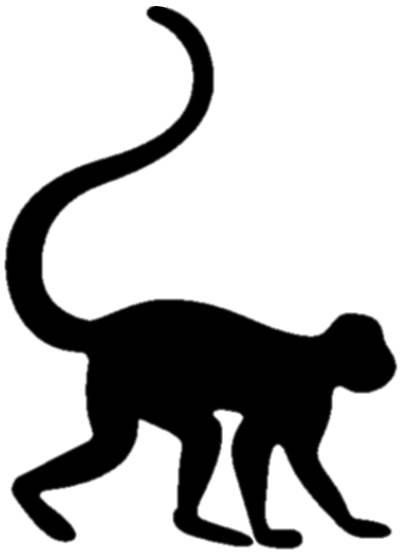
GOVERNMENT OF KARNATAKA

Kyasanur Forest Disease

A compendium of Scientific Literature

Prepared by NCDC, New Delhi



Department of Health & Family Welfare Services

OFFICE OF THE DEPUTY DIRECTOR

VIRUS DIAGNOSTIC

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Acronyms & abbreviations

AFI	Acute febrile illness
AHFV	Alkhurma haemorrhagic fever virus
BSL	Biosafety level
CCHF	Crimean-Congo Haemorrhagic fever
CDC	Centre for Disease Control and Prevention
CFR	Case fatality rate
DEET	N, N-Diethyl-meta-toluamide
DMP	Dimethyl phthalate
ELISA	Enzyme-linked immunosorbent assay
GHSA	Global Health Security Agenda
HI	Hemagglutination inhibition
ICD	International Classification of diseases
ICMR	Indian Council of Medical Research
ICTV	International Committee on Taxonomy of viruses
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IPM	Integrated Pest/Vector Management
JE	Japanese encephalitis
KFD	Kyasanur Forest Disease
KFDV	Kyasanur Forest Disease Virus
LIV	Louping ill virus
LGTV	Langat virus
MAHE	Manipal Academy of Higher Education
MBFV	Mosquito-borne flaviviruses
MIV	Manipal Institute of Virology
NCDC	National Centre for Disease Control (Delhi)
NKV	No Known Vector
NIV	National Institute of Virology (Pune)
OHFV	Omsk Haemorrhagic Fever Virus
PCR	Polymerase chain reaction
PI	Post infection
POWV	Powassan virus
PPE	Personal protective equipment
RNA	Ribonucleic acid
RSSE	Russian Spring Summer Encephalitis Virus
RT-PCR	Reverse transcription polymerase chain reaction
RVF	Rift Valley fever
TBE	Tick-borne encephalitis
TBFV	Tick borne flaviviruses
VDL	Viral Diagnostic Laboratory (Shimoga)
VRDLN	Virus Research And Diagnostic Laboratory Network
WHO	World Health Organization

Chapter. 1

Description of unusual illness

In the early summer months of 1957 (February), Kyasanur forest in Soraba taluk of Shimoga (now called as Shivamogga) reported unusual deaths of red-faced bonnet macaques and black-faced langurs. A few weeks later an outbreak of severe acute febrile illness (AFI) with encephalitis and haemorrhage was reported among the locals with a case fatality rate of 10% affecting 20 villages [1] [2].

How was KFD investigated?

Dr Telford Work, Director, VRC, Pune and his team investigated this outbreak and considered yellow fever a possibility for this outbreak. However, with the onset of the south-west monsoon, the cases decreased, and the probable diagnosis of the mosquito-borne illness was ruled out. Within the next few months, Dr Work and team isolated a new pathogen, and it was named Kyasanur Forest Disease Virus (KFDV) [3].

What was concluded?

KFDV was first isolated in March 1957 from black faced Hanuman langur monkey *(Semnopithecus entellus*) in Sorab taluk of Shimoga district of Karnataka, India. They found KFDV is closely related to Russian Spring-Summer Encephalitis Virus (RSSE) / Omsk Hemorrhagic Fever Virus (OHF). KFDV was later isolated from humans, ticks, and monkeys and Kyasanur Forest Disease (KFD) was classified under tick-borne viral hemorrhagic fever [4].

Overview of KFD

Kyasanur Forest Disease (KFD) is a tick-borne viral disease endemic to the south-western part of India. Kyasanur Forest Disease Virus (KFDV) is the causative organism, and it belongs to the Flaviviridae virus family. KFDV is transmitted to humans through the bite of infected hard ticks (*Haemaphysalis spinigera*) which act as a reservoir of KFDV or through contact with infected animals, especially ill or deceased monkey. No person-to-person transmission has been reported. Other common hosts for KFDV are rodents and shrews. Animals such as cows, goats, and sheep may get infected by KFDV, but their role in transmission is not clearly understood [5]. Approximately 400 to 500 cases occur each year with the case fatality of 3 to 5%. KFD has an incubation period of 3 to 8 days [6].

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[1] T H Work and H Trapido, 'Kyasanur Forest Disease. A New Virus Disease in India. Summary of Preliminary Report of Investigations of the Virus Research Centre on an Epidemic Disease Affecting Forest Villagers and Wild Monkeys of Shimoga District, Mysore', *Indian Journal of Medical Sciences*, 11.5 (1957), 341–42.

[2] Telford H Work and others, 'VIROLOGICAL EPIDEMIOLOGY OF THE 1958 EPIDEMIC OF KYASANUR FOREST DISEASE', *American Journal of Public Health and the Nations Health*, 49.7 (1959), 869–74

[3] Work and others.

[4] Work and others.

[5] CDC, 'CDC Fact Sheet, Kyasanur Forest Disease (KFD)'

[6] CDC.

Origin of KFD

Kyasanur forest disease is also known as "Monkey fever (*Manga-na-kayale, in the Kannada language*)" because of its close association with monkey deaths [7]. The KFD virus was first isolated in 1957 from sick monkeys commonly known as black-faced langurs in Kyasanur forest of Soraba taluk, Shimoga district in the Karnataka state of India. In March 1957, ICMR's Virus Research Centre investigated and described Kyasanur forest disease as an illness similar to Russian spring-summer viral aetiology. Viruses which are closely related to KFD are Omsk hemorrhagic fever virus in Siberia, Alkhurma hemorrhagic fever virus in Saudi Arabia, and Nanjianyin virus in China. Serological diagnosis of cases reported during the 1957 epidemic showed seasonal patterns notably during the spring and summer seasons in South India (January to June months). During 1956-57, around 500 cases were reported with nearly 10% of mortality. In the following year (1958) KFD affected 181 cases with 3% case fatality and several monkey deaths Kyasanur forest and this disease became well established in this region [8].

Before 1957

Retrospective epidemiological studies indicated the absence of similar illness in humans or monkeys before December 1955 and the first ever outbreak was reported from January to April 1956. The disease spread rapidly from four villages in 1956 to 20 villages in 1957, affecting both monkeys and humans. The virus was also isolated from *Haemaphysalis* ticks during the same time in Kyasanur forest. Few studies revealed the presence of specific neutralising antibodies to KFDV in rodents indicating a non-primate cycle which maintains the infection in the environment. However, the cause of its emergence in 1956 is not precisely known. Several theories have been proposed and described by the early researchers on its emergence in India.

[7] Mark Nichter, 'Kyasanur Forest Disease: An Ethnography of a Disease of Development', *Medical Anthropology Quarterly*, 1.4 (1987), 406–23.

[8] Work and others.

Chapter. 2

Subsequent outbreaks

1959 to 2001

Since the early epidemics in 1956 - 1958, every year, several human cases and monkey deaths have been reported in Shimoga district. During 1959 to 1966, the incidence of cases slowly extended to a broader range mainly towards the south and south-west regions from the initially infected area, which included around 72 villages and hamlets across Shimoga district. As per a surveillance study conducted from 1959 to 1966, a total of 322 human cases, with 4% mortality was reported during the given period. The year wise distribution of cases is given in the table below:

Table 1: Number of human cases of KFD reported from 1958 to 1966

Year	Number of cases
1958-59	56
1959-60	73
1960-61	1
1961-62	14
1962-63	52
1963-64	3
1964-65	16
1965-66	107
Total	**322**

The cases were calculated as per the KFD onset season, i.e., from September to August. The highest number of cases were reported from February to April with a peak during March. The increase in cases during 1966 was associated with improved surveillance and high exposure to the forest due to scanty rainfall during 1965 monsoon. Most of the localities having human cases had also reported monkey deaths in and around the nearby areas [9].

A similar surveillance study on the epizootiology of KFD in wild monkeys during the same period (1957-64) revealed a high prevalence of the disease among two species of monkeys, i.e., *Presbytis entellus* (Langur) and *Macaca radiata* (Bonnet). The study was done in 234 localities covering Soraba, Sagara, Shikaripura, and Hosanagara taluks of Shimoga district and Sirsi taluk of Uttar Kannada district, a total of 163 virus-positive monkeys were detected out of 394 monkeys autopsied and tested. The majority belonged to Sagar, Soraba, and Shikaripura taluks of Shimoga. The study recorded 1159 monkey deaths in which *Presbytis entellus* were 948, *Macaca radiata* was 165, and 46 were from unknown species. Deaths were recorded from January to May with a peak from February to March, which corresponded to the occurrence of human cases [10].
During the epizootics among wild monkeys recorded between 1964 and 1973, a total of 1046 monkey deaths from 213 localities were reported. Similar to previous surveillance study

[9] S Upadhyaya, DP Murthy, and CR Anderson, 'Kyasanur Forest Disease in the Human Population of Shimoga District, Mysore State, 1959-1966.', *The Indian Journal of Medical Research*, 63.11 (1975), 1556–63.
[10] M K Goverdhan and others, 'Epizootiology of Kyasanur Forest Disease in Wild Monkeys of Shimoga District, Mysore State (1957-1964).', *The Indian Journal of Medical Research*, 62.4 (1974), 497–510.

(1957-64) the deaths among *Presbytis entellus* (860) was comparatively higher than *Macaca radiata* (186). The seasonality and time trends of mortality were similar to the previous epidemics. However, the spatial distribution of the positive monkey deaths showed the extension of the disease transmission to surrounding newer places from the original epidemic foci. Five taluks of Shimoga, namely Sagara, Soraba, Shikaripura, Hosanagara and Thirthahalli and two taluks of Uttara Kannada district (Sirsi and Honnavara) reported virus positive monkey deaths. Similarly, localities with virus-positive monkeys had a higher incidence of the virus isolated from ticks. In 1982, a new-foci of epizootics was reported from Beltangady taluk of Dakshina Kannada district, which is situated 130 km south to the initial epidemic foci [11].

Table 2: Number of monkey deaths and virus-positive monkey autopsy samples reported during 1957-1973

Year	Number of Monkey Deaths	Number of Necropsied / Tested	Number of Virus Positives
1957 (Jan-Sep)	105	14	6
1957 – 58 (Oct –Sep)	92	19	5
1958 -59	290	111	42
1959 – 60	187	62	28
1960 – 61	80	27	5
1961 – 62	114	36	18
1962 – 63	147	69	36
1963 – 64	144	56	23
1964 – 65	109	38	15
1965 – 66	191	76	36
1966 – 67	126	31	11
1967 – 68	138	50	32
1968 – 69	135	26	15
1969 – 70	88	16	8
1970 – 71	75	30	4
1971 – 72	101	20	6
1972 – 73	83	21	4

The geographic extension of KFD included Shimoga district, parts of Uttar Kannada to Dakshin Kannada, Chikmagalur, and Udupi districts during 1980 to 2001.

Since 2001

A gradual increase in KFD outbreaks and sporadic cases were observed in the KFD endemic districts of Karnataka since 2001. A total of 3263 human cases of which 823 were lab confirmed and 28 deaths were reported from 2003 to 2012 in Karnataka state. The major outbreaks since 2000 have been given below in Table 3. Every year outbreaks and several

[11] MA Sreenivasan and others, 'The Epizootics of Kyasanur Forest Disease in Wild Monkeys during 1964 to 1973', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 80.5 (1986), 810–14.

sporadic cases were reported with a case fatality rate of 3 to 4% in Shimoga and adjoining districts [12] [13] [14] [15] [16] [17].

Table 3: Number of KFD confirmed human cases and deaths from 2000 to 2019

Year	Number of Human KFD cases	Human deaths
2000	130	9
2001	435	-
2002	98	6
2003	953	11
2004	153	5
2005	63	7
2006	99	2
2007-2008	50	-
2009-2010	64	1
2011-2012	61	2
2013-2014	106	-
2014-2015	100*	3*
2015-2016	256*	1*
2016-2017	244*	2*
2017-2018	121*	4*
2018-2019	142*	-

*AFI surveillance data

Serological evidence for KFD

There are reported serological evidence for KFD detected in humans in other parts of India, namely Kutch and Saurashtra regions of Gujarat state, Kingaon and Parbatpur of West Bengal state [18]. A seroprevalence study in Andaman and Nicobar islands in 2002 revealed a

[12] K Ajesh, B K Nagaraja, and K Sreejith, 'Kyasanur Forest Disease Virus Breaking the Endemic Barrier: An Investigation into Ecological Effects on Disease Emergence and Future Outlook.', *Zoonoses and Public Health*, 64.7 (2017), e73–80.

[13] Michael R. Holbrook, 'Kyasanur Forest Disease', *Antiviral Research*, 96.3 (2012), 353–62.

[14] Jeny Kalluvila John, 'Kyasanur Forest Disease: A Status Update', *Advances in Animal and Veterinary Sciences*, 2.6 (2014), 329–36.

[15] Gudadappa S Kasabi, Manoj V Murhekar, Pragya D Yadav, and others, 'Kyasanur Forest Disease, India, 2011-2012.', *Emerging Infectious Diseases*, 19.2 (2013), 278–81.

[16] Pragya D Yadav and others, 'Outbreak of Kyasanur Forest Disease in Thirthahalli, Karnataka, India, 2014', *International Journal of Infectious Diseases*, 26 (2014), 132–34.

[17] D. Arunkumar et al., 'REDRAWING THE BOUNDARIES OF KYASANUR FOREST DISEASE (KFD) IN INDIA-EARLY RESULTS OF GHSA-SUPPORTED ACUTE FEBRILE ILLNESS SURVEILLANCE', *AMERICAN JOURNAL OF TROPICAL MEDICINE AND HYGIENE*, 95.5 (2016), 200–201.

[18] Priyabrata Pattnaik, 'Kyasanur Forest Disease: An Epidemiological View in India', *Reviews in Medical Virology*, 16.3 (2006), 151–65.

high prevalence of HI antibodies against KFDV [19]. Also, earlier KFDV variant was isolated from Saudi Arabia and China [20].

The emergence of KFD outbreaks (2012 to 2018)

The emergence of KFD incidence in other regions away from the original foci was observed since Nov 2012, when 12 monkeys were found dead in Bandipur National Park, Chamarajanagar district of Karnataka state. Subsequently, six human clinical cases from Mole Hole village and Madhur colony in Bandipur tiger reserve who were reported to have handled the dead monkeys during incineration contracted the infection. Four out of six human samples and 3 out of 7 monkey autopsy sample were positive for KFDV. During the same season in January 2013, monkey autopsy samples were collected from Nilgiri Forest, Tamil Nadu state, and were tested positive for KFDV. It was followed by a human case in Noolpuzha of Wayanad district of Kerala, which is a neighbouring district to Karnataka and Tamil Nadu [21].

A new-foci of KFDV incidence was reported during May 2014 when a cluster of fever cases was investigated from Nagamala hills in Nedumkayam Reserve Forest of Malappuram district, Kerala state. The results revealed five positive cases (4 IgM by ELISA & 1 RNA by RT-PCR) among two clusters of suspected cases. The index case was positive for both IgM (acute sample) and IgG (convalescent sample) antibodies [22]. A major outbreak was reported in Wayanad and Malappuram districts of Kerala state from December 2014 to June 2015 which included 107 confirmed human cases with 14 deaths. During the same season, several monkey deaths were reported from the same region. All the monkey deaths and human KFD cases belonged to six villages which fall under Karulai forest range (Nilambur south forest division) and Kurichiyat forest range, close to Nilgiris forest range of the Western Ghats [23]. Goa state which is situated several km away from the primary KFD foci in the northwestern part of Western Ghats range witnessed a major outbreak in Pali village of Sattari taluk, North Goa in the early months of 2015. The outbreak claimed 18 confirmed cases and nine deaths. Since then, several outbreaks and sporadic cases have been reported throughout Sattari taluk of North Goa [24] [25]. The disease made its presence in Dodamarg taluk of Sindhudurg district of Maharashtra state in January 2016 (Ker village) [26]. Later with a regular AFI surveillance, more cases were detected from several villages of Dodamarg taluk. Every year several cases are reported during the cashew nut harvesting season, which coincides with the KFD seasonality in Sattari and Dodamarg taluks of Goa and Maharashtra respectively [27].

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[19] V S Padbidri and others, 'A Serological Survey of Arboviral Diseases among the Human Population of the Andaman and Nicobar Islands, India.', *Southeast Asian Journal of Tropical Medicine and Public Health*, 33.4 (2002), 794–800.

[20] Jinglin Wang and others, 'Isolation of Kyasanur Forest Disease Virus from Febrile Patient, Yunnan, China', *Emerging Infectious Diseases*, 15.2 (2009), 326–28.

[21] Devendra T Mourya and Pragya D Yadav, 'Spread of Kyasanur Forest Disease, Bandipur Tiger Reserve, India, 2012 - 2013', *Emerging Infectious Diseases*, 19.9 (2013), 1540–41.

