeddah and Makkah area of Saudi Arabia (Qattan et al., 1996; Zaki, 1997; Madani, 2005; Madani et al., 2011). This novel flavivirus was initially isolated from Alkhumra district, Saudi Arabia, hence named as Alkhumra virus infection. The nucleotide analysis of prototype strain of this virus from Saudi Arabia (strain 1176) and the KFDV reference strain from India (P-9605) showed 92% sequence similarity. Charrel et al. (2007) isolated Alkhumra virus from Ornithodoros savignyi.

Another variant of KFDV was isolated from a febrile patient in south western China initially referred as Nanjianyin virus. Later studies grouped the Nanjianyin virus in KFDV group. The sequence alignment and homology analysis conducted by Wang et al. (2009) revealed that Nanjianyin virus belongs to the KFD virus clade and the results of phylogenetic analysis of PrM–E gene and NS5 gene suggesting that both viruses are in same genetic cluster.

Studies suggesting that tick born flaviviruses spread through various geographical areas are comparatively very slow (Gould et al., 2001; Gould and Solomon, 2008). The diversity and evolution study of KFDV by Mehla et al. (2009) point out that the various isolates of KFDV from India, Saudi Arabia, and China share a recent common ancestor.

**HOST RANGE AND VECTOR INVOLVED**

KFDV infection was reported mainly from wild primates and humans. The natural host of KFDV mainly involves wild primates: black faced langurs (Semnopithecus entellus) and red faced bonnet monkeys (Macaca radiata) and various tick species of genus Haemaphysalis (Work and Trapido, 1957; Bhatt et al., 1966). Many wild animals serve as natural hosts, the Blanford rat (Rattus blanfordi), the striped forest squirrel (Funambulus tristriatus tristriatus) and the house shrew (Suncus murinus). These animals have sufficient viremic titers for the transmission (Trapido et al., 1959).

Wide host range of KFDV includes humans, tick species, rodents (shrews, forest rats, white tailed rat, and white belled rat), monkeys (grey langur, black–faced langur, and bonnet macaque), bats, ground dwelling birds, squirrels, Indian crested porcupines. In experimental infections with KFDV, high virus titers was noticed in black-napped hares, porcupines, flying squirrels, Malabar giant squirrels, three-striped squirrels, gerbils (Boshell, 1969). Domestic ruminants can maintain the infected tick population for long time.

The ticks of genus Haemaphysalis, mainly Haemaphysalis spinigera act as a major vector for KFD (Sreenivasan et al., 1986). Wide spread distribution of this species of tick in forests especially tropical and deciduous of southern and central India. KFDV has been isolated from various other species of ticks, H. turturis, H pauana kinnavari, H. kyasamurenisis, H. minuta, Dermacentor, Ixodes, Ornithodorus (adult), Hyalomma marginatum isuaci (Verma et al., 1960; Singh et al., 1964; Singh and Bhatt., 1968; Singh et al., 1968; Bhat and Naik, 1978; Bhat et al., 1978a).

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**Figure 1**: Transmission cycle of KFD

The ticks of genus Haemaphysalis, mainly Haemaphysalis spinigera act as a major vector for KFD (Sreenivasan et al., 1986). Wide spread distribution of this species of tick in forests especially tropical and deciduous of southern and central India. KFDV has been isolated from various other species of ticks, H. turturis, H pauana kinnavari, H. kyasamurenisis, H. minuta, Dermacentor, Ixodes, Ornithodorus (adult), Hyalomma marginatum isuaci (Verma et al., 1960; Singh et al., 1964; Singh and Bhatt., 1968; Singh et al., 1968; Bhat and Naik, 1978; Bhat et al., 1978a).
In the transmission of KFDV, human act as a dead end host, with no sufficient viremia for further transmission (Labuda et al., 1993). Neutralizing antibodies of KFDV have been found in cattle, buffaloes, goats, wild boars, porcupines, squirrels, flying squirrels, rats, mice, shrews, bats (Bhatt et al., 1978b) and a number of bird species. Amplification of virus occurs in monkeys (Boshell, 1969).

