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Kyasanur forest disease in India: A comprehensive update

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Abstract
Kyasanur forest disease (KFD) is a re-emerging tick-borne zoonotic hemorrhagic disease in humans caused by the Kyasanur forest disease virus. It is also known as monkey sickness or monkey fever because of its association with monkeys. The virus belongs to the *Flaviviridae* family, genus Flavivirus. It is transmitted to humans through the bite of an infected hard tick, *Haemaphysalis spinigera*, acting as a vector. KFD results in high-grade fever, frontal headache, nausea, vomiting, diarrhoea, and neurological and hemorrhagic symptoms have been reported in some cases. KFDV was first isolated in 1957 in the Shimoga District of Karnataka. KFDV is currently spreading to new regions in the Western Ghats and is endemic to many areas of India, namely Goa, Maharashtra, Tamilnadu and Kerala. Diagnosis can be made by collecting human blood samples, monkey guts, and ticks from the endemic regions. Samples are either tested for antigen during acute infection using real-time RT-PCR, or chronic cases are detected using IgM ELISA methods. Prevention and control of the disease can be done by monitoring human, monkey, and tick populations. The present review focuses on the different aspects of the disease, such as etiology, transmission, epidemiology, clinical symptoms, diagnosis and various prevention and control strategies.

Keywords: Kyasanur forest disease, KFD, monkey fever, tick borne disease**,** viral zoonosis

1. Introduction
Kyasanur forest disease (KFD) is a highly infectious, re-emerging, tick-borne viral zoonotic disease caused by the virus of the family *Flaviviridae* under the genus Flavivirus (Bhatt *et al*., 1966 [5]; Work and Trapido [56], 1957; Yadav *et al*., 2014 [58]). KFD, mainly focused in southern India, causes acute febrile haemorrhagic sickness in men and monkeys (Muraleedharan, 2016 [33]; Chakraborty *et al*., 2019 [9]). The disease was noticed in the Kyasanar forest of Shimoga in Karnataka, India, for the first time in 1957 (Work and Trapido, 1957 [56]; Bhatt *et al*., 1966 [5]). Kyasanur forest disease virus (KFDV) is classified in the risk group 4 pathogenic organisms (Carletti *et al*., 2010 [8]; Shah *et al*., 2018 [44]; Bhatia *et al*., 2020 [4]). The transmission of the virus occurs through the bite of an infected tick most commonly, *Haemaphysalis spinigera* (Work, 1958 [54]; Trapido *et al*., 1959 [48]) through various vertebrate species are involved in its transmission cycle where monkeys act as a sentinel host. This disease has an incubation period of two to eight days and the symptoms of viral infection include, high fever, conjunctivitis, frontal headache, prostration, and bleeding from the mouth, nose and gastrointestinal tract typical disease manifestations beyond acute fever are still controversial (Holbrook, 2012) [17]. Despite of the frequent occurrence of KFD in an endemic area, relatively less information is available about its pathogenic mechanisms or the response of the host to the infection even though they exhibit the same pathogenesis mechanisms as all flaviviruses (Pastorino *et al*., 2010) [36]. In India, an average of 400–500 KFD cases occur per year (Pavri, 1989) [38]. KFD has now spread to the states of Maharashtra, Tamil Nadu, Kerala, and Goa (Awate *et al*., 2016 [3]; Yadav *et al*., 2020 [60]). People who work or live in locations where virus-infected ticks are present have a high probability of acquiring the disease and outbreaks. The present review emphasizes epidemiology, prevalence, transmission, clinical manifestation and control of this disease.

2. Etiological agent
KFDV belongs to flaviviruses and has a positive-sense, single-stranded RNA genome of approximately 11 kb length encoding a single polyprotein (Yadav *et al*., 2020) [60]; which cleaves post-translationally into three structural (Capsid protein, Envelope Glycoprotein M and E) and seven non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) proteins (Dodd *et al*., 2011) [12].

E protein has a significant role in infection and the protective immune response (Füzik, 2018) [13]. The NS1 protein of flavivirus possesses immune evasive functions (Kuzmenko *et al*., 2016) [21].