[22] Babasaheb V Tandale and others, 'New Focus of Kyasanur Forest Disease Virus Activity in a Tribal Area in Kerala, India, 2014', *Infectious Diseases of Poverty*, 4 (2015), 12.

[23] C. Sadanandane, A. Elango, and others, 'An Outbreak of Kyasanur Forest Disease in the Wayanad and Malappuram Districts of Kerala, India', *Ticks and Tick-Borne Diseases*, 8.1 (2017), 25–30.

[24] Manoj V. Murhekar and others, 'On the Transmission Pattern of Kyasanur Forest Disease (KFD) in India', *Infectious Diseases of Poverty*, 4.1 (2015), 37.

[25] Arunkumar, G et al.

[26] P Awate and others, 'Outbreak of Kyasanur Forest Disease (Monkey Fever) in Sindhudurg, Maharashtra State, India, 2016.', *The Journal of Infection*, 72.6 (2016), 759–61.

[27] Arunkumar, G et al.

Table 4: Sequence of major KFD events since November 2012

Year	Events
Nov 2012	Incidence of 12 monkey deaths and six human clinical cases (4/6 human case positive & 3/7 monkey sample positive for KFDV) in Bandipur Tiger Reserve (National Park), Chamarajanagar district, Karnataka state.
Jan 2013	Incidence of KFDV in the monkey of Nilgiri forests, Tamil Nadu.
May 2013	The first case of Human KFD in Noolpuzha village, Wayanad district, Kerala.
May 2014	4 IgM, 1 IgG and 1 RT-PCR positivity among two clusters of cases in a tribal population of Nagamala hills in Nedumkayam Reserve Forest of Malappuram district, Kerala state.
Dec 2014 – Jun 2015	A major KFD outbreak (107 confirmed cases and 14 deaths) among the population of 6 villages in Karulai forest range (Nilambur south forest division) and Kurichiyat forest range of Wayanad and Malappuram districts, Kerala.
March 2015	The first incidence of KFD with a major outbreak in Pali village, Sattari taluk, North Goa (18 confirmed cases and 9 deaths).
December 2015- June 2016	Several outbreaks are reporting a high number of KFD cases distributed over maximum part of Sattari taluk, North Goa. Affected villages include Mauzi, Dhabe, Zarme, and Kopardem.
Jan 2016	The first case of KFD from Ker village, Dodamarg taluk, Sindhudurg district of Maharashtra detected by AFI surveillance. It was followed by repeated outbreaks in the subsequent years with a high number of cases covering many villages of Dodamarg taluk and Banda region (March 2017).
March 2016	Villages of Khanapur taluk of Belgaum district (Kapoli, Chapoli, Mudagai and Amte) reported 12 suspected cases of KFD [28]. The cases were migrants to KFD affected areas in Goa for cashew nut harvesting [29].
Jan - April 2017	KFD cases occurred in Gudalur taluk and Pandalur taluk of Nilgiris district, Tamil Nadu, among tea-plantation workers. 18 KFD cases were detected in this region through AFI surveillance.
December 2018	Aralagodu village in Shivamogga district reported cases of KFD for the first time. (Areas affected includes Bannumanae, Dombekai, and Kanchinkai)

[28] 'KFD Monkey Fever Reported in Forest Areas of Khanapur', *All About Belgaum* (Belgaum, 19 March 2016).
[29] D Y Patil and others, 'Occupational Exposure of Cashew Nut Workers to Kyasanur Forest Disease in Goa, India.', *International Journal of Infectious Diseases : IJID : Official Publication of the International Society for Infectious Diseases*, 61 (2017), 67–69.

Chapter. 3

Epidemiology of KFD

Virus classification (by ICTV)

According to the International Committee on Taxonomy of Viruses [30].

Group: Group 4 Arbovirus
Family: Flaviviridae
Genus: Flavivirus
Species: KFDV (Kyasanur Forest Disease Virus)
Biosafety Level:

KFDV is highly pathogenic pathogen classified under BSL- 4.[31] The US-CDC lists KFDV under category-4 pathogenic viruses. However, KFDV is category-3 pathogen on the European list [32]. According to "Regulations and guidelines on Biosafety of Recombinant DNA Research and Biocontainment 2017, KFDV is classified under the list of Risk Group 4 microorganisms. [33]

ICD classification

International Classification of diseases classified Kyasanur Forest Disease under ICD-10-CM A98.2. [34]

Vectors

Hard ticks

Principal Vector

Haemaphysalis spinigera

Reservoir Host

Porcupines, rats, squirrels, mice, shrews, cattle.

Amplifying Host

Red-faced Bonnet *(Macaca radiata)*

Black-faced Hanuman langur *(Semnopithecus entellus). Semnopithecus entellus* is the scientific name, and *Presbytis entellus* is a homotypic synonym [35].

Accidental Host

Human (Dead-end host. No Human to Human transmission has been reported) [36].

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[30] Claude Fauquet and others, *Virus Taxonomy - Eighth Report of the International Committee on the Taxonomy of Viruses*, 2005, LXXXIII.

[31] M Muraleedharan, 'Kyasanur Forest Disease (KFD): Rare Disease of Zoonotic Origin.', *Journal of Nepal Health Research Council*, 14.34 (2016), 214–18.

[32] MR Klein, *Classification of Biological Agents, RIVM Letter Report 205084002/2012*, 2012.

[33] *Regulations and Guidelines on Biosafety of Recombinant DNA Research and Biocontainment 2017*, 2017.

[34] 'ICD-10-CM, Chapter 1, Section A90-A99, ICD-10-CM Code A98.2 - Kyasanur Forest Disease', 2016.

[35] 'Taxonomy Browser (Semnopithecus Entellus)'.

[36] CDC.

Transmission

Transmission occurs by the bite of infected hard ticks or direct contact with infected or dead animals.

Affected states in India

The disease initially reported from Shimoga district of Karnataka which is a primitive sylvan territory in Western Ghats of India. The disease spread out to other districts of Karnataka involving districts of Chikkamagalore, Uttara Kannada, Dakshina Kannada, and Udupi districts, Chamarajanagar district (2012), Belagavi district (2016). In 2013, KFDV was detected in monkey autopsies from Nilgiris district of Tamil Nadu state. Monkey deaths and human cases have now been reported from three neighbouring states bordering Karnataka, i.e., Wayanad (2013) and Malappuram districts of Kerala (2014), North Goa district of Goa state (2015), and Sindhudurg district of Maharashtra (2016)[37] [38].

Table 5: States and districts affected by KFD (with population)

States	Districts	District Population (Census 2011) (Ref)
Karnataka	Shimoga	1752753
	Chikkamagalur	1137961
	Udupi	1177361
	Uttara Kannada	1437169
	Dakshina Kannada	2089649
	Hassan	1776421
	Kodagu	554519
	Mysore	3001127
	Chamarajanagara	1020791
	Belgaum	4779661
Kerala	Wayanad	817420
	Malappuram	4112920
Tamil Nadu	Nilgiris	735394
Goa	North Goa	818008
Maharashtra	Sindhudurg	849651
	Total population at risk	**2,60,60,805**

Risk factors and risk groups

The spill-over of this zoonotic disease happens at the crossroads of the animal-human-interaction, especially villages adjoining forest areas and inter-state borders. People who frequently visit the forest areas of the Western Ghats region such as forest guards and officials, range forest officer (RFO), forest watchers, shepherds, firewood collectors, dry leaf collectors, hunters, people who handle dead animal carcasses, travellers who camp in the forest areas, tribal communities living inside the forest areas (*Jenu kurubas and Betta kurubas*), cashew nut workers especially those who engage in cleaning the dry leaves before the harvest season (seen in Pali and Mauxi outbreaks, North Goa), and areca nut farm workers working in infected tick areas will have a high risk of acquiring KFD infection.

[37] Arunkumar, G et al.

[38] D T Mourya and P D Yadav, 'Recent Scenario of Emergence of Kyasanur Forest Disease in India and Public Health Importance', *Current Tropical Medicine Reports*, 3.1 (2016), 7–13.

People who live in the KFD endemic areas and refuse to take KFD vaccination are at risk in contracting the infection.

Agent

The KFD virus (KFDV) has high sequence similarity with Alkhurma Hemorrhagic Fever Virus (AHFV). This RNA virus is measuring about 25nm (40-60 nm) in diameter. 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, prM/M and E) and seven non-structural (NS1, NS2a, NS2b, NS3, NS4a, NS4b and NS5) proteins.

Natural hosts and reservoir

Several forest-dwelling small mammals like rodents, shrews, insectivorous bat and many birds maintain the natural enzootic cycle of the virus in the forest ecosystem. The wild primates, black-faced Hanuman langurs (*Presbytis entellus*), and red-faced bonnet monkeys (*Macaca radiata*) get the virus infection by a tick bite and are susceptible to the infection. Man is an incidental dead-end host. Cattle play a significant role in maintaining the tick population.

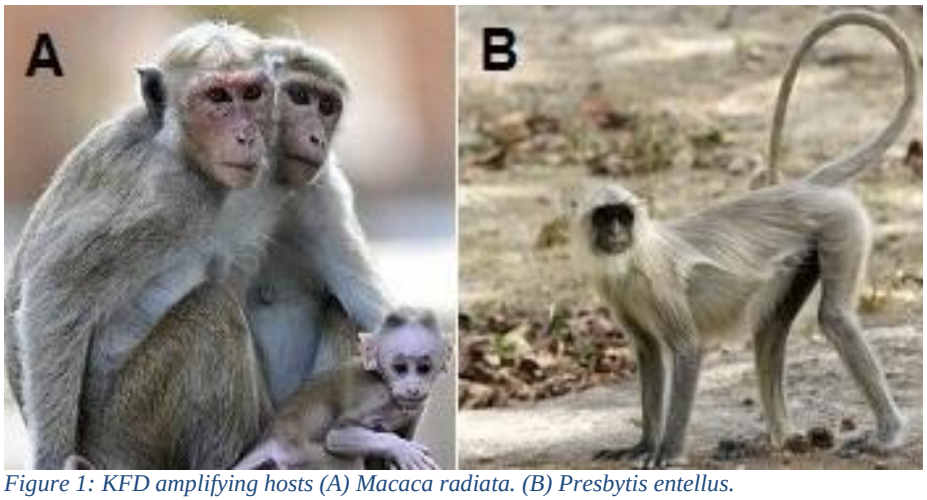

Figure 1: KFD amplifying hosts (A) Macaca radiata. (B) Presbytis entellus.

KFD vectors

KFDV is primarily transmitted by an infected tick-bite within primates (*Presbytis entellus* and *Macaca radiata*) and other wild reservoirs and accidentally to humans [39] [40][41]. Ticks of various genera within Ixodid families such as *Haemaphysalis* spp., *Dermacentor* spp., *Ixodes* spp., and *Riphicephalus* spp. are widely identified with KDV infections [42] [43]. While *Haemaphysalis spinigera* is the primary vector [44] [45], apart from that many other species of

[39] Work and Trapido.
[40] Pattnaik.
[41] Ajesh, Nagaraja, and Sreejith.
[42] G Geevarghese and A C Mishra, *Haemaphysalis Ticks of India* (Elsevier, 2011).
[43] M J Boshell and P K Rajagopalan, 'Preliminary Studies on Experimental Transmission of Kyasanur Forest Disease Virus by Nymphs of Ixodes Petauristae Warburton, 1933, Infected as Larvae on Suncus Murinus and Rattus Blanfordi', *The Indian Journal of Medical Research.*, 56.4 suppl (1968).
[44] Pattnaik.
[45] M G R Varma, H E Webb, and Khorshed M Pavri, 'Studies on the Transmission of Kyasanur Forest Disease Virus by Haemaphysalis Spinigera Newman', *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 54.6 (1960), 509–16.

Haemaphysalis spp. were recorded with KFDV in India, which includes *H. turturis*, *H. pauana kinneari*, *H. minuta*, *H. cuspidata*, *H. bispinosa*, *H. kyasanurensis*, *H. wellingtoni* and *H. aculeate* [46]. Additionally, some ticks of Argasidae *(Ornithodoros spp. and Argas spp.)* have demonstrated successful KFDV acquisition under laboratory conditions [47]. Hence there is always a possibility of bats and other bird's involvement in KFD transmission and maintenance [48].

Figure 2: Microscopic picture of female and male Haemaphysalis spinigera

Table 6: List of ticks associated with Kyasanur forest disease transmission in India

Ticks isolated with KFD in field condition	**Tick demonstrated with KFD in laboratory**
Haemaphysalis spinigera	*Rhipicephalus haemaphysaloides*
Haemaphysalis turturis	*Hyalomma marginatum issaci*
Haemaphysalis papuana kinneari	*Ornithodoros crosi*
Haemaphysalis minuta	*Argas persicus*
Haemaphysalis cuspidata	*Dermacentor auratus*
Haemaphysalis kyasanurensis	*Ixodes ceylonensis*
Haemaphysalis bispinosa	
Haemaphysalis wellingtoni	
Haemaphysalis aculeata	
Ixodes petauristae	

[46] Pattnaik; Geevarghese and Mishra; H R Bhat and others, 'Transmission of Kyasanur Forest Disease Virus by Haemaphysalis Kyasanurensis Trapido, Hoogstraal and Rajagopalan, 1964 (Acarina: Ixodidae).', *The Indian Journal of Medical Research*, 63.6 (1975), 879–87.

[47] D T Mourya and Yadav.

[48] Syed Z. Shah and others, 'Epidemiology, Pathogenesis, and Control of a Tick-Borne Disease- Kyasanur Forest Disease: Current Status and Future Directions', *Frontiers in Cellular and Infection Microbiology*, 8.May (2018).

Haemaphysalis species are the ticks of the temperate region, and they act as ectoparasites for more than one animal during their life-cycle [49]. A Haemaphysalis tick life cycle involves three life stages (Larvae, Nymph and Adult) and feeds on three different vertebrate hosts, as they require blood-source to either moult into next life-stage or for nourishing their eggs. Ticks usually inject its saliva into the host at the site of bite and virus enters the host along with saliva [50]. Tick bite and attachment while feeding on the host is generally painless and extend for a longer duration (several hours to sometimes days) enhancing their vector potential. Haemaphysalis ticks can be infectious only after it acquires an infection during their immature life stage (usually larval stage) and can be infectious through the rest of its life via transstadial transmission [51]. Even though there was no strong evidence of transovarial transmission; an Ixodid can act as a natural reservoir for KFD due to its longer life span (under unfed condition, a hard tick can survive years). Nymphs are the more infective life stage of KFD for both primates and humans as their host preferences are poorest during this stage [52]. Compared to adult ticks, the immature are non-specific in host selection and ends up frequently feeding on all immediately available living-hosts including humans. Otherwise, humans have no role in the maintenance of virus apart from being an accidental and dead-end host. Usually, a hard-tick detaches from its dead host in search of others to complete feeding procedure. Therefore entering the closer zone to infected dead monkey were suspected to be high risk to infected tick bites and KFD infection [53].

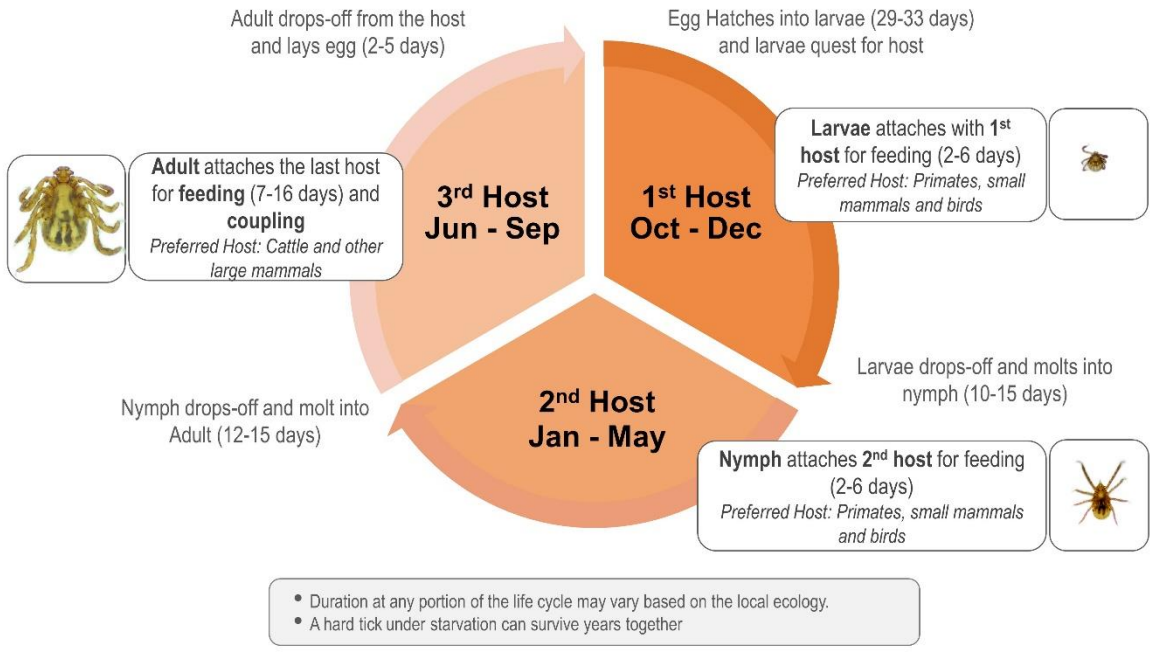

Figure 3: The Life cycle of tick (Haemaphysalis spinigera) responsible for the transmission of KFDV to humans

Haemaphysalis ticks are the most prevalent host-seeking tick of Western-Ghats region in India and especially highly abundant in the KFD reported areas [54]. Hence there is a high

[49] Geevarghese and Mishra.
[50] P A Nuttall and others, 'Adaptations of Arboviruses to Ticks', *J Med Entomol*, 31.1 (1994), 1–9.
[51] Ajesh, Nagaraja, and Sreejith.
[52] Pattnaik; D T Mourya and Yadav; Shah and others.
[53] D T Mourya and Yadav; Shah and others; Ajesh, Nagaraja, and Sreejith.
[54] N Naren Babu and others, 'Spatial Distribution of Haemaphysalis Species Ticks and Human Kyasanur Forest Disease Cases along the Western Ghats of India, 2017-2018', 77.3 (2019), 435–47; C. Sadanandane, M. D. Gokhale, and others, 'Prevalence and Spatial Distribution of Ixodid Tick Populations in the Forest Fringes of

chance of domestic or other local animals to transport the tick to human settlements, which again could elevate the extent of the disease spread [55]. The presence and prevalence of Haemaphysalis ticks depend on factors such as climatic and microclimatic conditions, vegetation and host availability/mobility [56]. The activity of Haemaphysalis nymphs are reported highest during the post-monsoon season (November to May), and so most of the human and primate infections occur during this period of a year[57]. Most of the wild vertebrates get ectoparasite infestation as a cluster, which usually composes of different species and stages of ticks or mites. Even if the vertebrate host is uninfected, ticks can acquire an infection during this mass feeding process directly from mouthpart of an infected tick to an uninfected one and the phenomenon is widely known as co-feeding transmission [58]. The co-feeding transmission is demonstrated in many of the tick-borne viral, bacterial and rickettsial diseases including CCHF. The phenomenon may play a potential role in KFD and is yet need to be investigated.