MODE OF TRANSMISSION
The mode of evolution of KFDV is unclear, although in a study by Boshell (1969) reported, a considerable increase in the human population in Shimoga district during 1950's. Increasing human needs for wood and land for agriculture leads to destruction of local forest areas (Boshell, 1969). Alteration of ecosystem occurred as a result of human intrusion may led the way for introduction of KFDV from its wild reservoir host to humans.

All tick–borne Flaviviruses share one general feature in its natural transmission cycle. Man having no role in virus transmission in any of these diseases. Humans do not develop adequate viremia to infect the ticks (Labuda et al., 1993). In KFD small mammals, mainly rodents have been considered as the reservoir. For the survival of many viruses, a reservoir is very essential. Rodents are best maintenance hosts; because of their short generation time, always provide a group of new animals. In ticks, virus is maintained throughout life, the virus is passed to next generation through transstadial and trans–ovarial transmission. Co–feeding of mammalian host offer a more convenient means for virus transmission among ticks than feeding a viremic animal (Boshell and Rajagopalan, 1968; Randolph, 2011).

In enzootic areas, the KFDV was maintained and circulated in small mammals especially rodents, shrews, ground birds and ticks (Pattnaik, 2006). Infection of wild monkeys occurs through the bite of infected ticks and further spread to other non infected ticks and monkeys. Severe febrile illness was noticed in some of the KFDV infected monkeys. Human's contact infection mainly through the bite of infected nymph and also by contact with infected animals especially monkeys. Horizontal transmission between humans not reported. Persons visiting forest for recreation or for collecting wood will contract infection by accidental tick bite (CDC, 2013) (Figure 1).

In primate host, black–faced langurs were found highly vulnerable to the virus (Sreenivasan et al., 1986). Tick population hike during dry season (December to May), results in all major epizootics during this period (Rajagopalan et al., 1968a, b). Viremic birds play an important role in distant spread of virus and may also carry tick infected with virus (Gould and Solomon, 2008).

RISK OF EXPOSURE
The KFD was found endemic in various districts of Karnataka, Tamil Nadu and Kerala. Additionally related KFDV was isolated from Saudi Arabia and China. Persons will get bite from infective ticks while visiting forest areas in Karnataka for recreation, hunting or for collecting wood and herbs. The disease has a seasonal occurrence mainly during dry periods (November–June). Visiting the forest areas of Karnataka during this period without adequate protective measures increases the risk of exposure. The environmental conditions favours the tick multiplication, makes an area endemic for KFD (Parola and Raoult, 2001). In recent years, increased occurrence of tick–transmitted diseases has been reported around the globe (Piesman and Eisen, 2008; Nicholson et al., 2010). Grazing of cattle in forest areas with infected ticks led to a introduction of these ticks to new areas (Chomel et al., 2007).

EPIDEMIOLOGY
In the forest area of Shimoga district Karnataka, India during 1956 large number of monkey mortality were reported followed by acute, febrile haemorrhagic disease in humans nearby (Work et al., 1959). Research on this leads to the isolation of a new Flavivirus from the autopsied samples from monkeys. Later, an analogous virus was isolated from Ixodid ticks population in the affected forest areas (Work and Trapido, 1957). The name Kyasanur Forest disease was given after the forest where the first viral isolate was obtained (Kyasanur forest) (Dobler, 2010). Transmission is mainly by the tick of genus Haemaphysalis. Natural host of the virus are small wild mammals, become viremic and are infested by various stages of ticks (Trapido et al.,1959). Around 400–500 cases of KFD are reporting from India every year (Work et al., 1957).

KFD is endemic in 5 areas of Karnataka, India mainly Shimoga, Chikamagalore, Uttara Kannada, Dakshina Kannada, and Udupi. In every season of epidemic, around 500 cases are reporting from these areas (Sreenivasan et al., 1986; Pattnaik, 2006). Outbreaks in human population of Shimoga district, Mysore State were reported from 1959–1966 (Upadhyaya et al., 1975). Similarly reports of non human primates are from 1957 – 1964 by Goverdhana et al. (1974) and 1964–1973 by Sreenivasan et al. (1986). Report of Upadhyaya et al. (1975), mainly centered on endemic areas, whereas the report of Sreenivasan (1986) spread out the area to non endemic places also and reported 1046 monkey mortality during the peak season of tick activity. More cases around 50% are reported from black faced langurs.