3. Epidemiology

KFDV was first isolated during an outbreak of febrile illness of people and unusual deaths of black-faced langur (*Semnopithecus entellus*) and red-faced bonnet monkeys (Macaca radiata) in the Kyasanur forest area of the Shimoga district in the Karnataka, India (Work and Trapido, 1957) [56]. Affected persons were those who had a history of the visit to the affected forest at that period (Mehla *et al*., 2009) [26]. Heavy mortality of monkeys was usually noticed from December to May, the active phase of the nymphal stage of *Haemaphysalis* ticks (Pattnaik, 2006) [38]. Human intrusion in the affected region increases the chance of contact with infected ticks. Human activities for harvesting paddy, and gathering firewood and forest products usually increase during post-monsoon (Upadhyaya *et al*., 1975) [50]. At present, the annual outbreak of KFD among human beings ranges from 200–500 numbers (Chakraborty *et al*., 2019) [9]. It is estimated that from the year 1957 to 2017, there were 9,594 reported cases of KFD in 16 Indian districts. The most important human outbreaks were during the year1957–1958 (681 No's), 1983–1984 (2,589 No's), 2002–2003 (1,562 No's), and 2016–2017 (809 No's).

The reported KFD cases increased to more than 600 during 2002 and reached 900 in the year 2003. The total number of human KFD cases reported from Karnataka from 2003 to 2012 was 3263, with 823 confirmed reports and 28 deaths (Holbrook, 2012) [17]. In recent years, KFD has no longer remained limited to Karnataka. KFD in monkeys has been reported from the neighbouring states of Karnataka, *viz*., Tamil Nadu State and Kerala State (Murhekar *et al*., 2015) [15]. In May 2013, the first human KFD case in Kerala was reported from the Wayanad district, sharing borders with Karnataka. In 2014, confirmed cases were noticed from two more districts of Kerala, *viz*., Malappuram and Alappuzha. An outbreak resulting in the death of several monkeys and 18 human KFD cases was recorded from Wayanad in 2015. In 2015, nine human deaths due to KFD cases were observed in the Sattari taluk of Goa (Patil *et al*., 2017) [37]. In the meantime, a viral presence was established in the Sindhudurg of Maharashtra State in 2016 (Awate *et al*., 2016) [3]. These outbreaks confirm the endemicity of this disease in newer areas (Chakraborty *et al*., 2019) [9].

The tick, *Haemaphysalis spinginera*, is extensively dispersed in the deciduous and evergreen forest areas of Sri Lanka and India (Sreenivasan *et al*., 1986) [46]. Since 1957, many sporadic outbreaks have been recorded yearly in Karnataka (Pattnaik, 2006) [38]. Various isolates of the virus obtained from KFD outbreaks in Karnataka during 1957–1972 were kept in the viral culture depository of NIV in Pune, India (Muraleedharan, 2016) [33]. KFDV extended to far-away places, from the original hotspots that are Shimoga district in Karnataka (Sarkar and Chatterjee, 1962) [43] and at present anti KFDV antibodies were detected in those people from Kutch and Saurashtra of Gujrat. Forest workers from the Bandipur Tiger Reserve were affected by KFD during 2012 and the virus was isolated from ticks and monkeys in Nilgiri and Wayanad in the same period (Mourya and Yadav, 2016) [28]. Antibodies against this virus have also been found in people from West Bengal and Andaman and Nicobar Islands (Padbidri *et al*., 2002) [35]. KFDV infection in India has been tabulated in Table 1.

Table 1: Reports of KFDV infection in India

Year	Areas of outbreak	Reference
1957	Kyasanur Forest of shimoga district, Karnataka	Work and Trapido., 1957 [56]
1957-1964	Shimoga, Karnataka	Goverdhan *et al*., 1974 [14], Upadhaya *et al*.,1975 [50]
1964-1972	Southeast forest of Sagar town, Northwest sorab town, Karnataka	Sreenivasan *et al*.,1986 [46]
1972-2002	Chikmagalur, Udupi, Uttar and Dakshina Kannada	Pattnaik *et al*., 2006 [38]
2003-2012	Karnataka-3263 human cases were reported; out of these, 823 were laboratory confirmed.	Holbrook, 2012 [17]
2012-2013	Bandipur Tiger Reserve, Wayanad district of Kerala	Mourya *et al*., 2012 [31]
2014-2015	Shimoga, Karnataka, Wayanad, and Malappuram districts of Kerala	Munivekatappa *et al*., 2018 [32], Thippeswamy and Kiran, 2017 [47]
2016	Dodamarg Taluka, Sindhurga district, Maharashtra, Kerala and Goa	Patil *et al*., 2017 [37]
2017	Karnataka, Maharashtra, Goa	Patil *et al*., 2017 [37],
2018	Kerala, Karnataka, Maharashtra, Goa	Gaurav *et al*., 2018 [16]
2019	Kerala, Karnataka	IDSP, 2019 [18]
2020	Kerala, Karnataka	IDSP, 2020 [19]