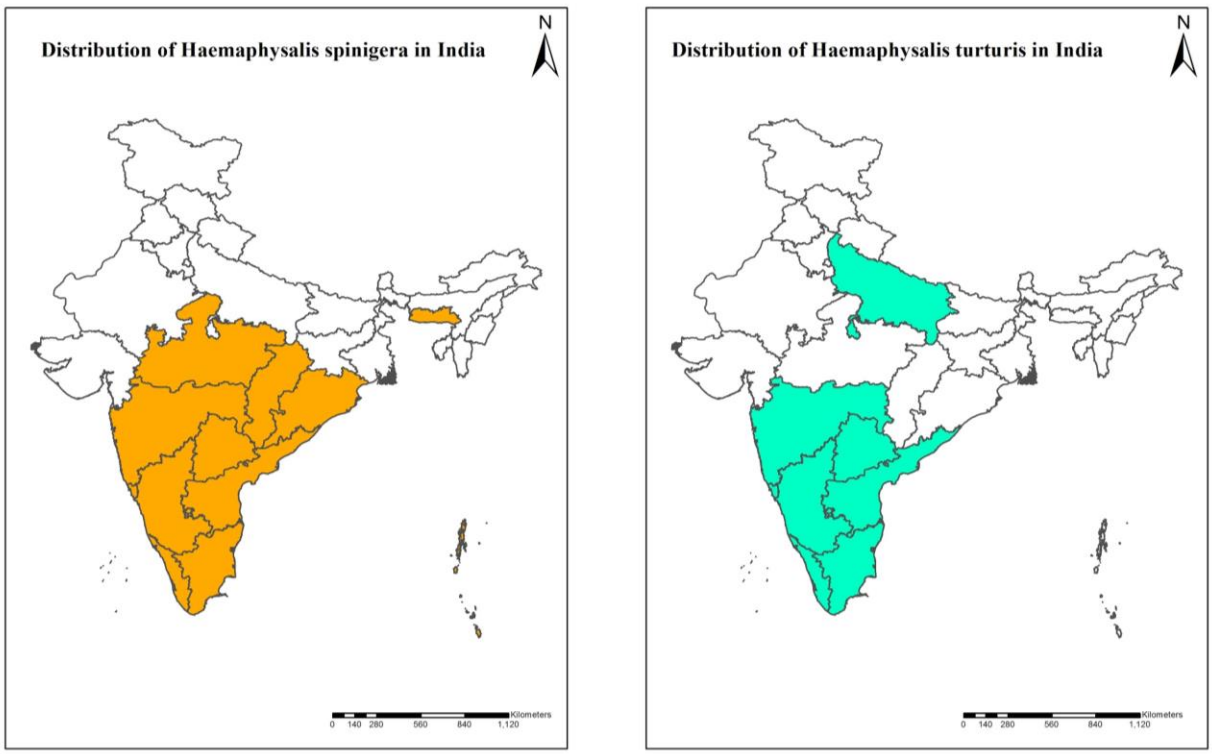

Figure 4: Distribution of Haemaphysalis spinigera and Haemaphysalis turturis in India

Environmental factors

KFD shows seasonality, the epidemic period usually begins in November and peaks from January to April, then declines by May and June. The epidemic/outbreaks relate to the activity of nymphs, which is very high during November to May. Adult fed female ticks lay eggs, which hatch to larvae under the leaves. They further infest small mammals and monkeys, as well as accidentally infect humans, and feed on their hosts. Subsequently, they

Western Ghats Reported with Human Cases of Kyasanur Forest Disease and Monkey Deaths in South India', *Experimental and Applied Acarology*, 75.1 (2018), 135–42.

[55] Murhekar and others.

[56] Pattnaik; Shah and others.

[57] Pattnaik; Work and Trapido.

[58] K L Mansfield and others, 'Emerging Tick-Borne Viruses in the Twenty-First Century', *Front Cell Infect Microbiol*, 7 (2017), 298; S E Randolph, 'Transmission of Tick-Borne Pathogens between Co-Feeding Ticks: Milan Labuda's Enduring Paradigm', *Ticks Tick Borne Dis*, 2.4 (2011), 179–82.

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 lifecycle.

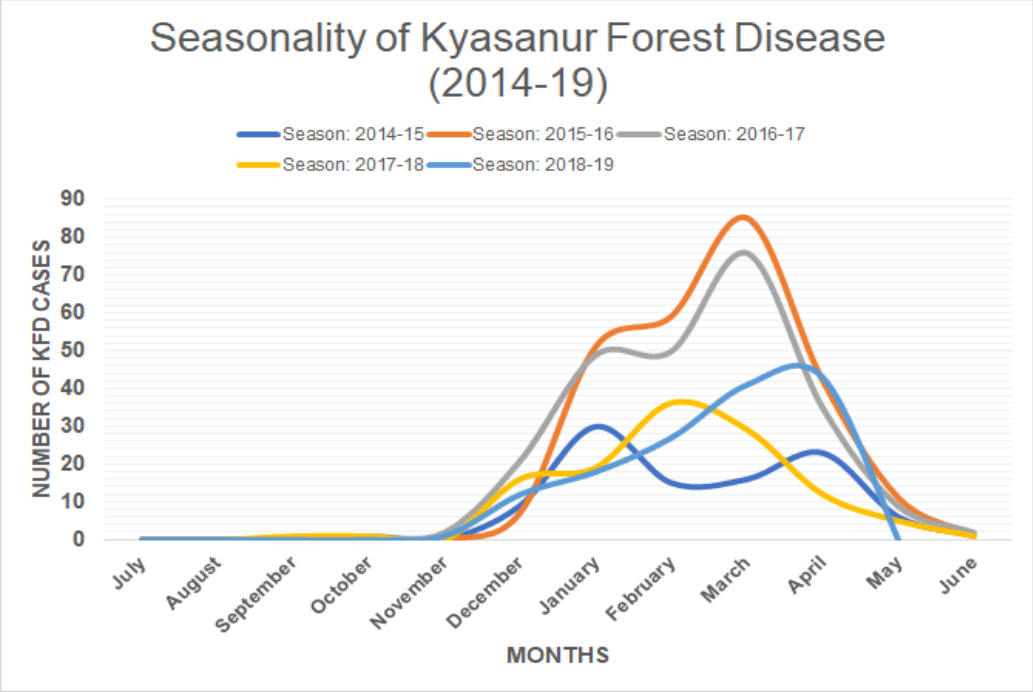

Figure 5: Seasonality of KFD with laboratory confirmed cases detected through AFI Surveillance (2014-19)

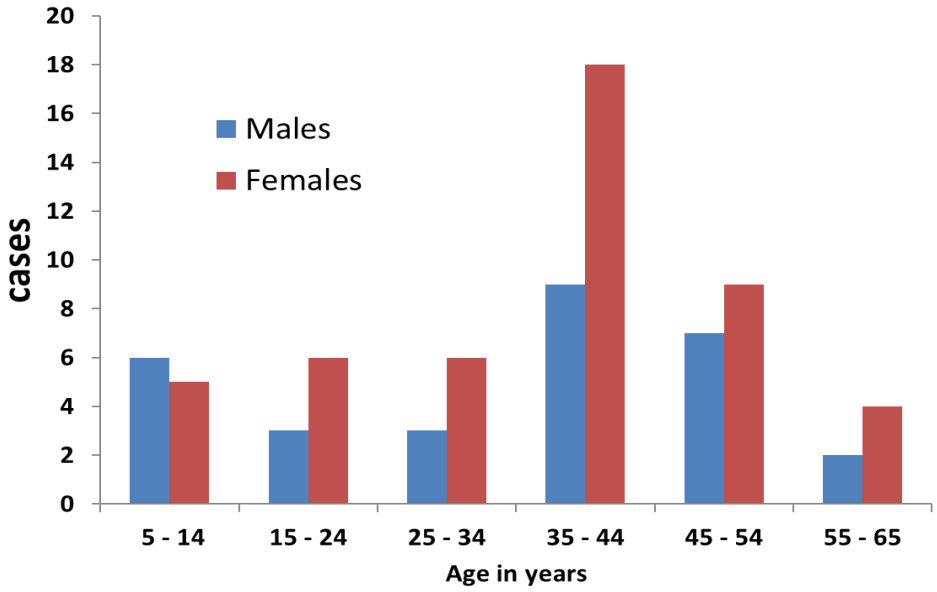

Figure 6: Age-wise and gender-wise distribution of laboratory confirmed cases through AFI surveillance (2014-15) (n = 111)

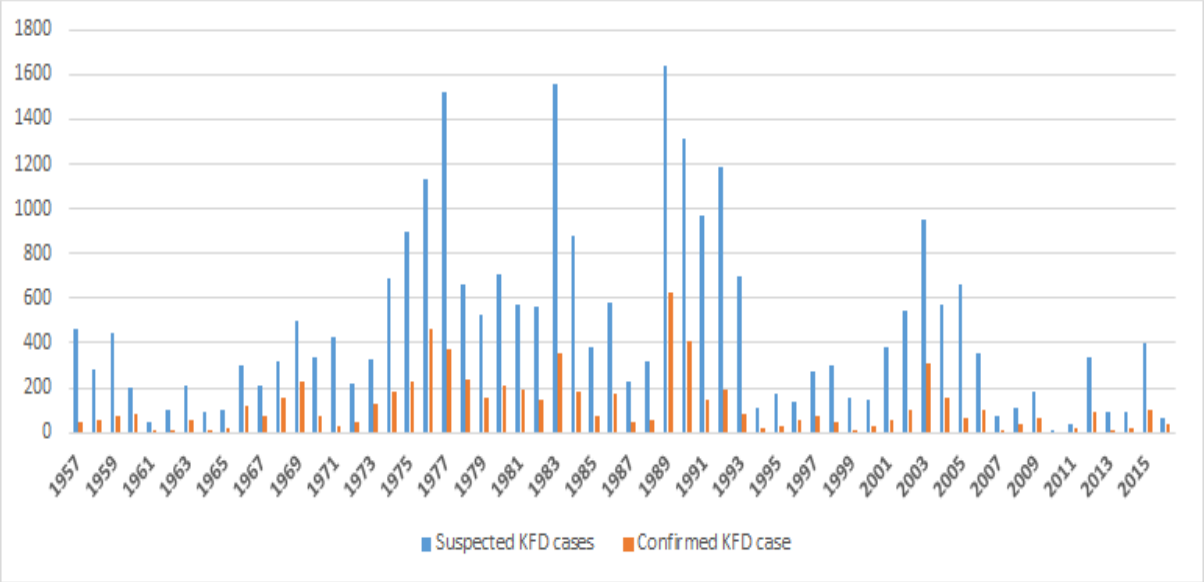

Figure 7: Year-wise distribution of KFD suspected / confirmed cases (1957 – 2016)

(**Source:** VDL Shimoga Data, MCVR data and Antiviral Res. 2012 December ; 96 (3): 353 – 362)

Transmission of KFDV

KFDV is transmitted by the bite of an infected tick, especially nymphal stages. The wild monkeys *Semnopithecus entellus and Macaca radiata*, gets the disease through the bite of infected ticks. The infection causes a severe febrile illness in most of the monkeys. When infected monkeys die, the ticks drop from their body, thereby generating "hot spots" of infectious ticks that further spread the disease. Humans can get the disease from an infected tick bite or by contact with an infected animal. Human-to-human transmission does not occur.

Virus ecology

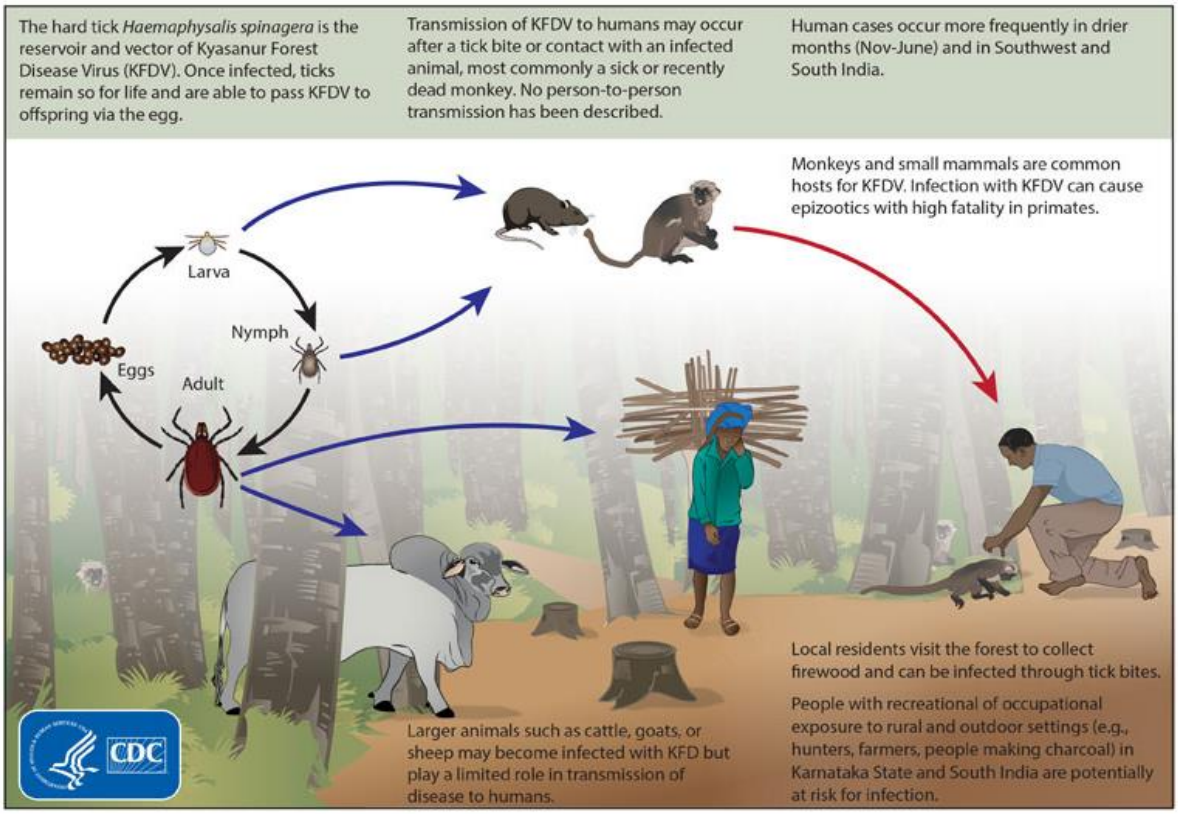

Figure 8: Kyasanur Forest Disease (KFD) ecology (Source: CDC)

Figure 8. shows the ecological cycle for Kyasanur Forest Disease virus. The hard tick *Haemaphysalis spinigera* is both the reservoir and the vector for the virus. Transmission to humans can occur directly through contact with ticks or through contact with infected monkeys and small animals. Larger animals may become infected, but play a limited role in the transmission of disease to humans.

Incubation period

3 to 8 days after the bite of an infective hard tick.

Chapter. 4

Clinical features

After an incubation period of 3-8 days, the symptoms of KFD begin suddenly with chills, fever, and headache. Severe muscle pain with vomiting, gastrointestinal symptoms and bleeding problems may occur 3-4 days after initial symptom onset. Patients may experience abnormally low blood pressure, and low platelet, red blood cell, and white blood cell count. After 1-2 weeks of symptoms, some patients recover without complication. However, the illness is biphasic for a subset of patients (10-20%) who experience a second wave of symptoms at the beginning of the third week. These symptoms include fever and signs of neurological manifestations, such as severe headache, mental disturbances, tremors, and vision deficits. The estimated case-fatality rate is from 3 to 5% for KFD. The disease progress with a biphasic presentation with initial phase lasts for 10 -14 days. The clinical spectrum begins with rapid inception of fever, chills, headache, and generalised myalgia, especially of the neck, upper and lower back and extremities [59]. Upon physical examination of febrile patients, severe prostration is noticed [60].