During 2003, a total of 953 suspected cases were reported from human patients, out of which 306 were confirmed and 132 suspected cases from non human primates and out of that 11 were confirmed as KFD cases from Karnataka. During 2004, out of 568 suspected cases, 153 were confirmed in humans and out of 86 cases reported, 8 were confirmed as KFD in non human primates. From 2005–2008, a total of 1208 cases were reported from Shimoga district out of which 212 were positive for KFDV in humans. Between 2009 and 2011, a total of 225 suspected cases from humans, 83 were confirmed. The case fatality rate from 2003–2012 is around 3.4%. During this period, maximum cases reported during 2003 and the least in 2007 and 2010. Case fatality rate of KFD in non human primates from 2003–2012 in Shimoga district is 1.4% (Holbrook, 2012).

In a study by Kasabi et al. (2013) reported 215 suspected cases from different villages of Shimoga from December 2011–March 2012, in that 61 were KFD positive. More cases are reported from adult males of those areas. In 2012 from Bandipur National Park, Karnataka State, 12 out of 21 suspected cases in humans, 4 monkeys (total death 12) and 2 out of 14 tick pools were confirmed as KFDV cases (Mourya et al., 2012). This study confirmed the spread of KFDV to new loci. Detection of KFDV in Tamil Nadu and Kerala State of India, pointing out the presence of the virus
in several tropical forest areas of India. Serological evidences are there for the probable existence of KFDV in different states of India (Sarkar and Chatterjee, 1962; Pattnaik, 2006).

KFDV variants have been isolated from Saudi Arabia and China (Zaki, 1997; Wang et al., 2009). During 1995 and 2001, a novel flavivirus was isolated from haemorrhagic fever patients in Jeddah and Makkah area of Saudi Arabia (Zaki, 1997; Madani, 2005; Alzahrani et al., 2010). This novel flavivirus was initially isolated from Alkhurma district, Saudi Arabia, hence named as Alkhurma virus infection. Another variant of KFDV was isolated from a febrile patient in south western China initially referred as Nanjianyin virus. Later studies grouped the Nanjianyin virus in KFDV group.

Incidences of KFD in monkey were also confirmed in Nilgiris district of Tamilnadu. One incidence of Human was confirmed in Kerala State from Noolpuzha–Aalathoor colony in Wayanad district in 2013. Later in April 2014, the dreaded KFD has been diagnosed among monkeys of the temple compound at Vallikkavu near Chengannur in Kerala’s Alappuzha district. Distribution of KFD in India and world are depicted (Figure 2a and Figure 2b)
CLINICAL SIGNS AND PATHOLOGY IN HUMANS

The incubation period of KFDV ranges from a few days up to 2 weeks (Work and Trapido, 1937). KFD is endemic in 5 areas of Karnataka, India mainly Shimoga, Chikkamagalore, Uttara Kannada, Dakshina Kannada, and Udupi. (Pavri, 1989). The disease has various stages in development. The initial prodromal stage lasts for around one week, with sudden onset of fever, chills, headache, gastro intestinal disturbances, insomnia, sore throat, decreased blood pressure and heart rate, pain in muscles, extremities and conjunctivitis. Humans infected with KFDV have low platelet, white blood cells and red blood cells count. Ophthalmic manifestations of KFD are haemorrhages in conjunctiva, retina and vitreous humour, keratitis, opacity of lens, mild iritis (Iyer et al., 1959; Grard et al., 2007)

The next haemorrhagic stage is characterised by irregular epistaxis with blood in vomitus and faeces, blisters on mouth, haemorrhages from the gum and nose. The haemorrhagic stage is followed by a long convalescent stage. Frequently, a second febrile stage (relapse phase–10–20%) of around 2 weeks with same clinical manifestations of first phase along with various neurological complications was reported. Abnormal reflexes, confusion and tremors noticed as neurological complication (Pavri, 1989; Adhikari Prabha et al., 1993; Heymann, 2004; Pattnaik, 2006).