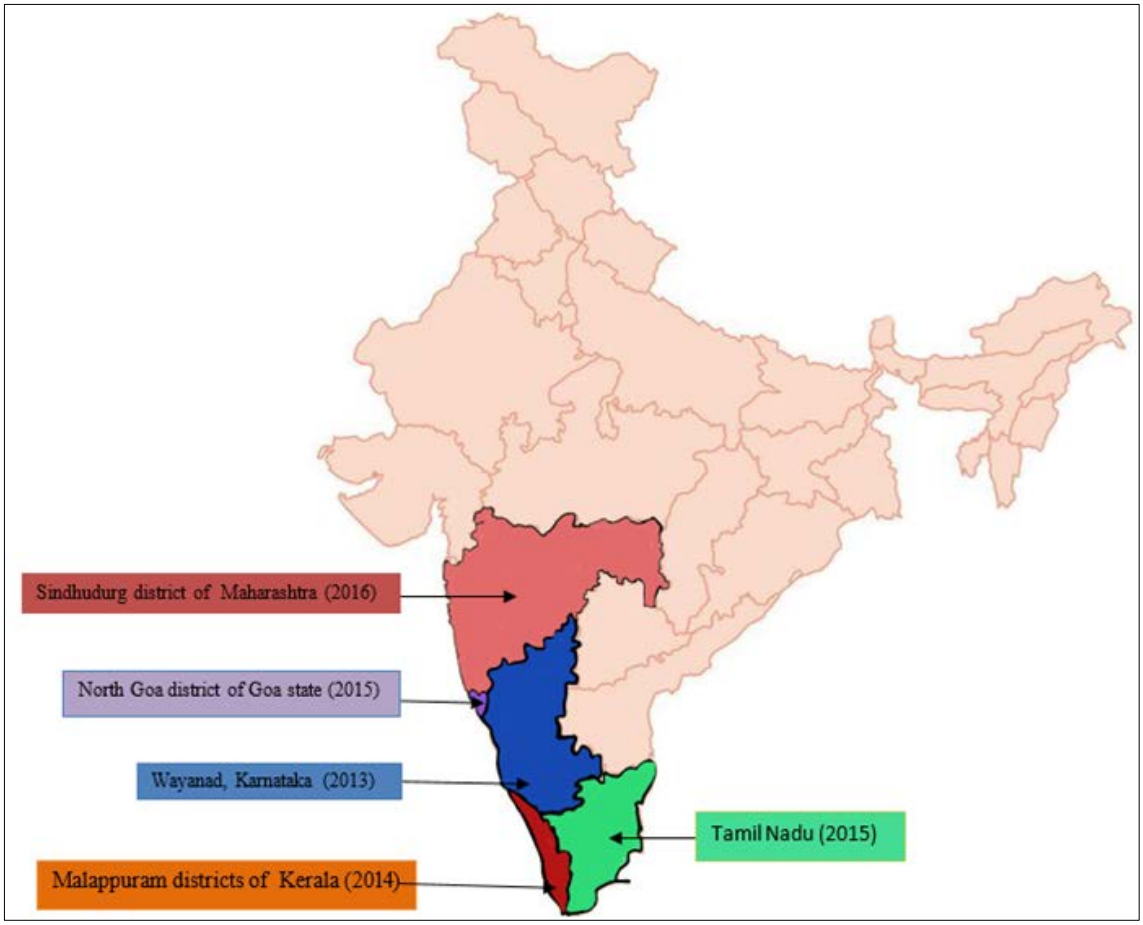

Fig 1: KFDV affected areas in India

4. Transmission

KFDV is circulated between a tick vector and a vertebrate host (Bhatia *et al*., 2020) [4]. Ticks act as vectors and the main reservoirs of this virus (Sadanandane *et al*., 2017) [41]. The Haemophysalis ticks spread the virus to a non-human vertebrate host (birds or mammals) in the natural cycle of transmission of KFDV (Ajesh *et al*., 2017) [2] Humans are considered dead-end hosts and usually become infected by infected nymphal bites, as unfed nymphs are extremely anthropophilic (Pattnaik, 2006) [38].