Table 7: KFD clinical course

Clinical Course	Period	Signs and Symptoms
First Phase	7-12 days post incubation period	Sudden onset of continuous high-grade fever, diarrhoea, vomiting, severe prostration, myalgia, and headache.
Second Phase (Occurs in a subset of 10 to 20% of the cases)	2-12 days after an afebrile period of 1-2 weeks	Meningeal signs, altered sensorium, seizures, bleeding manifestations, and prolonged convalescent period (may last for a few months).

KFD progression

The progression of disease during the early phase of illness associated with gastrointestinal symptoms including vomiting, abdominal pain and diarrhoea [61]. Occasional epistaxis with blood in vomitus and faeces also noticed [62]. Severe dehydration may result due to lack of fluid intake [63]. Decreased in heart rate (Bradycardia) and fall in blood pressure are seen. Lymphadenopathy and hepatomegaly are also noticed. Ocular signs involve photophobia, conjunctivitis, keratitis, iritis, haemorrhages in the retina and vitreous humour, and opacity of lens [64] [65].

After 3 - 4 days of the initial development of signs, hemorrhagic phase starts which comprises inflammation of oral mucosa and maculopapular eruptions over both soft and hard

[59] Ashok Munivenkatappa and others, 'Clinical & Epidemiological Significance of Kyasanur Forest Disease', *Indian Journal of Medical Research*, 2018, 145–50

[60] Khorshed Pavri, 'Clinical, Clinicopathologic, and Hematologic Features of Kyasanur Forest Disease', *Reviews of Infectious Diseases*, 11.Ii (1989), S854–59

[61] Munivenkatappa and others.

[62] John.

[63] Munivenkatappa and others.

[64] Pavri.

[65] John.

palate, haemorrhages from the gum and nose [66] [67]. Pulmonary involvement also sometimes noticed with persistent cough and blood-tinged sputum [68]. A small proportion of patients develop coma or bronchopneumonia before death [69].

By the end of the second week, most of the patients recovered without any complications. However, nearly one-tenth of patients develop a second phase of illness with neurological manifestations such as severe headache, drowsiness, transient disorientation, confusion, rarely convulsions and loss of consciousness which lasts for another two weeks [70] [71]. The patient is unable to straighten hamstring and ankle. In the convalescent period, occasional tremors, body weakness is seen in survivors extending up to a month. The case fatality rate is approximately 3-5% [72].

Hypotension in KFD could be of myocardial origin, whereas encephalopathy could be due to a metabolic cause probably of hepatic origin and lung signs due to intra-alveolar haemorrhage and secondary infections [73].

Pathogenesis

Transmission of KFDV to a vertebrate host probably occurs either via contact with an infected animal or a tick bite that injects the virus and saliva components into the skin site of feeding [74].

[66] Munivenkatappa and others.
[67] John.
[68] Pavri.
[69] Pattnaik.
[70] CDC.
[71] Munivenkatappa and others.
[72] CDC.
[73] M R Adhikari Prabha and others, 'Clinical Study of 100 Cases of Kyasanur Forest Disease with Clinicopathological Correlation.', *Indian Journal of Medical Sciences*, 47.5 (1993), 124–30.
[74] Shah and others.

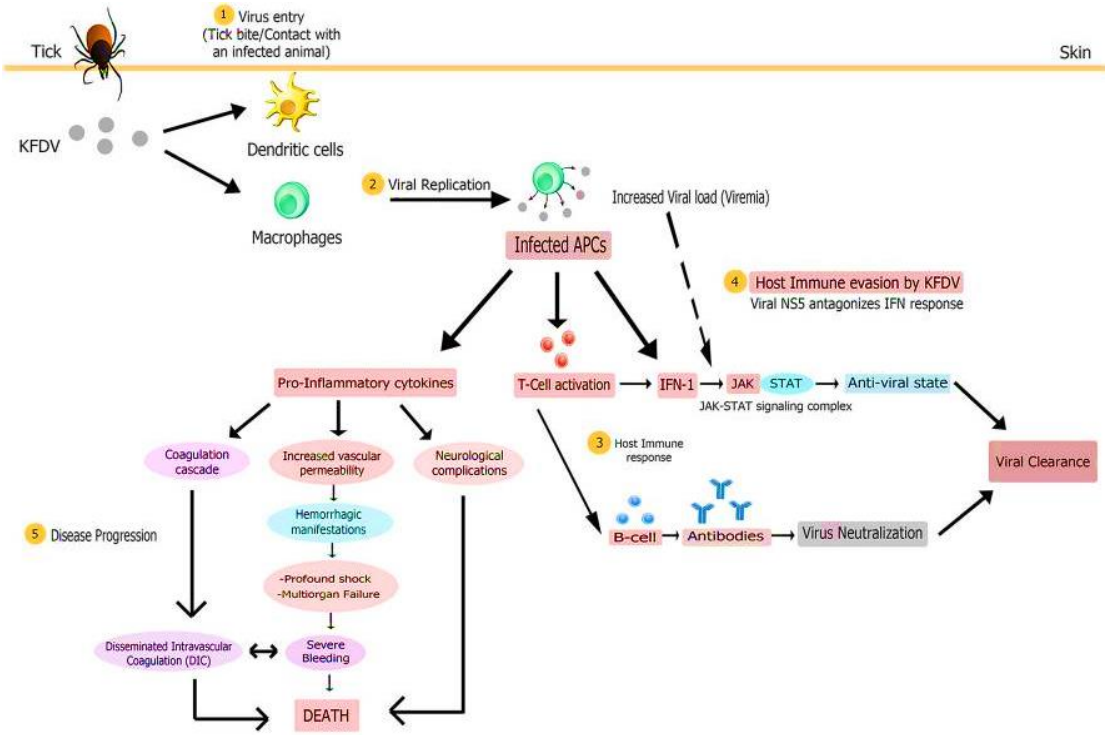

Figure 9: Proposed pathogenesis model of Kyasanur Forest Disease

Figure 9: Virus enters (1) in the body on tick bite or through contact with an infected animal. The virus initially targets macrophages and dendritic cells. Multiplication of virus (2) in these host cells yields high viremia, leading to systemic spread of the virus to spleen, liver, and other replication sites to produce disease symptoms. The infected antigen presenting cells (APCs), that present viral antigens to T cells could release large amounts of pro-inflammatory cytokines early after infection and also modulate host immune response (3) via type 1 interferon production. Antigen positive (activated) T cells could also produce IFN-1. Subsequent activation of the JAK-STAT signalling induces an antiviral state for alleviating virus burden. Humoral immune response via the production of antibodies by activated B cells might also assist in viral clearance from the body. To counter the host immune response, KFDV employs its NS5 non-structural protein to antagonise IFN response (4) by inhibiting JAK-STAT pathway, possibly bringing about uncontrolled viral replication and inadequate immune response. The multi-systemic illness might be attributed to the pro-inflammatory cytokine storm that could contribute to immunosuppression and disease progression (5) by inducing disseminated intravascular coagulation (DIC), neurological complications and vascular dysfunction that leads to hemorrhagic manifestations, multi-organ failure and shock. These complications altogether finally result in death.

Pathological findings

Macrophage and lymphocyte infiltration in liver, kidney and spleen. Liver necrosis & tubular damage in the kidney are consistent findings. Brain: Cerebral edema and inflammatory cells in brain tissue in few cases noticed.

Laboratory findings

Humans infected with KFDV have low platelets, white blood cells and red blood cells count [75]. Blood counts were on the lower side (leucopenia, eosinopenia with lymphopenia) during the first week of illness. Leukopenia is a constant feature in KFD patients and was due to a reduction in both neutrophils and lymphocytes. In most cases, the neutrophil count drops below 2000 cells/ml [76]. Lymphopenia was usually observed within the first week of illness and significant eosinopenia during the first or early in the second week. In several patients, lymphocytosis was also observed between the third and the fifth week [77].

[75] John.
[76] Pattnaik.
[77] Pavri.

The virus can easily be isolated from human sera. The level of virus in blood circulation is considerably high (3.1×106), especially during the period of 3–6 days after the onset of illness [78]. Low levels of serum albumin, slightly increased levels of y-globulin, moderately raised levels of alkaline phosphatase, slightly increased levels of bilirubin, and elevated zinc sulfate turbidity were recorded as the usual abnormal pattern. The values for blood urea nitrogen, nonprotein nitrogen, and serum chloride were always normal. A consistent finding is the presence of atypical lymphocytes in peripheral blood in most of the patients at some stage of the disease. The haematology picture becomes normal during the time of discharge [79].

Thrombocytopenia of various degrees is a frequent finding. Thromboagglutinins were detected between the third and 30th day of illness, when the peripheral platelet count ranged from 26,400 to 251,000 cells/µL (mean, 86,000 cells/µL) [80]. Studies by Sathe et al. found that levels of circulating IFN in the acute samples (GM 216.3 +/_8.7) collected between 4–7 post-onset day (POD) were significantly higher (p less than 0.001) than the convalescent samples (GM 13.19 +/_1.6) collected between 30–90 POD [81].

[78] Pattnaik.
[79] Pavri.
[80] Pavri.
[81] Pattnaik.

Chapter. 5

How was KFD vaccine developed?

1960

Formalin-inactivated RSSEV (mouse brain) vaccine was used. It was not very effective.

1965

Chick embryo based KFDV vaccine was developed and used. It failed due to poor immunogenicity.

1966

Formalin-inactivated chick embryo fibroblast cell culture based vaccine and was successful. It is manufactured at the Institute of Animal Health & Veterinary Biologicals (IAH & VB), Hebbal, Bengaluru, Department of Health & Family Welfare, Government of Karnataka, India. Currently licensed for use in KFD endemic areas for 6 to 65 years of age.

Requires multiple doses. Two doses of the vaccine are administered to individuals aged 7–65 years at an interval of one month. As the immunity conferred by the vaccination is short-lived, booster doses are recommended within 6–9 months after primary vaccination and repeated for five consecutive years after the last confirmed case in the area.

The vaccine for KFDV consists of formalin-inactivated KFDV. The vaccine has a 62.4% effectiveness rate for individuals who receive two doses. 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 the vaccine was around 62% in individuals received initial 2 doses and 83% in individuals who received further boosters [82].

[82] Gudadappa S Kasabi, Manoj V Murhekar, Vijay K Sandhya, and others, 'Coverage and Effectiveness of Kyasanur Forest Disease (KFD) Vaccine in Karnataka, South India, 2005-10.', ed. by Daniel G. Bausch, *PLoS Neglected Tropical Diseases*, 7.1 (2013), e2025.

Chapter. 6

Prevention and Control measures

Tick Vector Control

Haemaphysalis ticks are naturally capable of parasitizing both wild and domestic animals [83]. Population control of such ticks with wide host range are practically difficult compared to an one-host tick [84]. Acaricides application, biological control, reproductive host reduction or exclusion, host-targeted acaricides to tick reproductive or pathogen reservoir hosts, landscape and habitat modifications, and anti-tick vaccines are the common approaches practised globally against ticks [85]. However, control of ticks are based on chemical method (use of acaricides), while the methods such as vegetation and host management may remotely help [86]. Availability, high-cost, residual capacity in the environment and importantly resistant development among targeted tick population poses a great dis-advantage on chemical approaches [87]. Encouragingly personal protection method could be effectively used at large scale for tick-borne disease control [88]. Tick vector control strategies can be broadly divided into; a). Reducing the tick abundance and b). Personal protection measures [89].

Reducing the tick abundance

Physical control

Controlled burning of the dry leaves and bushes in the forest boundaries, premises of human habitats.

Chemical Control

Acaricide can be used in multiple ways to control tick, and a suitable method should be adopted based on the conditions and requirements.

Targeted application

Insecticides can be applied upon a targeted habitat or animal host. Indoor, peri-domestic areas, animal shelters and areas around suspected dead-animals can be applied with insecticides such as; DDT (5%), lindane (0.5%), propoxur (1%), bendiocarb (0.25–0.48%), pirimiphos methyl (1%), diazinon (0.5%), malathion (2%), carbaryl (5%), chlorpyrifos (0.5%). The residual sprays were usually applied on floors, walls, furniture and fences. Domestic animal which acts as vehicle for the tick to reach human from the wild condition. Those animals can be treated with insecticides such as; malathion (5%), dichlorvos (0.1%), carbaryl (1%), dioxathion (0.1%), naled (0.2%), coumaphos (1%) through Dipping, washing or spray-on procedures and carbaryl (5%), coumaphos (0.5%), malathion (3–5%),

[83] Geevarghese and Mishra; Frans Jongejan and Gerrit Uilenberg, 'Ticks and Control Methods', *Revue Scientifique et Technique-Office International Des Epizooties*, 13.4 (1994), 1201–42.
[84] Jongejan and Uilenberg.
[85] Jongejan and Uilenberg; Kirby C Stafford III, Scott C Williams, and Goudarz Molaei, 'Integrated Pest Management in Controlling Ticks and Tick-Associated Diseases', *Journal of Integrated Pest Management*, 8.1 (2017), 28; Jan A Rozendaal, *Vector Control: Methods for Use by Individuals and Communities* (World Health Organization, 1997).
[86] Jongejan and Uilenberg.
[87] S Ghosh, P Azhahianambi, and M P Yadav, 'Upcoming and Future Strategies of Tick Control: A Review', *Journal of Vector Borne Diseases*, 44.2 (2007), 79.
[88] Rozendaal.
[89] Stafford III, Williams, and Molaei.

trichlorphon (1%) through Insecticidal powder dusting procedure. The back, neck, belly and the back of the head in animals are the common sites for tick attachment, which all need to be concentrated while insecticide application [90].

Area spraying

During outbreak conditions, outdoor area spraying is handy. Insecticides of organophosphorus, carbamate and pyrethroids insecticide compound groups can be used for the purpose. The treatment may be lost for a month or more based on the size and condition of the area covered. Large areas can be treated with ultra-low-volume spraying using aircraft, while a compression pump or mist blower could be useful for smaller areas [91].

Personal protection

These approaches are used to protect an individual or a group from tick-bite. It interrupts the contact between infected ticks with humans. These methods could some-time used by a large number of individuals in a community to make an impact on transmission control.

Avoidance of tick habitats

Usually, a host-seeking tick quest for wandering hosts by climbing the edges of plant leaves grass blades and leaf litters. Avoidance of entering wild conditions and bushy peri-domestic areas which are potential habitats for tick activities could be a simple personal protective measure against tick-bite. This method could be useful during outbreak/epidemic situations for the control of rapid disease spread. Activities such as trekking, leaves or firewood collection, camping, gardening, hunting, sleeping on the floor of affected forest areas need to be entirely avoided during outbreak conditions [92].

Protective clothing

In case of entering forested areas, proper clothing could protect from tick exposure. Gum-boots, trousers tucked in boots, long -leeved shirts tucked in trousers are the basic protective clothing recommended. Once after every visit to the forest, the clothing should be examined for ticks and should be removed if present (Light-coloured cloths enables quick spotting of ticks). Addition clothing could be treated with pyrethroid insecticides such as 0.5% permithrin or cyfluthrin. The treatment can be made either by spraying or soaking, and they may remain effective for several weeks to months based on usage and washing frequency [93].

Tick Removal

Once returned form tick-infested areas, the whole body should be examined for tick attachment. Check especially under the arms, in and around the ears, inside the belly button, back of the knees, in and around the hair, between the legs, and around the waist. Tick attached with the skin should be removed using fine-pointed forceps or tick removal tool. The removing action should be slow and constant towards the upward direction and always remove the tick closer to the point of attachment (i.e., as close as possible from the mouth, to

[90] Rozendaal.
[91] Rozendaal.
[92] Rozendaal.
[93] Rozendaal.

avoid squeezing of the abdomen). Care should be taken not to break off the embedded mouthparts, as they may cause irritation and secondary infection [94].

Repellents

Repellents such as DEET (N, N-Diethyl-meta-toluamide), DMP (dimethyl phthalate), benzyl benzoate, dimethyl carbamate, indalone, picaridin, PMD (para-menthane-diol), and 2-undecanone, could be used effectively used against tick exposure. These repellents could be used either on skin or clothing. Repellents on clothing may be effective for a more extended period (even up to some days), than in skin (Usually between 15 min to 10 hours depending on the repellent used). In temperate condition, the effective may reduce further due to constant perspiration, and it is recommended to repeat the application frequently. Repellents should be applied sparingly to all exposed skin, especially the neck, wrists and ankles. The surroundings of the eyes or mucous membranes (nose, mouth) should not be treated. Repellents should not be sprayed on the face directly but can be applied by spraying on to the hands. Some natural repellents of aromatic plants, leaves, flowers and tree bark oil or extracts were used against tick bites, but their effectiveness is yet to be verified [95].

Future strategies for tick-control

The current methods of tick control having disadvantages of chemical resistance, residues, environmental pollution and high cost. Effective alternatives are needed to embed along with the current methods in future. Integrated Pest/Vector Management (IPM) could be a potential approach for tick control in future. The approach facilitates to target multiple vector/pest species at a time with a rational and complementing usage of multiple control methods. Anti-tick vaccines, new generation or herbal acaricides and transgenic approaches are other upcoming tick-control methods which could enhance the IPM approaches [96].

Table 8: List of options for integrated ticks and tick-borne disease management in-specific to Kyasanur Forest Disease (KFD)

Approaches	Methods
Personal protection measures	Avoid tick habitats
	Protective clothing
	Tick checks and prompt tick removal
	Synthetic chemical repellents
	Natural product-based repellents
	Insecticide-treated clothing
Treatment/vaccination humans	Screening for infection after a tick bite
	Human vaccine

[94] Rozendaal.
[95] Rozendaal.
[96] Stafford III, Williams, and Molaei; Ghosh, Azhahianambi, and Yadav.