Gross and histopathological lesions in KFD are not pathognomonic. Nephrosis, hepatomegaly with degenerative changes, pneumonia with haemorrhage in the lung parenchyma, haemorrhages in gastro intestinal tract, distinct reticulo–endothelial cells in liver and spleen, with noticeable phagocytosis of RBC in spleen (Pattnaik, P. (2006). No clear brain lesions were found on autopsy examination except cerebral oedema in few cases (Work et al., 1957).

CLINICAL SIGN

KFD in animals is always fatal with an acute onset. Mortality in animals is noticed during the high viremic stage. Case fatality rate of around 100% noticed in experimental infections (Kenyon et al., 1992). Clinical signs and pathology of KFD in bonnet macaque are similar to that of humans (Webb and Chaterjea, 1962). Neurological signs were noticed in second febrile stage in bonnet macaques in experimental infection with KFDV (Webb and Burston, 1966). Even though Rhesus macaque has a related viremic phase like that of bonnet macaque, there is no clinical illness/mortality noticed in this species (Work, 1958).

DIAGNOSIS

The clinical signs of KFD are similar to many other viral/haemorrhagic fevers. There should be a reliable and fast differential diagnostic test for confirmation of KFD. The disease should be differentially diagnosed from various types of influenza’s, typhoid and from various rickettsial fevers (Mourya et al., 2014). Earlier for KFD detection, virus isolation and some antibody based detection methods such as hemagglutination inhibition (HI), complement fixation (CF) and neutralization test (NT) were used (Upadhyaya and Murthy, 1967; Pavri and Anderson, 1970). With advancement of technologies, laboratories developed various molecular diagnostic methods for diagnosis. Due to aerosol transmission of KFDV many cases were reported among the laboratory technicians. A BSL–3 facility is required for handling and working with KFDV, so during initial periods of outbreaks, little studies were conducted on KFD (Mourya et al., 2014). In India, many flavivirus infections are prevalent, cross reactivity between these viruses may create problems in diagnosis.

Blood and serum samples should be collected aseptically from patients with necessary protective measures for the personnel’s collecting samples. Complete blood picture analysis should be carried out in blood samples of suspected patients. Also various liver and kidney function tests (Mourya et al., 2014). The virus isolation can be done from blood during febrile period or from organ samples collected during autopsy. Paired sera sample can be used for serological examination.

Virus Isolation

Virus isolation of KFDV can be done in BHK–21, Vero E6 cell lines, embryonated chick cell or in mice (Mehla et al., 2009; Wang et al., 2009). In BHK–21, KFDV will produce characteristic cytopathic effect. Intra–cerebral inoculation of virus in 3 day old mice will cause mortality in all. Similar findings were obtained after intra–peritoneal inoculation in 50 day old mice (Wang et al., 2009). Mice (3 day old) are highly recommended for virus isolation than all other methods due to its high vulnerability to virus (Mourya et al., 2014).

Serological Methods

By HI test and neutralization test, KFDV antibodies were demonstrated from many states of India especially from south western states such as Gujarat and Maharashtra, also from West Bengal and Andaman and Nicobar Islands. In Andaman and Nicobar Islands, prevalence of KFD is around 12% when detected by neutralization test. (Padbidri et al., 2002). Mourya et al., (2012) developed IgM capture ELISA for detection of infection during acute phase.

Molecular Diagnosis

RT–PCR and real time PCR provides a very rapid and accurate diagnosis (Mackay et al., 2002; Mehla et al., 2009; Mourya et al., 2012). The RT–PCR reactions are highly specific and sensitive compared to other conventional methods (Eldadah et al., 1991; Tanaka, 1993; Fulmali, 2012). Mourya et al., (2012) developed nested RT–PCR, real–time RT–PCR for the rapid detection of KFD during acute phase infection. The flaviviruses specific NS–5 region was targeted for primer designing.