H. spinigera is extensively distributed in India, Vietnam, and Sri Lanka, and about ninety-five per cent of KFDV isolates are from this tick species (Varma *et al*., 1960 [51]; Bhatia *et al*., 2020 [4]). *H. spinigera* is commonly seen in Karnataka. Ixodes species of ticks can also act as a significant reservoir (Boshell and Rajagopalan, 1968a) [7]. Other tick vectors in disease transmission include *H. cuspidata*, *H. aculeate*, *H. formosensis*, *H. papuanakinneari*, *H. bispinosa*, *H. kyasanurensis* and *H. wellingtoni* (Work *et al*., 1957) [57] and *H. turturis.* Ticks can be infected at any stage of their life. The virus is transmitted to succeeding stages of ticks and mature tick progeny through the transstadial and/or transovarial routes. Co-feeding of ticks is another form of transmission between ticks (Randolph, 2011 [40]; Mansfield *et al*., 2017 [23]).

KFDV is circulated and maintained mainly in small mammals, *viz*., rodents, shrews, ground birds and ticks. The rodents are the best maintenance hosts as they have a very short generation period. Indian crested porcupines, bats, squirrels, monkeys and ground-dwelling birds can also act as hosts (Boshell and Rajagopalan, 1968b) [6], but red-faced bonnet monkeys and black-faced langurs are also highly prone to KF viral infection (Sreenivasan *et al*., 1986) [46]. The infected ticks drop down from monkeys' carcasses after their death, thus creating new hot spots for affected ticks. Those human beings visiting such forest areas are highly prone to KF viral infection. The direct transmission of this disease to humans from wild animals can also be possible, as deforestation is often reported as a reason for KFD outbreaks (Walsh *et al*., 1993 [53]; LaSala and Holbrook, 2010 [22]). The role of asymptomatic carriers in disseminating this disease has not been fully elucidated until now (Shah *et al*., 2018) [44].