Landscape/vegetation management	Xeroscaping/hardscaping
	Remove leaf litter and brush, mow the grass
	Remove rodent harborage
Target host-seeking ticks	Synthetic chemical acaricides
	Botanically-based acaricides
	Biological agents and biopesticides (entomopathogenic fungi, nematodes, and other pathogens)
	Acaricides with semiochemicals as lures or decoys
Rodent-targeted approaches	Topical acaricide bait boxes
	Oral tick growth regulator
Monkey-targeted approaches	Use of insecticides in about a radius of 50 m circling the dead monkey
	Disposal of the infected dead monkey
	Topical acaricide self-treatment bait stations
	Systemic acaricides
	Oral tick growth regulator
	Anti-tick vaccine

Disposal of monkey carcasses

Proper disposal of monkey carcasses is very crucial. Considering the gravity of infection, the incineration method is generally preferred above other methods to prevent further transmission from the source as this method eliminates the pathogen and the attached ticks surrounding the carcasses. Based on resource availability, cost, local environment, and social norms, the disposal method has to be chosen. It should be done in the presence of technical veterinarian along with forest officials. While selecting disposal site care should be taken on the nearby water channel, human habitation, and contagious nature of the disease. Open air burning and fixed incinerator facility should be done as per the local requirement [97] [98].

KFD Surveillance

NCDC recommends three forms of surveillance for Kyasanur Forest Disease. They are as follows:

[97] Government of India NDMA, *National Disaster Management Guidelines Management of the Dead in the Aftermath of Disasters*, 2008.
[98] OIE, *Chapter 4.13, Disposal of Dead Animals, OIE - Terrestrial Animal Health Code*, 2019.

Human surveillance

Early detection of patients, prompt laboratory diagnosis and proper management of patients are very essential. Passive routine surveillance and routine review of the surveillance data to be done under IDSP to detect impending outbreaks of KFD. Event-based surveillance of unusual suspected KFD cases/deaths to be done in the control and containment.

Monkey surveillance

The surveillance for the death of monkey/ monkeys in non-endemic as well as endemic areas of KFD to be carried out regularly in real time manner in collaboration with Forest and Veterinary Department. Human cases can be suspected in case of unusual monkey death.

Tick surveillance

Tick surveillance and tick mapping for identifying hotspots and tick incrimination studies in KFD prone areas for monitoring tick positivity for KFD to be carried out regularly on a periodic basis.

Tick surveillance

Along with human case surveillance, vector surveillance facilitates the control of vector-borne diseases [99]. Tick surveillance activities will be supportive in the early detection of potential (High-risk) areas for human KFD outbreak [100]. Arthropods are typically collected, sent to an appropriate laboratory alive, or preserved in ethanol (70%), and assayed for identification and infection. For surveillance purposes, ticks are trapped, identified, sorted by life-stage, sex, physiological type, counted and stored for later assays [101]. Tick surveillance may be implemented either in the active or passive approach [102].

Active tick surveillance:

Active tick surveillance involves effective monitoring of prevalence, distribution, and infection rate among the vector ticks in a targeted geographical area [103]. Different methods are demonstrated successfully on tick surveillance, and the efficiency of each method might vary based on the tick species, developmental stage, and host-seeking behavior [104].

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[99] Salima Gasmi, Nicholas H Ogden, Patrick A Leighton, Ariane Adam-Poupart, and others, 'Practices of Lyme Disease Diagnosis and Treatment by General Practitioners in Quebec, 2008–2015', *BMC Family Practice*, 18.1 (2017), 65; *Contingency Pest and Vector Surviellance, Armed Forces Pest Management Board Technical Guide No. 48*, Guide to O (Mary Land: Information Services Division (ISD), Armed Forces Pest Management Board (AFPMB), 2013), XLVIII; Lee W Cohnstaedt and others, 'Arthropod Surveillance Programs: Basic Components, Strategies, and Analysis', *Annals of the Entomological Society of America*, 105.2 (2012), 135–49.

[100] Pattnaik; Sadanandane, Gokhale, and others; Naren Babu and others.

[101] *Contingency Pest and Vector Surviellance, Armed Forces Pest Management Board Technical Guide No. 48*, XLVIII.

[102] *Contingency Pest and Vector Surviellance, Armed Forces Pest Management Board Technical Guide No. 48*, XLVIII; Cohnstaedt and others; Marion Ripoche and others, 'Passive Tick Surveillance Provides an Accurate Early Signal of Emerging Lyme Disease Risk and Human Cases in Southern Canada', *J Med Entomol*, 55.4 (2018), 1016–26; Nicholas H Ogden and others, 'Active and Passive Surveillance and Phylogenetic Analysis of Borrelia Burgdorferi Elucidate the Process of Lyme Disease Risk Emergence in Canada', *Environmental Health Perspectives*, 118.7 (2010), 909–14.

[103] *Contingency Pest and Vector Surviellance, Armed Forces Pest Management Board Technical Guide No. 48*, XLVIII; Cohnstaedt and others; Ripoche and others.

[104] Călin M Gherman and others, 'CO 2 Flagging-An Improved Method for the Collection of Questing Ticks', *Parasit Vectors*, 5.1 (2012), 125.

Methods used in active tick surveillance approach are as follows

1. Dragging, flagging, and dry ice-baited traps are the few collection methods, which targets hosts seeking tick population.
2. Collection of tick parasitizing live host.
3. Leaf litter sampling method.

Dragging and flagging methods

Dragging and flagging methods involve in sliding a cloth upon tick questing areas, which mimics host exposure to tick-bite in the natural environment [105]. Tick drags are performed with a rectangular sized white flannel cloth of standard measurement (Usually 1.5 X 1 m), attached with a solid rod (1.1 m) along one side of the cloth and a rope of 4 m length tied connecting both ends of the rod [106]. Tick flags are also needed to be performed with a rectangular sized white flannel cloth of standard measurement (Usually 1m X 0.75 m), while a long solid rod (Usually 1.5 m) attached with the cloth at one side [107]. The drag method can be effective in plains, while flags are useful in the vegetation of different heights [108]. Bushes, dry leaves, grasslands, animal trails, forest fringes, and areas with animal-human-tick interaction should be targeted during flagging or dragging procedure [109]. The tick-abundance is expressed as the number of ticks collected per man-hour, while tick-density is expressed as the number of ticks collected per area covered (Usually per 1000 m2 area) [110].

Dry ice baited method

Dry ice baited method of tick collection involves in attract-catch of the host-seeking tick with the help of slow release CO2 evaporation from dry ice [111]. The CO2 mimics the excretion of host respiration, which is an olfactory-cue for ticks to seek a host for feeding [112]. The ticks approaching towards the CO2 bait can be collected using a white spread-sheet or a pitfall trap or a sticky trap [113]. The tick-abundance is expressed as the average number of ticks attracted towards a bait per hour (or day) per area covered (Usually per 1000 m2 area).

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[105] *Contingency Pest and Vector Surviellance, Armed Forces Pest Management Board Technical Guide No. 48*, XLVIII.

[106] *Contingency Pest and Vector Surviellance, Armed Forces Pest Management Board Technical Guide No. 48*, XLVIII; Cohnstaedt and others; RC Falco and D Fish, 'A Comparison of Methods for Sampling the Deer Tick, Ixodes Dammini, in a Lyme Disease Endemic Area', *Experimental and Applied Acarology*, 14.2 (1992), 165–73; Howard S Ginsberg and Curtis P Ewing, 'Comparison of Flagging, Walking, Trapping, and Collecting from Hosts as Sampling Methods for Northern Deer Ticks, Ixodes Dammini, and Lone-Star Ticks, Amblyomma Americanum (Acari: Ixodidae)', *Exp Appl Acarol*, 7.4 (1989), 313–22.

[107] *Contingency Pest and Vector Surviellance, Armed Forces Pest Management Board Technical Guide No. 48*, XLVIII; Cohnstaedt and others; Falco and Fish; Ginsberg and Ewing.

[108] Ginsberg and Ewing; Cohnstaedt and others.

[109] Naren Babu and others; Sadanandane, Gokhale, and others.

[110] *Contingency Pest and Vector Surviellance, Armed Forces Pest Management Board Technical Guide No. 48*, XLVIII; Cohnstaedt and others; Falco and Fish; Ginsberg and Ewing.

[111] *Contingency Pest and Vector Surviellance, Armed Forces Pest Management Board Technical Guide No. 48*, XLVIII; Cohnstaedt and others; Gherman and others; Falco and Fish.

[112] Trevor N Petney and others, 'A Look at the World of Ticks', in *Progress in Parasitology* (Springer, 2011), pp. 283–96.

[113] *Contingency Pest and Vector Surviellance, Armed Forces Pest Management Board Technical Guide No. 48*, XLVIII; Falco and Fish.

Collection of tick parasitising live host

Collection of tick parasitising live host is commonly performed on wild animal, rodent and bird population using capture and screen technique. The method can also be performed on domestic animals on a need basis. This method of tick surveillance provide us with basic information on host, pathogen, and tick relationships. Fine needle forceps or a special tick removal tool can be used for tick removal from the host. Muzzle, head, pinna, neck, front leg, hind leg, sternum, abdomen, tail and rest of the body are the common sites need to be checked for tick infestation. Sometimes, the combing method can be adapted for effective tick collection in animals especially on the rodent population. Other option for a small animal can be, after the animals have been trapped, they are transferred to holding cages over water. Ticks detaching from the animals are collected from the water each morning and evening [114].

Leaf litter sampling method

Leaf litter sampling method involves the collection of leaf litter in the suspected tick habitats, followed by processing leaf litter for tick presence. Leaf litter are either assessed visually for ticks or placed in a Berlese-Tullgren funnel below an incandescent light. The arthropods move away from the heat and get trapped in a collection vial containing 70 % ethanol below the funnel [115].

Passive surveillance

Passive tick surveillance involves the voluntary submission of ticks found on humans or pets via participating medical and veterinary clinics, providing a signal of the presence of ticks in the environment [116]. Ticks found on humans and submitted through medical clinics also provide a direct measure of human exposure to ticks and to the pathogens they carry [117].

[114] *Contingency Pest and Vector Surviellance, Armed Forces Pest Management Board Technical Guide No. 48*, XLVIII; Cohnstaedt and others; Falco and Fish.

[115] *Contingency Pest and Vector Surviellance, Armed Forces Pest Management Board Technical Guide No. 48*, XLVIII; Cohnstaedt and others; Falco and Fish.

[116] Jules K Koffi and others, 'Passive Surveillance for I. Scapularis Ticks: Enhanced Analysis for Early Detection of Emerging Lyme Disease Risk', *J Med Entomol*, 49.2 (2012), 400–409.

[117] Salima Gasmi, Nicholas H Ogden, Patrick A Leighton, L Robbin Lindsay, and others, 'Analysis of the Human Population Bitten by Ixodes Scapularis Ticks in Quebec, Canada: Increasing Risk of Lyme Disease', *Ticks Tick Borne Dis*, 7.6 (2016), 1075–81.

Chapter. 7

Outbreak detection and Management

Case definition(s) for KFD

Presumptive case

A patient of any age presenting with acute onset of high grade fever with any of the following: Headache/ Myalgia/ Prostration/ Extreme weakness/ Nausea/ Vomiting/ Diarrhea/ Occasionally neurological/ haemorrhagic manifestations.
AND/ OR

- Rule out common etiologies of acute febrile illness prevalent in the area (Dengue/DHF, typhoid, malaria etc.,)
- History of exposure to tick bite
- Travel and/ or Living in and around forest area where laboratory confirmed KFD cases have been reported previously or an area where recent monkey deaths have been reported*

Confirmed case

A presumptive case, which is laboratory confirmed by any one of the following assays:

- Detection of KFDV-specific viral RNA by reverse transcription polymerase chain reaction (RT-PCR) or real-time RT-PCR from blood or tissues.
- Isolation of KFDV in cell culture or in a mouse model, from blood or tissues.
- Positive for immunoglobulin M (IgM) enzyme-linked immunosorbent assay (ELISA) for KFD. (Considered Lab Confirmed for Operational Purposes)

Note: Suggestive case definitions are provided for the reference. However, local public health experts may be consulted.

As per State Government of Karnataka policy, a area in a radius of 5 km from where recent monkey deaths have been reported, is considered as potential exposure zone. Local authorities may decide the operational zone as per their own requirements.

Treatment

There is no specific treatment for KFD, but early hospitalization and supportive therapy is important. Supportive therapy includes the maintenance of hydration and the usual precautions for patients with bleeding disorders. Unwanted referral of KFD patients to higher centres can prevent mortality.

Various stakeholders in KFD prevention and management

KFD has multidimensional risk factors for its transmission and sustenance. Looking at various aspects of KFD epidemiology, inter-sectoral coordination is vital to implement various preventive & control measures effectively. Health department, Veterinary Public Health Department, Forest and Wildlife departments, Vector control division, District administration, Tribal welfare, Fire control departments, and many more are the key stakeholders in its control. Each of the stakeholders has to be clear about their roles and responsibilities. Meticulous division of labour amongst all these departments is essential to have more coordinated efforts. State & district authority should chalk out responsibilities of various departments.

Chapter. 8

Laboratory techniques

Currently, the methods of laboratory diagnosis of KFDV include real-time RT-PCR assay, nested RT-PCR assay, anti-KFD IgM, and anti-KFD IgG ELISA. None of the diagnostic assays are currently available commercially [118].

Molecular diagnosis

RT–PCR and real time PCR provides a very rapid and accurate diagnosis and this is the first line of tests for the diagnosis of KFD (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. Viremia period in humans prolonged up to 12 days after post onset of symptoms, viremia levels peaks during the period of 3-6 days after the onset of illness [119] [120]. Viremia in human was comparable with that of in monkey experimentally [121]. Current KFD vaccine is not completely protect against KFDV infection, among vaccinated individuals virema period is shorter compared to unvaccinated cases. *(MIV unpublished data).* The present real time assay is very sensitive and nearly as sensitive as detecting up to 10 copies of viral RNA [122].

Serological diagnosis

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).

By HI test and neutralization test, KFDV antibodies were demonstrated in man and animals 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 (Padbidri et al., 2002).

KFD IgM antibodies was determined by enzyme-linked immunosorbent assay (ELISA). KFD IgM antibody can be detected from 5th day of onset of symptoms till 3 months. Currently in house KFDV IgM and IgG test available in National institute of virology, Pune, and Center for Disease Control and Prevention, USA.

Sequencing

Another advantage of the RT-PCR assay in comparison to realtime RT-PCR assay is that the amplicon obtained after RT-PCR amplification can be used

[118] Devendra T Mourya and others, 'Diagnosis of Kyasanur Forest Disease by Nested RT-PCR, Real-Time RT-PCR and IgM Capture ELISA.', *Journal of Virological Methods*, 186.1–2 (2012), 49–54.
[119] 'CD ALERT, Kyasanur Forest Disease a Public Health Concern', *National Centre for Disease Control, Directorate General of Health Services, Delhi*, 2018.
[120] S Upadhyaya, D P Narasimha Murthy, and B K Yashodhara Murthy, 'Viraemia Studies on the Kyasanur Forest Disease Human Cases of 1966.', *The Indian Journal of Medical Research*, 63.7 (1975), 950–53.
[121] H E WEBB and J B CHATERJEA, 'Clinico-Pathological Observations on Monkeys Infected with Kyasanur Forest Disease Virus, with Special Reference to the Haemopoietic System.', *British Journal of Haematology*, 8 (1962), 401–13.
[122] Mourya and others.

for sequencing and phylogenetic analysis for conclusive confirmation of positivity of the clinical sample.

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 (Mourya et al., 2014). Virus isolation from KFDV positive samples should be carried out in BSL-3 laboratory.

KFD serology (Mice-inoculation techniques to RT- PCR)

Pre 2010

Suckling mice intra-cerebral inoculation was used for diagnostics before 2010.

Post 2010

Molecular diagnosis such as PCR and RT-PCR techniques became available for KFD diagnostics.

Limitations of lab diagnosis

PCR positivity is limited to 10 days.
During the second phase of illness, rarely PCR will be positive.

Sample collection and transportation

Collection of serum from suspected patients

Collect 4-5 ml blood in a plain vial. Separate the serum following standard biosafety precautions. Paired sera sample can be used for serological examination [123].

Collection of Monkey viscera

Collect Brain, Lungs, Heart, Liver and Kidney specimens from the dead monkey following standard biosafety precautions [124].

Tick collection

Collect nymph tick and keep in a sterilised polypropylene container. The tubes should be airtight and sealed in plastic bags so that vial should not open during transportation and infected ticks spread in newer areas [125].

Sample Storage

Keep serum of human cases/ viscera of monkeys/ tick samples refrigerated (2 - 8°C) if it is to be processed (or sent to a reference laboratory) within 48 hours. Keep frozen (-20°C to -10°C), if it is to be processed after a week. The sample can be preserved for extended periods [126].

[123] 'CD ALERT, Kyasanur Forest Disease a Public Health Concern'.
[124] 'CD ALERT, Kyasanur Forest Disease a Public Health Concern'.
[125] 'CD ALERT, Kyasanur Forest Disease a Public Health Concern'.
[126] 'CD ALERT, Kyasanur Forest Disease a Public Health Concern'.