LABORATORY HAZARDS

Inhalation of aerosols may be the most frequent way of acquiring infection between persons handling the infected materials. Other means of transmission includes while conducting post mortem examination, accidental parental inoculation, spilling out of contents from broken glasswares or accidental ingestion (Morse et al., 1962; Banerjee et al., 1979). One should follow the WHO guideline while shipping of samples for diagnosis (WHO, 2013).

TREATMENT

Currently, no specific antiviral treatment exists for KFDV in humans; early hospitalization and supportive treatment becomes more essential. Supportive therapy includes the maintenance of normal blood cell counts, blood pressure...
and hydration (CDC, 2013; Mourya et al., 2014). Also, pain reliefs, antipyretics, blood transfusion, and antimicrobial therapy for secondary infections, corticosteroids and anticonvulsants for nervous disorders (Adhikari Prabha et al., 1993; Boria et al., 2002).

PREVENTION AND CONTROL
Tick borne diseases are emerging as a result of changes in public health policy, acaricide resistance, climatic changes, and emergence of new variant of pathogen. Measures need to be taken for reversal of these conditions (Dhama et al., 2013a). Prevention strategies such as quarantine, vaccination, early diagnosis, tick control will restrict the entry of virus to new areas.

The KFDV has been classified as risk group IV pathogen. Vaccination is one of the main control strategies for KFD. Formalin inactivated tissue culture vaccines are available for immunization against KFDV in endemic areas. Other control strategy includes wearing protective clothing while handling infectious materials and tick control. Strictly prohibit the visit to affected forest areas during outbreak time. If visit is inevitable, use protective clothing’s and gum boots to cover the whole body and apply some insect repellent to exposed body part (Ghosh et al., 2006; Piesman and Eisen, 2008; Kilpatrick et al., 2012; Mourya et al., 2014). The concept of ‘one world, one health and one medicine’ should be kept in mind while combating zoonotic infections (Dhama et al., 2013b).

VACCINES
An inactivated/killed tissue culture vaccine has been used in endemic areas of Karnataka, India since 1990. Initially 2 doses were used in persons of 7–65 years of age, in an interval of 4 weeks. Revaccination is required after 6–9 months for five years (Kasabi et al., 2013). Before introduction of formalin inactivated tissue culture vaccine, several other live and inactivated vaccines prepared in tissue culture, formalin–inactivated Russian Spring Summer Encephalitis (RSSE) virus (Aniker et al., 1962; Shah et al., 1962) and mouse brain were used in control programs (Bhatt and Anderson, 1971; Upadhyaya et al., 1979).

Incidence of KFD has been reported even in vaccinated individuals. The main drawback of these vaccines is poor area coverage, not taking boosters and poor storage conditions. Utilizing new technologies of vaccine production, develop better vaccine will combat the infection (Dhama et al., 2008; Paul-Pierce, 2009; Dhama et al., 2013c).

Vaccines against KFDV were initially produced in Shimoga district of Karnataka. Later, the unit was moved to Bangalore (Institute of Animal Husbandry and Veterinary Biologies, Hebbal). In a study conducted by Kasabi et al., (2013) noticed low coverage of vaccine in affected areas even less than half of the target population and the efficiency of vaccine was around 62% in individuals received initial 2 doses and 83% in individuals who received further boosters. In earlier studies, reported the efficiency around 79% in persons with one dose and 94% in those received 2 doses (Dandawate et al., 1994) and about 59% in those had 2 doses during 1970–1971 (Upadhyaya et al., 1979, Dandawate et al., 1980).

CONCLUSION
Kyasanur Forest Disease (KFD) is an emerging zoonotic viral tick borne disease affecting mainly monkeys. Every year from South India hundreds of human cases are reporting. Being a tick borne disease, strict measures should be taken for controlling the tick population. Formalin inactivated tissue culture vaccine are available for immunization in affected areas. Regular vaccination should be carried out for consecutive five years with increased area coverage. Even though an effective vaccine is available, KFD is still widespread and remain as a source of infection for humans. There is urgent need of effective control strategies so that this type of tick borne infections can be controlled.

REFERENCES