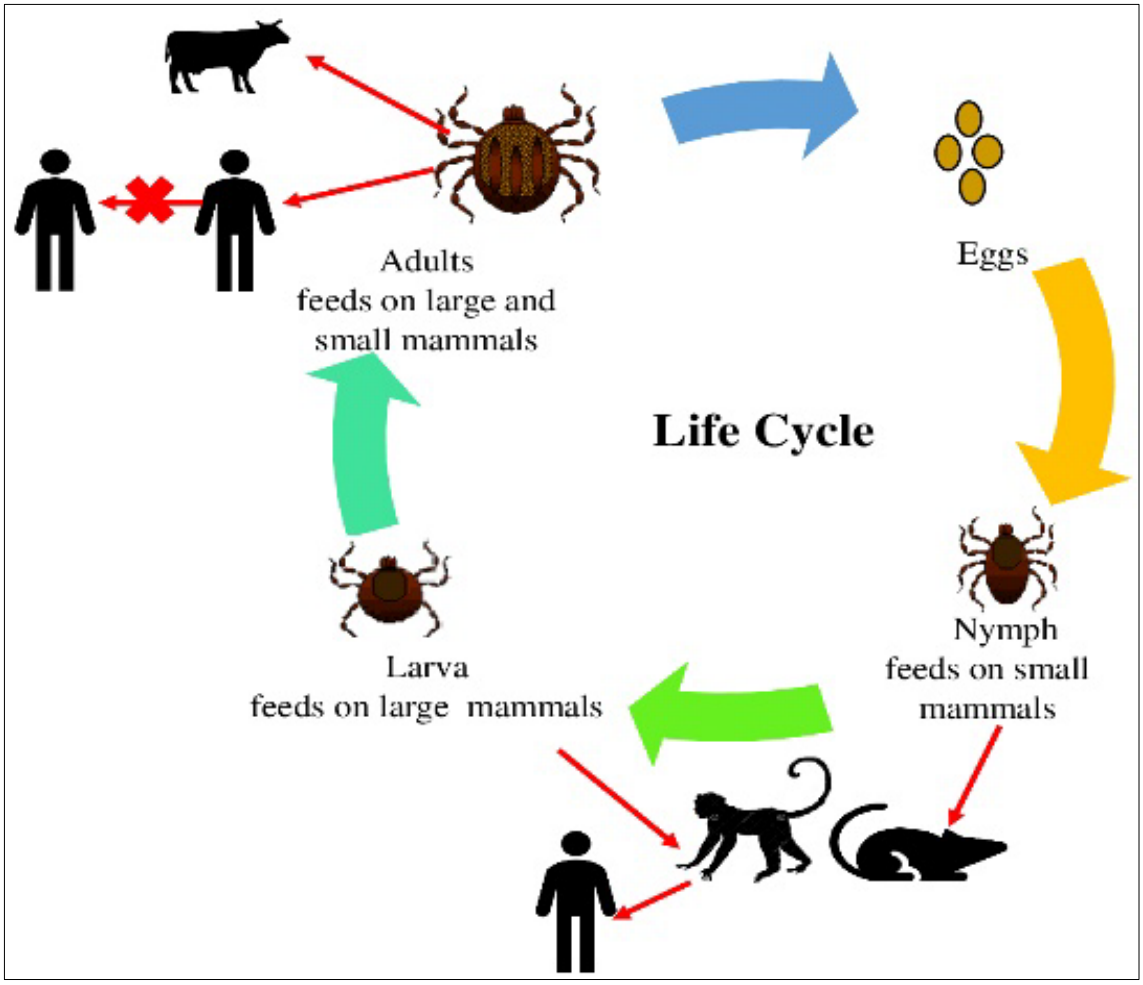

Fig 2: Transmission cycle of KFD Virus summary

5. Disease in human

The infectious dose for KFDV is currently unknown, and the incubation period of the disease ranges from 2 to 7 days. Case fatality rate ranges from 3 to 10%. The clinical symptoms usually commence with high fever (about 40 ^{0}C), headache, anorexia, diarrhoea, vomiting, insomnia, myalgia, cough, photophobia and symptoms such as bleeding from the gastrointestinal tract, nose, or gums resulting in hemoptysis and melena (Adhikari *et al*., 1993[(1)]; Mourya *et al*., 2013; Muraleedharan *et al*., 2016 [33]; Munivenkatappa *et al*., 2018 [32]). Other symptoms include frontal headache, Papulovesicular eruption on the soft palate, severe Prostration (Mourya *et al*., 2014; Shiji *et al*., 2016 [45]; Chandran *et al*., 2016 [10]), insomnia, decreased blood pressure, haemorrhages in the conjunctiva, vitreous humour, retina, the opacity of lens (Grard *et al*., 2007) [15]. Lymphocytosis has been seen in the 3rd and the 5th week after the onset of the disease. After the onset of ailments, the virus can be isolated from serum samples (Work *et al*., 1959 [55]; Bhatt *et al*., 1966 [5]). Though long-term sequelae are uncommon, some patients convalescing from KFDV may have tremors or unsteady gait for many weeks, which ultimately cures (Wadia, 1975) [52]. Bonnet monkeys and langurs, though, exhibit the same symptoms as humans, with a high mortality rate reaching up to 85% (Dobler, 2010) [11].

6. Diagnosis and treatment

Early detection of KFD always leads to a better prognosis, as viremia shoots to a very high level (3×10^6 CFU/ml) shortly after infection and remains at that level for two weeks. The first line diagnosis for KFDV infection is RT-PCR of blood samples targeting the NS5 region of the viral genome (Memish *et al*., 2012) [27], which can detect a very low virus load of ten copies/mL. Enzyme-linked immunosorbent assay (ELISA) is also used to detect KFDV/AHFV-specific antibodies in plasma or serum (Yadav *et al.*, 2019 [59]; Padbidri *et al*., 2002 [35]). IgM antibodies can be noticed from the 5th day after the commencement of symptoms to 90 days in the blood (Yadav *et al*., 2019) [59]. KFD should be differentially diagnosed from dengue, typhoid, malaria and other haemorrhagic fever. Symptomatic, supportive therapy is usually followed for treating KFD, as no approved antiviral drugs exist. Maintenance of normal blood pressure, blood cell counts, and hydration is essential and is to be monitored at 4-hour intervals. NSAIDs are not advised for treating KFD, but broad-spectrum antibiotic is used in those with Neutropenia.

7. Prevention and Control

Prevention policies include vaccination, control of ticks, timely diagnosis and quarantine. The primary strategy for management is vaccination. A formalin-inactivated vaccine generated by propagating the P 9605 strain of this virus in chick embryo fibroblast cell line is currently used for vaccination in endemic areas (Mansharamani *et al*.,1967 [25]; Mansharamani and Dandawate, 1967 [24]) though its efficacy is low (Kasabi *et al*., 2013) [20]. Two doses of vaccines at an interval of four weeks, followed by a booster dose at 6–9 months, are administered to get protective antibodies against this disease. Administering an annual booster dose of vaccine should be continued for five years after the last confirmed case in that area. Monitoring mortality and morbidity due to KFD in both domestic and wild animals will help in the early

detection and prevention of this disease. Being a tick-borne disease, avoid the visit to tick-infested areas and use protective clothes and tick repellents DEET (N, N-Diethyl-meta-toluamide), DMP (dimethyl phthalate), benzyl benzoate, dimethyl carbamate, and alone, picaridin, PMD (para-menthane-diol), and 2-undecanone) to deter tick bites will protect human beings from getting infected.

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