Transportation of the sample to the reference laboratory

Always use a triple-layer packaging and ship within 48 hours of collection under cold chain (dry ice or at least with cooling gels). The original samples should be packed, labelled, and marked. Always include the completely filled out clinical and epidemiological record [127].

Designated laboratory for KFDV diagnosis

The designated laboratory for diagnosis and isolation of KFDV in humans, monkey necropsy samples, and ticks sample [128].

(1) National Institute of Virology (NIV)
Microbial Containment Complex
130/1 Sus Road. Pashan, India,
Pune-411021.
Tel.No: 91-020-26006390
Fax No.: 91-020-25871895

Other designated laboratories for diagnosis of KFDV in human samples are as follows:

(1) Virus Diagnostic Laboratory (VDL)
Opp. Scout Bhawan, B H Road,
Shimoga, Karnataka, India.
Tel: +91-0812-222050
Email ddvdlsmg@gmail.com.

(2) Manipal Institute of Virology (MIV)
Manipal Academy of Higher Education (Deemed to be University),
Madhav Nagar, Manipal - 576 104, Karnataka State, India.
Tel: +91 820 2922663
Fax: +91 820 2922718
Email virology@manipal.edu.

The samples for diagnosis of the disease in suspected human cases can be sent to the above mentioned designated laboratories.

Biosafety

In the U.K and USA, KFD virus is a Class 4 pathogen. However, for sensitive single step RT-PCR assay for the detection of KFD viral RNA, this can be easily used in any BSL-2 laboratory for the screening of KFD suspected cases. In the absence of Biosafety Level 4 facility in smaller laboratories, detection of KFD viral RNA can be performed after inactivating the patient/monkey/ticks sample with phenol, or its variants like TRIzol. Virus isolation and conventional serological techniques such as neutralization assay, haemagglutination can generate aerosol and therefore should be performed in more secure laboratories.

[127] 'CD ALERT, Kyasanur Forest Disease a Public Health Concern'.
[128] 'CD ALERT, Kyasanur Forest Disease a Public Health Concern'.

Chapter. 9

Redrawing the boundaries of Kyasanur forest disease in India

AFI surveillance:

Manipal Centre for Virus Research (MCVR) (currently Manipal Institute of Virology), Manipal Academy of Higher Education, established 33 sentinel sites across 10 Indian states as part of its Acute Febrile Illness (AFI) surveillance project under the Global Health Security Agenda (GHSA). The project provides real-time laboratory-based disease statistics to the national disease surveillance programmes in the country on a weekly basis. AFI sentinel surveillance sites function in collaboration with the government health care facilities i.e. Primary health centers (PHC), community health centers (CHC), taluk hospitals, sub-district hospital, and district hospitals. Samples are collected by trained laboratory technicians from inpatients suffering from acute febrile illness. A case of AFI is defined as a sick case older than 1 year and younger than 65 years of age admitted to one of the participating hospitals with reported fever of ≤ 14 days and/or documented fever ≥38°C upon admission. The project "Hospital-based surveillance of Acute Febrile Illness (AFI) in India" conducted by Manipal Institute of Virology detected KFDV from new geographic location which was not known previously [129].

Table 9: State-wise distribution of KFD detected through AFI surveillance (2014-18)

Variables	Karnataka (n=245) N (%)	Kerala (n=52) N (%)	Tamil Nadu (n=31) N (%)	Goa (n=400) N (%)	Maharashtra (n=137) N (%)	Total Cases (n=865) N (%)
Age Group						
1 to 4	1 (0.4)	0 (0)	0 (0)	2 (0.5)	0 (0)	3 (0.3)
5 to 9	11 (4.5)	1 (1.9)	0 (0)	2 (0.5)	0 (0)	14 (1.6)
10 to 14	4 (1.6)	4 (7.7)	0 (0)	9 (2.3)	3 (2.2)	20 (2.3)
15 to 24	33 (13.5)	4 (7.7)	0 (0)	39 (9.8)	17 (12.4)	93 (10.8)
25 to 34	28 (11.4)	11 (21.2)	4 (12.9)	66 (16.5)	19 (13.9)	**128 (14.8)**
35 to 44	62 (25.3)	16 (30.8)	7 (22.6)	106 (26.5)	38 (27.7)	**229 (26.5)***
45 to 54	67 (27.3)	12 (23.1)	11 (35.5)	95 (23.8)	37 (27)	**222 (25.7)**
55 to 65	39 (15.9)	4 (7.7)	9 (29)	81 (20.3)	23 (16.8)	156 (18)
Gender						
Male	131 (53.5)	15 (28.8)	9 (29)	186 (46.5)	54 (39.4)	395 (45.7)
Female	114 (46.5)	37 (71.2)	22 (71)	214 (53.5)	83 (60.6)	**470 (54.3)***
(% of Pregnancy)	2 (1.6)	1 (2.4)	0 (0)	2 (1)	0 (0)	5 (1.1)
Season (July to June)						
2014-15	63 (25.7)	39 (75)	0 (0)	0 (0)	0 (0)	100 (11.6)
2015-16	23 (9.4)	7 (13.5)	0 (0)	216 (54)	10 (7.3)	**256 (29.7)**
2016-17	62 (25.3)	0 (0)	15 (48.4)	97 (24.3)	70 (51.1)	244 (28.3)
2017-18	15 (6.1)	0 (0)	13 (41.9)	61 (15.3)	32 (23.4)	121 (14)
2018-19	82 (33.5)	6 (11.5)	3 (9.7)	26 (6.5)	25 (18.2)	142 (16.5)
Occupation						
Agriculturist/Farmer	145 (59.2)	32 (61.5)	13 (41.9)	115 (28.8)	56 (40.9)	**361 (41.7)***
House-wife	20 (8.2)	5 (9.6)	3 (9.7)	138 (34.5)	56 (40.9)	222 (25.7)
Others	9 (3.7)	6 (11.5)	6 (19.4)	57 (14.3)	1 (0.7)	79 (9.1)
Skilled labourer	4 (1.6)	0 (0)	2 (6.5)	24 (6)	6 (4.4)	36 (4.2)
Student	28 (11.4)	6 (11.5)	0 (0)	23 (5.8)	10 (7.3)	67 (7.7)
Unemployed	2 (0.8)	2 (3.8)	1 (3.2)	38 (9.5)	8 (5.8)	51 (5.9)
Unskilled labourer	37 (15.1)	1 (1.9)	6 (19.4)	5 (1.3)	0 (0)	49 (5.7)
Socio-Economic Status						

[129] Arunkumar, G et al.

(Dec 2015 Onwards)						
Low	54 (30)	10 (76.9)	30 (96.8)	261 (66.8)	66 (48.2)	**421 (56)***
Middle	126 (70)	3 (23.1)	1 (3.2)	129 (33)	71 (51.8)	330 (43.9)
High	0 (0)	0 (0)	0 (0)	1 (0.3)	0 (0)	1 (0.1)
Deaths	8	0	0	1	1	**10***
Patient reffered to Higher Centre	43/210 (20.5)	10/36 (27.8)	3/31 (9.7)	54/317 (17)	44/123 (35.8)	**154/717 (21.5)**

*category with high frequency

Table 9. shows the state-wise distribution of KFD detected through AFI surveillance (2014-18). Majority (54.3%) of KFD cases were females, 41.7% of the cases were agricultural laborers, 56% belong to low socio-economic status.10 deaths were reported during this period. The median age of male patients was 42 (IQR: 30-50) years. The median age of female patients was 40 (IQR: 33-50) years. The mean duration of hospitalization of KFD cases were 4.5 ± 2 days.

Apart from the AFI surveillance, Manipal Institute of Virology also does routine surveillance for KFD as MIV it is part of the ICMR's VRDLN. Diagnostic work up is done for samples received from various district health departments and private hospitals.

Table 10: State-wise distribution of KFD positives through routine surveillance

State	Period of Sample Collection	No. of KFD positives
Goa	Jan-16 to Apr-19	178
Karnataka	Jan-16 to Apr-19	269
Kerala	May-13 to Dec-16	10
Maharashtra	Dec-15 to Apr-19	324
Tamil Nadu	Feb-17 to May-17	4
Total		**785**

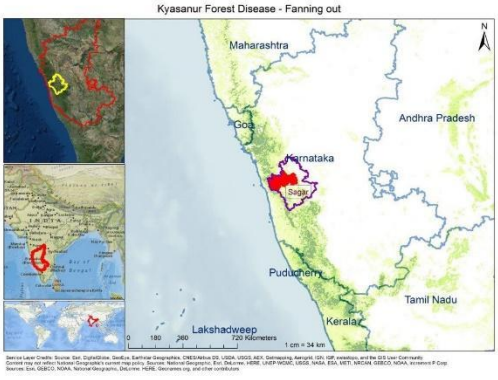
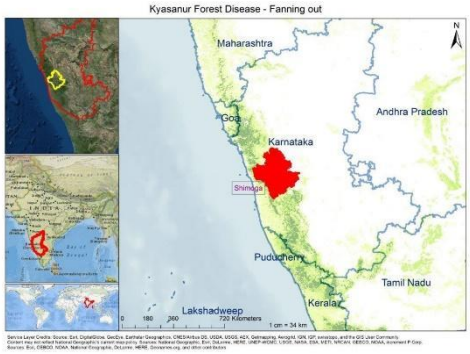
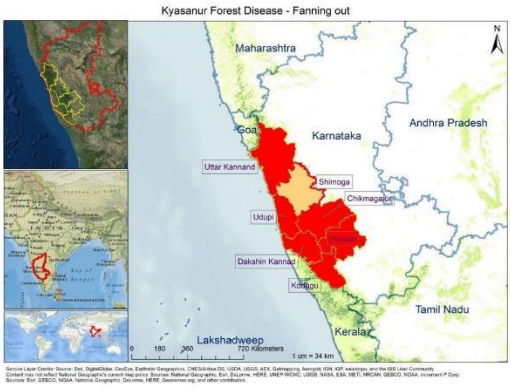
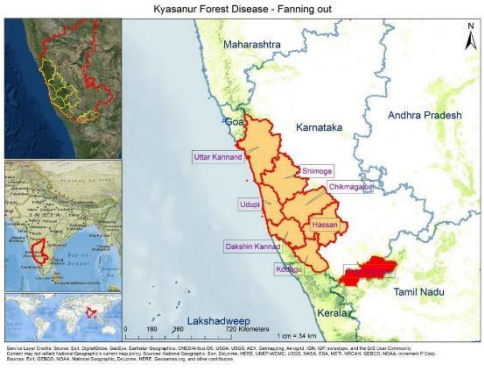
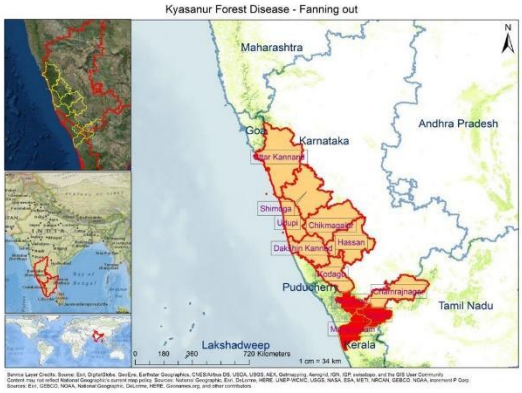
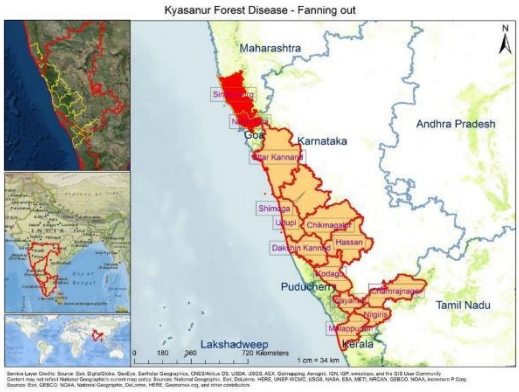
1957	1958
	
1961	**2012**
	
2013	**2016**
	

Figure 10: Geographic distribution of Kyasanur Forest Disease (1957- 2016)

Chapter. 10

Other Animals and Birds as reservoir

Animal Models

To study the pathogenesis of KFD, a number of animals such as squirrels, porcupines, shrews, rats, bonnet macaques, and mice have been experimentally infected with KFDV among which disease symptoms were observed in rodents, squirrels and bonnet macaque, and pathological studies were only carried out in bonnet macaques and mice. Among different species of bats very low level of viremia or not detected at all [130].

Table 11: Host species found to be susceptible to KFDV or to carry KFDV specific neutralizing antibodies

Species: Scientific name (commonly known name)	Remarks
Rattus blanfordi (whitetailed rat)	Experimental transmission Neutralization antibody positive
Suncus murinus (shrew)	Experimental transmission Neutralization antibody positive
Funanbulus tristriatus tristriatus (jungle stripped squirrel)	Experimental transmission Neutralization antibody positive
Rattus rattus wroughtoni (field rat)	Neutralization antibody positive, Virus isolation
Rattus rattus rufescens	Neutralization antibody positive
Golunda ellioti	Neutralization antibody positive
Mus booduga (field mouse)	Neutralization antibody positive
Vandeleuria oleracea	Experimental infection, Virus isolation
Funanbulus tristriatus numarius	Neutralization antibody positive
Funanbulus pennanti (northern palm squirrel)	Neutralization antibody positive
Tetera indica (Indian gerbil)	Neutralization antibody positive
Petaurista petaurista philippensis (giant flying squirrel)	Experimental infection
Rousettus leschenaultia (frugivorous bat)	Neutralization antibody positive
Eonycteris spelaea (frugivorous bat)	Neutralization antibody positive
Cynopterus sphinx (frugivorous bat)	Experimental infection, Neutralization antibody positive
Rhinolophus rouxi (insectivorous bat)	Neutralization antibody positive
Hipposideros lankadiva (insectivorous bat)	Neutralization antibody positive
Hipposideros speoris (insectivorous bat)	Neutralization antibody positive
Miniopterus schreibersi (insectivorous bat)	Neutralization antibody positive

[130] Pattnaik.

Mus platythrix	Experimental infection, Neutralization antibody positive
Lepus nigricollis (black-naped hare)	Experimental transmission
Tephrodornis virgatus	HI antibody positive.
Megalaima zeylanica	HI antibody positive.
Chalcophaps indica	HI antibody positive.
Treron pompadora	HI antibody positive.
Rhoppocichla atriceps	HI antibody positive.

Clinically monkey reported to develop anemia, hypotension, thrombocytopenia, diarrhea, leukopenia and encephalitis. Haematological changes include abnormally low lymphocytes level and anaemia. Histopathological changes is noticed in GI tract and lymphoid organs. Alterations in the fatty deposition in the liver, resulting in depletion of lymphocytes along with occasional necrosis of lymphoid organs, as well as the loss of GI tract architecture without any evidence of neurologic involvement. Among various KFDV-infected macaques, peripheral and visceral lymph nodes, spleens, and all mucosal lymphoid tissues showed moderate to severe follicular involution in addition to a variable degree of depletion of lymphocytes within the T-cell- dependent zones. There was a mucosal erosion leading to a reduced surface area of the luminal epithelium in the stomach and large intestine in addition to villus blunting and ultimately fusion in the small intestine [131].

In case of mice, no gross lesions seen in major organs. Encephalitis and interstitial pneumonitis are significant changes in mice pathogenicity. Histopathological finding in the brain showed vascular necrosis with lymphocyte infiltration in vessel walls. Spongiform lesions in vessel walls and vascular lesions progressed along with sickness with the onset of paralysis. Necrosis of neurons followed by the complete disappearance of neuron cells. Haemorrhages seen in lungs alveoli. The liver of mice does not show any inclusion such as observed in monkeys [132].

Current studies (Unpublished) have reported that severe dehydration resulting in polydipsia has been observed among sick monkeys which make them move towards the nearby water source.

[131] Shah and others.

[132] M Nayar, 'Histological Changes in Mice Infected with Kyasanur Forest Disease Virus.', *The Indian Journal of Medical Research*, 60.10 (1972), 1421–26.

Chapter. 11

KFD immunology

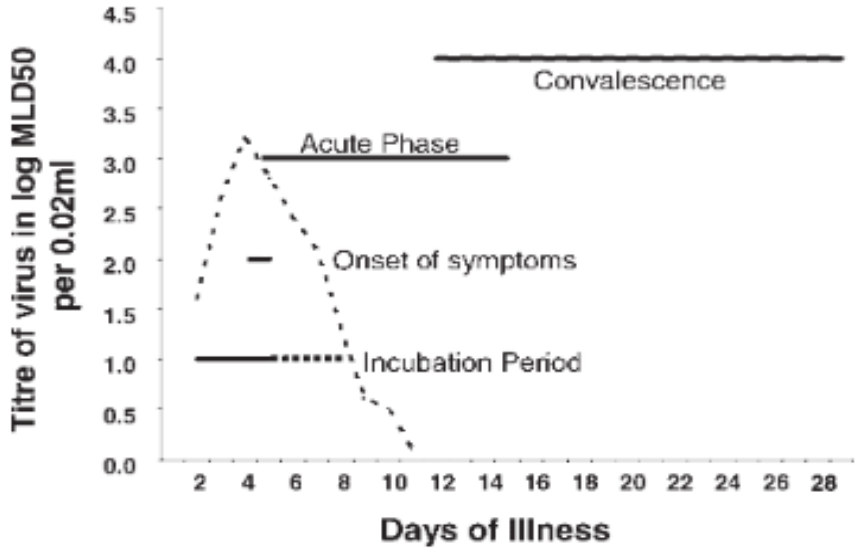


Figure 11: Viremia (.....) during the clinical course of KFD

KFD virology

Structure of KFDV

Single stranded positive sense RNA genome of 10,774 nucleotides. Icosahedral nucleocapsid surrounded by lipid bi-layer with two surface proteins.

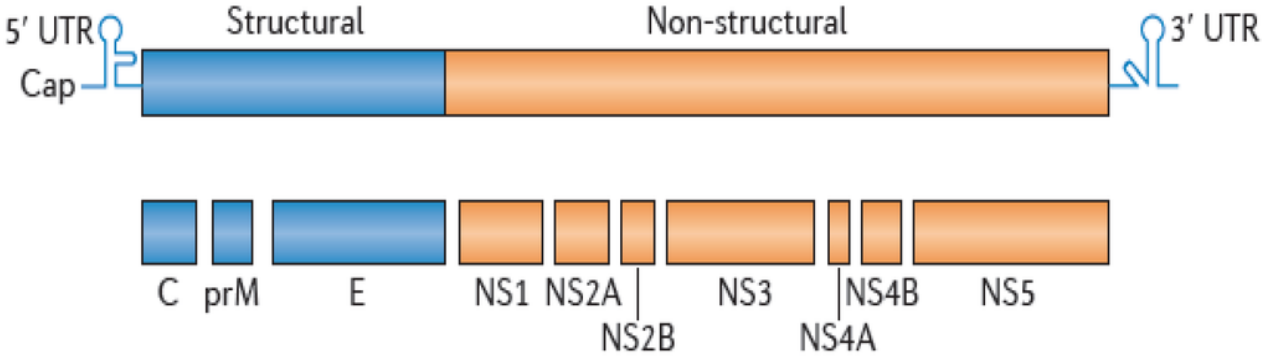


Figure 12: Structure of KFDV (Knipe and Howley, 2013)

Figure 13: Phylogenetic position of KFDV

Genetic diversity

A monophyletic lineage of the Flaviviruses are divided into three main groups: the tick-borne flaviviruses group (TBFV), the mosquito-borne flaviviruses (MBFV) and the No Known Vector (NKV) flavivirus group. The groups were further subdivided based on the phylogenetic analysis that generally correlates with the vector responsible for transmission, the host reservoir and the disease association [133]. The twelve recognized species of TBFV are divided into two groups, the mammalian tick-borne virus group (M-TBFV) and the seabird tick-borne virus group (S-TBFV) [134] [135].

The evolutionary characteristics displayed by TBFV has important consequences for their antigenic relationships, genetic diversity and geographical distribution which are largely

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determined by their modes of transmission [136] [137] [138]. The Louping ill virus (LIV), Tick borne encephalitis virus (TBEV), Omsk hemorrhagic fever virus (OHFV), Langat virus (LGTV), Kyasanur Forest disease virus (KFDV) and Powassan virus (POWV) are the six human and animal pathogens of the mammalian tick-borne flavivirus group. OHFV and KFDV species are the exceptions among all encephalitic viruses of M-TBFV that cause haemorrhagic fever in humans and have been assigned to biosafety class 4. Alkhurma hemorrhagic fever virus (AHFV), has been recommended for inclusion as a subtype of KFDV [139]. It appeared unexpectedly in Saudi Arabia in 1992 and found as closely related haemorrhagic virus. The comparison of the complete genome of a KFDV isolates from India with that of an isolates from Saudi Arabia has reported a diversity of 8% [140].

The genome of KFDV is a linear, non-segmented, positive-sense strand of RNA of approximately 11,000 bases. The single open reading frame genome encodes a single 3416 amino acid polyprotein which constitutes of three structural (capsid, membrane, and envelope) and seven non-structural (NS1, NS2A, NS2B, NS3, NS4A, NS4B, and NS5) genes flanked by untranslated regions (UTR) at both 5' and 3' ends [141] [142].

The envelope glycoprotein of KFDV shares 80% amino acid sequence homology with that of TBEV. Moreover, around 38–40% sequence homology with dengue virus, Japanese encephalitis, West Nile and yellow fever viruses with positionally conserved cysteines. Whereas the partial complementary DNA sequence of the NS5 region of KFDV, which acts as an RNA dependent RNA polymerase, shares 99% sequence similarity with that of Alkhurma virus, followed by a homology of 94% with TBEV; 93% with OHF, Langat, RSSE, Negishi viruses; 88% with Meaban virus; 87% with Kadam, Saumarez Reef, Tyuleniy viruses; 81% with Koutango and Alfuy viruses; 80% with Japanese encephalitis, West Nile, Apoi viruses and 76–78% with dengue and other related viruses [143] [144] [145]. Due to lack of larger amount of data, the divergence analysis spanning a longer time period has its limits.

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Chapter. 12

Alkhurma hemorrhagic fever (AHF)

Alkhurma hemorrhagic fever (AHF) is caused by a zoonotic virus, Alkhurma hemorrhagic fever virus (AHFV), a tick-borne virus of the *Flavivirus* family. AHFV is a variant of Kyasanur Forest Disease virus (KFDV) and was initially isolated from Saudi Arabia in 1995 [146]. Subsequent AHF cases have been documented among the tourists in Egypt [147]. The persistence of AHF virus within tick populations and the role of livestock in the transmission are not well understood. AHF cases peaks during the spring and summer months and several hundred cases have been reported so far. Transmission of AHFV is not very clear. Host ticks responsible for AHF are *Ornithodoros savignyi* (soft tick) and the *Hyalomma dromedari* (hard ticks). Transmission happened through the bite of an infected tick bite or while crushing infected ticks. No human-to-human transmission or transmission through non-pasteurized milk has been documented. Epidemiologic studies show contact with livestock may increase the risk of AHF infection, however, livestock play a minor role in transmitting AHFV to humans. Contact with livestock, slaughtering of animals, with tick exposure are risk factors for humans and it is possible that infected animals can develop viremia without obvious clinical signs. Clinical diagnosis is difficult due to similarities between AVHF, Crimean-Congo Hemorrhagic fever (CCHF), and Rift Valley fever (RVF), which occur in similar geographic areas. Laboratory diagnosis of AHF can be made in the early stage of the illness by molecular detection by PCR or virus isolation from the blood. Later, serologic testing using enzyme-linked immunosorbent serologic assay (ELISA) can be performed. There is no specific treatment for the disease, however, patients will require supportive therapy such as maintaining patient's fluid and electrolytes, maintaining oxygen status, blood pressure, and treatment for any complications. Case fatality for AHF can vary from 1 to 20%, however, later studies have shown CFR to be less than 1% [148].

AHF has a short incubation period of 2 to 4 days [149]. The disease presents initially with non-specific flu-like symptoms, including fever, anorexia, general malaise, diarrhoea, and vomiting. A second phase has appeared in some patients which includes severe neurologic and hemorrhagic symptoms. Multi-organ failure precedes fatal outcomes. Evidence suggests that a milder form may exist, where hospitalisation is not required. Thrombocytopenia, leukopenia, and elevated liver enzymes are nearly always observed in patients who have been hospitalised.

Prevention of AHFV includes avoiding tick-infested areas and to limit contact with livestock and domestic animals. Individuals should use tick repellants on skin and clothes and check the skin for attached ticks, removing them as soon as possible. Tick collars are available for domestic animals, and dipping in acaricides is effective in killing ticks on livestock. People working with animals or animal products in farms or slaughterhouses should avoid

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unprotected contact with the blood, fluids, or tissues of any potentially infected or viremic animals.

AHFV was discovered in 1994, Jeddah, Saudi Arabia(Dr.Ali Zaki et al). Transmission of AHFV is by soft tick (*Ornithodoros savignyi*) and hard ticks (*Hyalomma dromedary*). Transmission bite of an infected tick, crushing infected ticks or contact with infected animal's blood. Risk group are meat handlers and butchers. No human-to-human transmission of AHF has been documented [150].

Similarities between Kyasanur forest Disease Virus (KFDV) and Alkhurma Hemorrhagic Fever Virus(AHFV)

Kyasanur Forest Disease Virus (KFDV) and Alkhurma Hemorrhagic Fever Virus(AHFV) are tick-borne positive-stranded RNA viruses [151] and belong to the genus Flavivirus, classified into mammalian tick-borne virus group known as the tick-borne encephalitis (TBE) serocomplex of flaviviruses.
(http://www.searo.who.int/publications/journals/seajph/seajphv3n1p8.pdf).

KFD was first reported in March 1957 when there were a high number of monkey deaths in the Kyasanur forest of Shimoga district, Karnataka State, India [152] [153]. AHFV was detected from Saudi Arabia in 1994 [154]. In 1989, "Nanjianyin virus" was isolated from Yunnan province of China was nearly identical to some strains of KFDV [155]. The genome of these viruses possesses single positive-sense RNA of approximately 11 kb in length with nucleocapsid surrounded by a lipid bilayer with two surface proteins [156]. KFDV and AHFV the aetiology of significant morbidity and mortality in humans with case fatality rates of 2-10% for KFDV and less than 1% for AHFV. They share high sequence homology(>92% nucleotide similarity) and cause similar clinical presentation in people ranges from the acute onset of fever, myalgia, arthralgia to severe life-threatening condition such as hemorrhagic fever and encephalitis [157].

Human cases of KFD have reported in over five states across the Western Ghats region of India and AHF in across Saudi Arabia. However, KFDV and AHFV diverged more than 700 years ago and maintained distinct geographical locations in India and Saudi Arabia [158]. Despite KFDV and AHFV differ only 8% in nucleotide level, the vectors and host range for

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both viruses are distinct [159]. The tick *Hemaphysalis spinigera* identified as the most common vector for KFDV [160] but Ornithodoros savignyi is the principal vector for AHFV [161]. The Phylogenetic analysis of isolates of KFDV from India, Saudi Arabia and China share a recent common ancestor. This finding strongly points out that long-range shift of tick-borne Flaviviruses [162]. Several recent reports have highlighted the importance of surveillance to monitor the potential spread of KFDV into the newer geographical area throughout the western ghat region of India and AHF across Saudi Arabia. Significantly, within the past five years, confirmed cases of KFD had recorded for the first time in Kerala, Tamil Nadu, Goa and Maharashtra states. Mehla et al speculated that AHFV was introduced to Saudi Arabia when camels are transported from India through camel ticks via silk road or by ship [163]. The spread of KFDV was greatly influenced by human activities and increased bird migration [164].

Deforestation, climate change, expanding human population, increased migratory bird population contributed a major role in changing the epidemiology of emerging and re-emerging zoonotic viral diseases including KFD [165]. The black faced langurs *(Semnopithecus entellus)* and red faced bonnet monkeys *(Macaca radiata)* are the two monkey species and the tick, Hemaphysalis spp involved in the natural cycle of KFD. Small rodents, shrews and birds also circulate KFDV [166] [167]. *Hemaphysalis spinigera* is the principal vector for KFD, because it accounts for 95% of the KFDV isolations and there is also evidence that this vector transmits to humans the most [168] [169]. In addition to H. spinigera, 16 other species of Haemaphysalis ticks also showed the capability of KFDV transmission [170] [171]. Laboratory transmission of KFDV was demonstrated in many species of Haemaphysalis and Ixodes ticks [172].

Neutralizing antibodies against KFD have been found in many rodents, cattle, buffalo and number of avian species [173]. Rodent to human direct transmission is possible [174] [175] but person to person transmission has not been reported [176].

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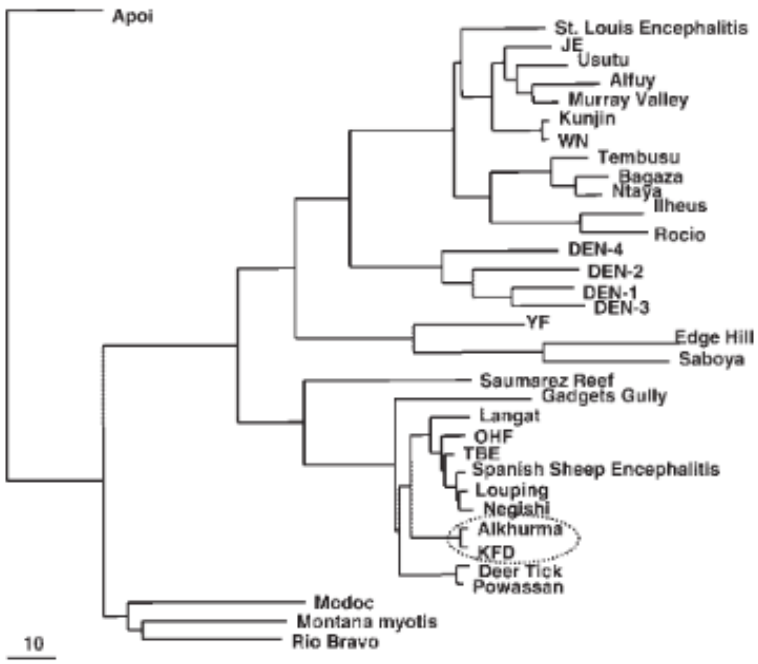

Figure 14: Close lineage of KFDV and Alkhurma virus suggests their co-evolution from the common ancestral origin

Current understanding / Knowledge Gap

KFD is originally described and classified as a VHF. But as early as 1959, it was noticed that KFD was more of encephalitis than hemorrhagic fever. Currently, hemorrhage is seen only in few cases whereas CNS manifestations in nearly 10-20% cases. Diarrhoea and abdominal pain very prominent in the initial days of illness leading to misdiagnosis as acute diarrheal diseases. Prolonged convalescence in nearly 30% of cases. Extreme weakness / prostration is a prominent feature in KFD. Geographic distribution of KFDV is continualy expanding. Early case detection and treatment is critical in clinical case management. KFDV and its ecology is still not fully understood. Epidemiology and pathogenesis of KFD need detailed studies. Immune response in KFDV is unexplored. New vaccine strategies required for KFDV. Is KFDV and AHFV are same? Is KFDV a BSL-4 agent?

Information, education, and communication (IEC)

NCDC recommends routine IEC activities by field staff to educate people about the disease as well as convince them for KFD vaccination. IEC for KFD should be focused on the vaccination campaign, conduct regular annual sensitization program for veterinary department, forest department officials, ASHA, education department, and gram panchayat officials. Pre-vaccination IEC campaigns should be intensified involving all possible media (25) .

Do’s

- Report monkey deaths to animal husbandry / forest officials and / health authority.
- Persons clothing is recommended for people visiting or working in tick-infested areas in the forest.
- Apply tick repellents like DMP oil to the exposed parts before going into the forest.
- Wash the clothes and body with hot water and soap after returning from the forest.

- Report of incidence of the disease / deaths, which occurs as high fever with severe headache and body ache to nearest health facility.
- Educate the villagers to avoid the forests areas where monkeys have died.
- Bring to the notice of the Health Department or Department Hospitals or Private Hospitals, regarding any serious cases in the villages or from KFD affected areas, which require immediate symptomatic treatment.
- Ectoparasite (tick) control in cattle and domestic animals will help in reducing the density of tick's population.

Don'ts

- Don't bring the leaves of trees from KFD infected area to the village for cattle bedding material.
- Don't visit the area where recent monkey death have been reported, especially an area where case of KFD has been reported in the past.
- Don't handle the infected monkey carcass by bare hand without personal protective equipment.

Factsheet

Key facts

- KFD virus causes severe viral fever outbreaks during the summer season.
- KFD is endemic to the western Ghats regions of India and cases have been reported from Karnataka, Kerala, Tamil Nadu, Goa, and Maharashtra.
- Transmission happens through the bite of infected hard ticks *(H. spinigera)* or direct contact with infected or deceased animal. Monkeys are the amplifying host. No person-to-person transmission.
- The incubation period of KFD virus is nearly 3- 8 days in humans.
- No specific treatment is available for KFD only symptomatic management.
- KFD vaccination is available to the endemic regions in India.
- Case fatality rate is 3 to 5%.

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Annexure

I. List of villages affected from Kyasanur Forest Disease (AFI surveillance data 2014 – 19)

Table A. List of villages affected by Kyasanur Forest Disease in Goa (AFI surveillance data)

District	Taluk	Village	2014-15	2015-16	2016-17	2017-18	2018-19	Total Cases
North Goa	Bardez	Tivim		1		1		2
		Paliem		1				1
		Revora			1			1
	Bicholim	Latambarcem		2	2	2		6
		Sanquelim (M Cl)		1				1
		Velguem		1				1
	Pernem	Virnora		4				4
		Pernem			2			2
		Querim		2				2
		Pernem (M Cl)				1		1
	Ponda	Ponda (M Cl)					1	1
		Usgao (CT)		1				1
	Satari	Mauzi		33	5			38
		Choraundem		28	2			30
		Dabem		22	7			29
		Querim		15	14			29
		Compordem		21	1			22
		Morlem		19	2			21
		Carambolim-Bozruco			1	19		20
		Caranzol		1		17	1	19
		Velguem		1	5	4	2	12
		Ivrem-Buzruco		4	7			11
		Sonal		2		9		11
		Cotorem		3	4	2		9
		Valpoi (M Cl)			3		6	9
		Zormen		7	1			8
		Siroli			6			6
		Pale		2	3			5
		Davem		3	1			4
		Saleli		3	1			4
		Sanvordem		1		3		4
		Malpona			1		2	3
		Nagargao		2			1	3
		Birondem		2				2
		Buimpal		2				2
		Golauli		2				2
		Guleli		1	1			2
		Rivem			2			2
		Assodem					1	1
		Codqui			1			1
		Cudcem					1	1
		Damocem		1				1
		Derodem			1			1
		Maloli			1			1
		Naguem		1				1
		Naneli					1	1
		Nanorem			1			1
		Ravona		1				1
		Siranguli			1			1

		Sirsodem			1			1
		Vainguinim			1			1
	Tiswadi	Carambolim		1				1
South Goa	Sanguem	Sancordem					1	1
		Grand Total	**0**	**191**	**79**	**58**	**17**	**345**

CT = Census Town; M CI = Municipal Council.

Table B. List of villages affected by Kyasanur Forest Disease in Karnataka (AFI surveillance data)

District	Taluk	Village	2014-15	2015-16	2016-17	2017-18	2018-19	Total Cases
Shimoga	Hosanagara	Haridravati				1		1
		Ryave		1				1
		Talale					1	1
	Sagar	Aralagodu				1	14	15
		Jog Kargal (TP)		1		1	1	3
		Sagar (CMC)					2	2
		Banumane					1	1
		Keladi					1	1
		Sasaravalli					1	1
	Shimoga	Gajanuru State Forest					1	1
	Sorab	Guddekoppa		3				3
		Yalavalli					1	1
	Tirthahalli	Kudumallige		1	35	1	5	42
		Bejjavalli	8		10		3	21
		Mahishi					19	19
		Kukke	3	4	2	6	1	16
		Virupapura	9					9
		Hedduru					7	7
		Shedgar	1				5	6
		Aralapura	4	1				5
		Biluvehariharapura	5					5
		Kunda	4		1			5
		Singanabidare					5	5
		Bandya			1	3		4
		Guddekoppa	1	2			1	4
		Guthiyadehalli	4					4
		Kannangi		2			2	4
		Hallusale	3					3
		Kudige			3			3
		Neralakoppa			3			3
		Thuduru	3					3
		Dabbanagadde					2	2
		Kanaboor	2					2
		Konandur	2					2
		Kuchhalu	2					2
		Kuduvalli			1		1	2
		Malur	1				1	2
		Melinakuruvalli		1		1		2
		Thotadakoppa		1			1	2
		Agasadi	1					1
		Araga		1				1
		Arehalli			1			1
		Attigadde		1				1

		Balagatte	1					1
		Basavanagadde		1				1
		Bhandigadi	1					1
		Demlapura			1			1
		Devangi	1					1
		Halumahishi					1	1
		Hanagere					1	1
		Honnetalu			1			1
		Karekoppa	1					1
		Katagaru					1	1
		Malali		1				1
		Malalur					1	1
		Melige	1					1
		Mulubagilu		1				1
		Nellisara			1			1
		Neraturu	1					1
		Suruli	1					1
		Talale					1	1
		Theerthahalli (Rural)			1			1
		Tirthahalli (TP)			1			1
		Triyambakapura	1					1
		Tyarandoor				1		1
		Udukere	1					1
Mysore	Piriyapatna	Bylakuppe					1	1
Uttara Kannada	Siddapur	Hejani		1				1
		Grand Total	**62**	**23**	**62**	**15**	**82**	**244**

CMC = City Municipal Council; TP = Town Panchayat.

Table C. List of villages affected by Kyasanur Forest Disease in Kerala (AFI surveillance data)

District	Taluk	Village	2014-15	2015-16	2016-17	2017-18	2018-19	Total Cases
Wayanad	Mananthavady	Thirunelly					3	3
		Thrissilery					2	2
	Sulthanbathery	Pulpalli	14	2				16
		Padichira	2	3				5
		Sulthanbathery	5					5
		Irulam	4					4
		Kuppadi	4					4
		Kidanganad	3					3
		Noolpuzha	1	1				2
		Ambalavayal	1					1
	Vythiri	Kalpetta (M)	1	1				2
		Grand Total	**35**	**7**	**0**	**0**	**5**	**47**

M = Municipality.

Table D. List of villages affected by Kyasanur Forest Disease in Maharashtra (AFI surveillance data)

District	Taluk	Village	2014-15	2015-16	2016-17	2017-18	2018-19	Total Cases
Sindhudurg	Dodamarg	Kasai		2			8	10
		Kudase			2	4	4	10
		Panturli				5		5

		Kumbral			3	1		4
		Adali					3	3
		Ghotge			3			3
		Ker		3				3
		Morgaon			1	2		3
		Sateli Bhedshi			3			3
		Zolambe		3				3
		Ghotgewadi		2				2
		Kolzar			2			2
		Konal		1		1		2
		Mangeli				1	1	2
		Talekhol		2				2
		Talkat			2			2
		Terwanmedhe			2			2
		Usap		2				2
		Hewale			1			1
		Kalane				1		1
		Kendre Bk.				1		1
		Maneri				1		1
		Morle		1				1
		Parme			1			1
		Patye		1				1
		Pikule		1				1
		Sasoli					1	1
		Shirwal			1			1
		Zare				1		1
	Sawantwadi	Banda (CT)		2	35		1	38
		Dongarpal			1	12		13
		Dingne			9	2	1	12
		Galel			10			10
		Bhalawal					7	7
		Degave		4	2	1		7
		Tamboli					4	4
		Konas					3	3
		Nigude			3			3
		Chaukul					1	1
		Danoli			1			1
		Insuli			1			1
		Madkhol			1			1
		Majgaon (CT)		1				1
		Nemale				1		1
		Netarde			1			1
		Satose			1			1
		Vilavade				1		1
		Wafoli			1			1
		Grand Total	**0**	**25**	**87**	**35**	**34**	**181**

CT = Census Town.

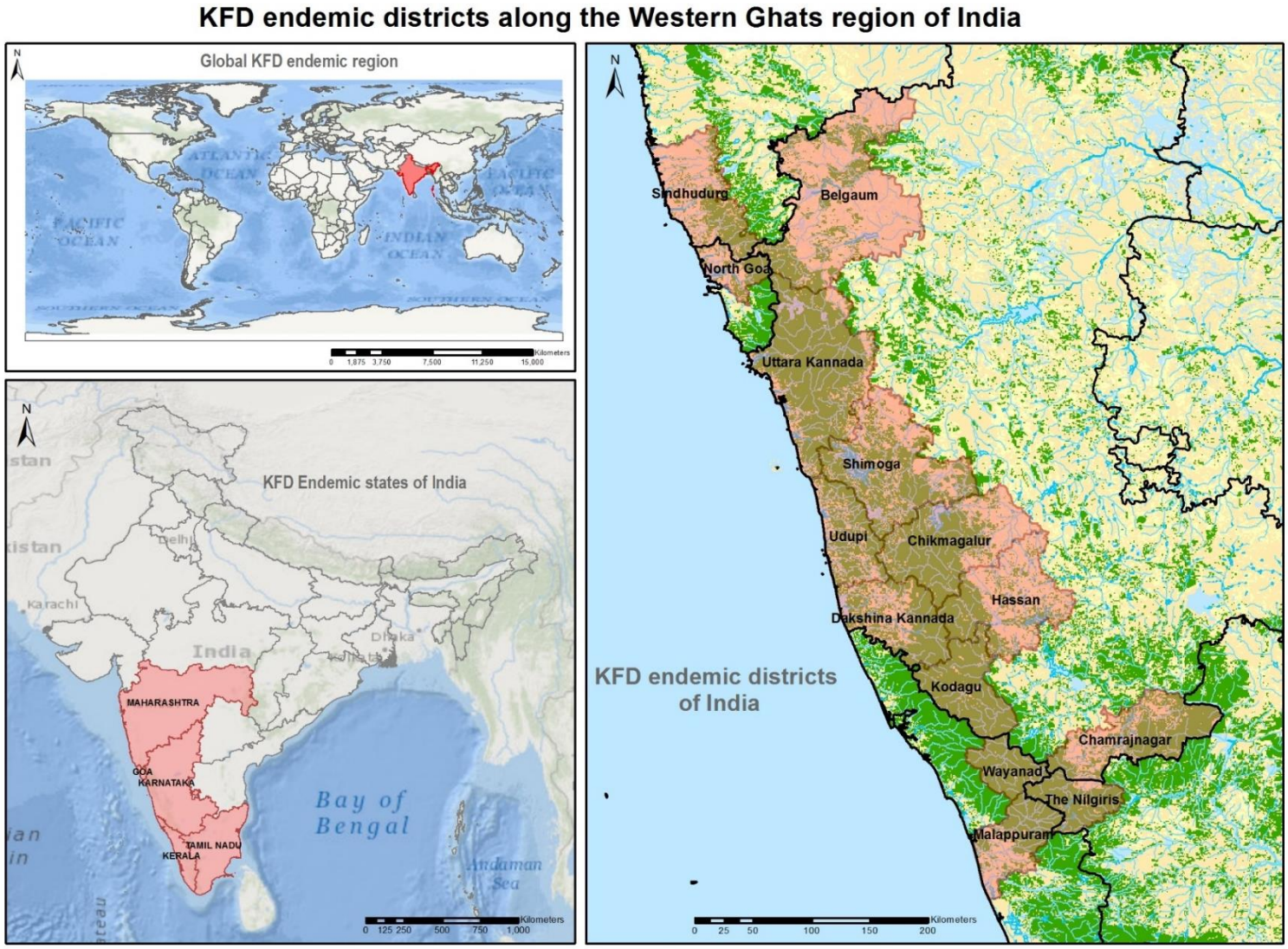
Table E. List of villages affected by Kyasanur Forest Disease in Tamil Nadu (AFI Surveillance data)

District	Taluk	Village	2014-15	2015-16	2016-17	2017-18	2018-19	Total Cases
The Nilgiris	Gudalur	Gudalur (M)				3	1	4
		Devarshola (TP)				1		1
		O' Valley (TP)	1					1
		Srimadurai				1		1
	Panthalur	Nelliyalam (M)			3	8	2	13
		Nelliyalam			12			12
		Grand Total	**1**	**0**	**15**	**13**	**3**	**32**

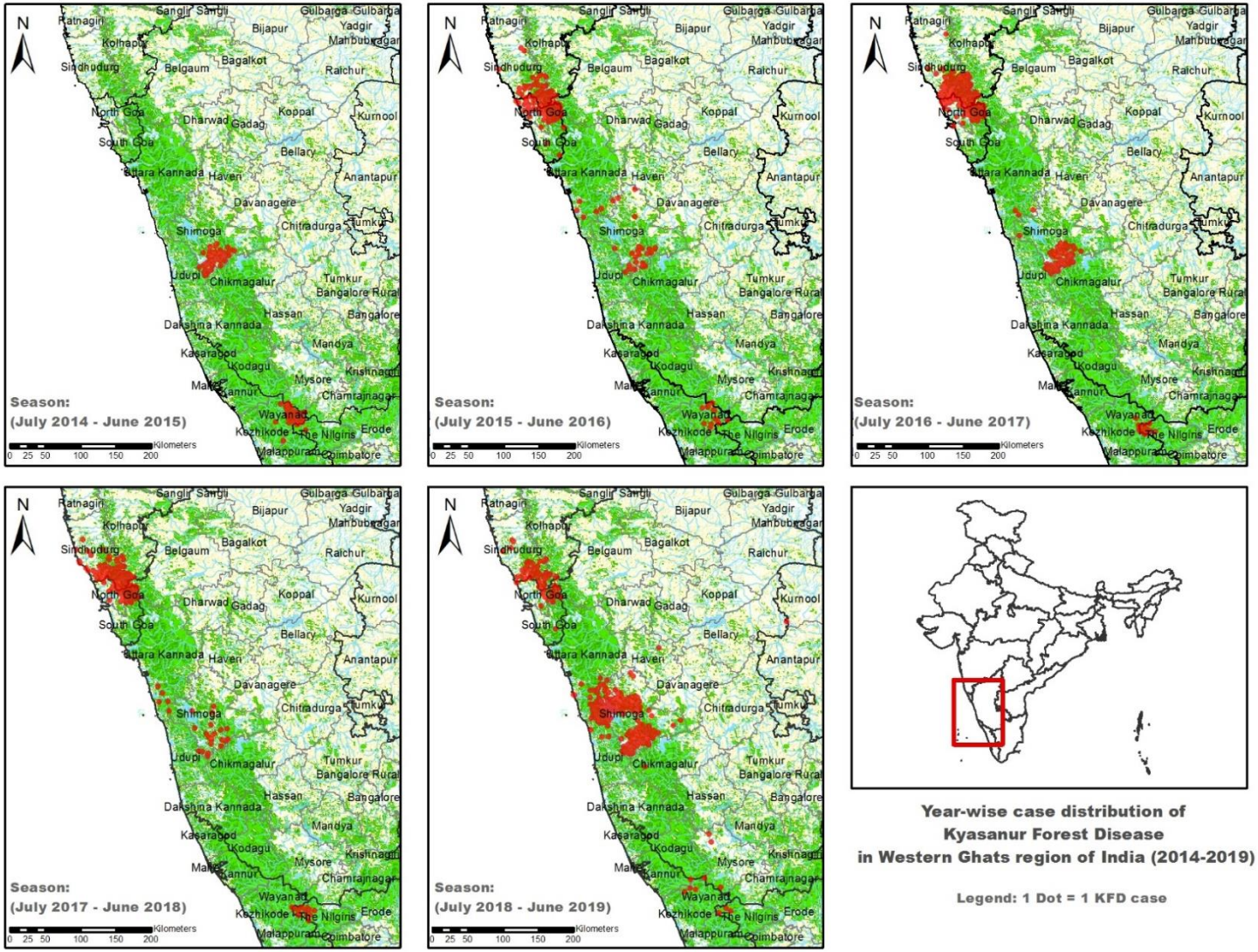
TP = Town Panchayat; M = Municipality.

Annexure

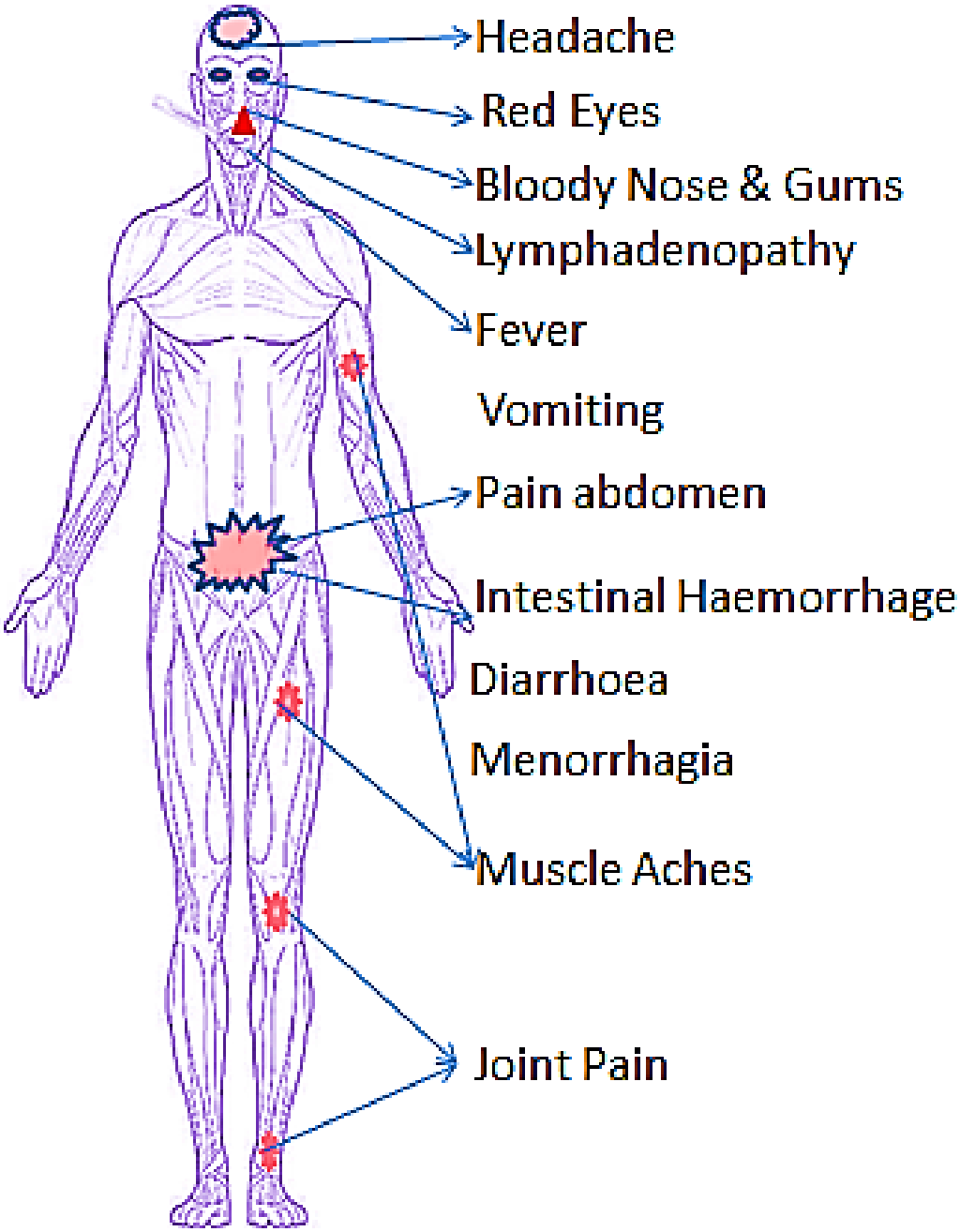
II. Map showing KFD endemic districts along the Western Ghats region of India



Annexure III. Year-wise case distribution of Kyasanur Forest Disease in Western Ghats region of India (2014 – 19)



Signs and Symptoms of Kyasanur forest disease



Tick Lifecycle