lacked adjustment for confounding, we think that the results of Bacci et al. (*1*) should be interpreted with caution. Furthermore, a large clinical study from 2008 concluded that *C. difficile* type 078, which is the most frequently found binary toxin positive non-027 strain, was not associated with a high all-cause mortality rate (*3*). A more recent publication confirmed this finding (*4*). Therefore, in our opinion, there is currently no convincing epidemiologic proof that binary toxin is a marker for infection with virulent *C. difficile.*

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References

1. Bacci S, Mølbak K, Kjeldsen MK, Olsen KEP. Binary toxin and death after *Clostridium difficile* infection. Emerg Infect Dis. 2011;17:976–82. http://dx.doi.org/10.3201/eid1706.101483
2. Hensgens MP, Goorhuis A, Dekkers OM, van Benthem BHB, Kuijper EJ. All-cause and disease specific mortality in hospitalized patients with *Clostridium difficile* infections; a multicenter cohort study. Clin Infect Dis. 2013 Jan 13 Epub ahead of print.
3. Goorhuis A, Bakker D, Corver J, Debast SB, Harmanus C, Notermans DW, et al. Emergence of *Clostridium difficile* infection due to a new hypervirulent strain, polymerase chain reaction ribotype 078. Clin Infect Dis. 2008;47:1162–70. http://dx.doi.org/10.1086/592257
4. Walk ST, Micic D, Jain R, Lo ES, Trivedi I, Liu EW, et al. *Clostridium difficile* ribotype does not predict severe infection. Clin Infect Dis. 2012;55:1661–8. http://dx.doi.org/10.1093/cid/cis786

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Spread of Kyasanur Forest Disease, Bandipur Tiger Reserve, India, 2012–2013

To the Editor: Kyasanur Forest disease virus (KFDV; family *Flaviviridae*, genus *Flavivirus*) was first recognized in 1956 in Shimoga District, Karnataka State, India (*1*). The natural cycle of KFDV involves 2 monkey species–black-faced langurs (*Semnopithecus entellus*) and red-faced bonnet monkeys (*Macaca radiata*)–and various tick species (genus *Haemaphysalis*). Monkeys become infected with KFDV through the bite of infected ticks; the virus is then transmitted to other ticks feeding on infected monkeys. KFDV infection causes severe febrile illness in some monkeys. When infected monkeys die, ticks drop from the body, thereby generating hot spots of infectious ticks that further spread the virus. In the enzootic state, KFDV circulates through small mammals (e.g., rodents, shrews, ground birds) and ticks (*2*).

Humans can also be infected with KFDV. In humans, the disease causes high fever, frontal headache, and severe myalgia, followed by bleeding from the nasal cavity, throat, gingivae, and, in some cases, gastrointestinal tract (*3*). In the natural KFDV cycle, humans are dead-end hosts.

KFD is unique to 5 districts (Shimoga, Chikkamagalore, Uttara Kannada, Dakshina Kannada, and Udupi) in the Malnad region of Karnataka State, India, where each year during January–May, 100–500 persons are affected by the disease (*2*,*4*). During December 2011–March 2012, a total of 215 suspected KFD case-patients were identified in 80 villages in Shimoga District; laboratory testing confirmed that 61 (28%) were infected with KFDV (*5*).

In November 2012, the deaths of 12 monkeys in Bandipur National Park, Chamarajanagara District, Karnataka State, were reported. At the same time, 6 humans from Mole Hole village and Madhur colony in the Bandipur Tiger Reserve who handled and incinerated the sick monkeys were reported to have clinical signs and symptoms typical of KFD (online Technical Appendix Figure 1, wwwnc.cdc.gov/EID/article/19/9/12-1884-Techapp1.pdf). The monkey handlers (20–55 years of age) were admitted to the local hospital in Gundlupet Taluk. Monkey autopsy specimens, serum samples from suspected human case-patients, and tick pools were collected by staff from the Virus Diagnostic Laboratory in Shimoga. The samples were sent to the National Institute of Virology in Pune for determination of the etiologic agent. Additional samples from humans with suspected KFDV infection, monkeys, and tick pools were received from Chamarajanagar District and adjoining border areas of Tamil Nadu State and Kerala State (Table).

Monkey brain and liver and tick pools were sonicated in 600 mL of Minimum Essential Media (GIBCO/BRL, Life Technologies, Grand Island, NY, USA), and 400 mL of media was added to the homogenate. TriPure Isolation Reagent (Roche Diagnostics, Indianapolis, IN, USA) was used to perform RNA extraction as described (*6*).

Samples were tested for KFDV by nested reverse transcription PCR (RT-PCR) and real-time RT-PCR as described (*6*); 12 of 21 human samples and 4 monkey samples were positive (Table). Two of 14 tick pools screened for KFDV by real-time RT-PCR were positive; however, 1 was weakly positive (Table). The PCR-amplified products were purified by using the QIAquick Gel Extraction Kit (QIAGEN, Hilden, Germany) and then sequenced. KFDV sequences from the samples showed 95.8%–98.1% similarity with prototype strain KFDV P9605. This finding supports the earlier conclusion that a high level of conservation exists for KFDV sequences (*7*). The phylogenetic tree formed 2 clades: the first

Table. Real-time reverse transcription PCR and nested reverse transcription PCR results for specimens screened for Kyasanur Forest disease virus, India, November 2012–May 2013*

Date of sample collection	Location of sample collection	No. samples positive/no. total		
		Human	Monkey	Tick pools
2012 Nov	Maddur Forest Range, Bandipur Tiger Reserve, Chamarajanagara District, Karnataka State	4/6	3/7	–
2013 Jan	Chamarajanagara District, Karnataka State	7/13	–	0/7
2013 Jan	Nilgiri, Tamil Nadu State	0/1	1/2	0/5
2013 Feb	Chamarajanagara District, Karnataka State	–	–	1/2
2013, May	Wayanad District, Kerala State	1/1	–	–
Total no. positive samples		12/21	4/9	1/14

*–, no samples from the area.

included mainly KFDV sequences from 1957–2006, the second included KFDV sequences (human and monkey) from Chamarajanagara District (online Technical Appendix Figure 2).

KFDV has not been detected previously in Chamarajanagara District, the location of Bandipur National Park. Affected areas in the district share a border with Mysore District (Karnataka State), Kerala State, and Tamil Nadu State. In addition,we subsequently found monkey samples from Nilgiri, Tamil Nadu, to be positive for KFDV.

The human case-patients from Chamarajanagara District were mainly forest workers involved in the incineration of the dead monkeys. Infection among these workers indicates that they did not follow appropriate biosafety procedures while handling the infected animals.

Our findings confirm that KFD has occurred outside the districts in Karnataka State where KFDV is known to be endemic. A hemagglutination inhibition antibody survey conducted during December 1988–January 1989 (*8*) indicated the possible existence of this disease in other regions of India. The presence of KFD becomes noticeable when enzootic infections occur and sentinel animals, like monkeys, start dying (*9*). Detection of KFDV in Chamarajanagara District, Tamil Nadu State (Nilgiri), and Kerala State indicates the presence of the virus in many evergreen and semi-evergreen forest areas of India. Infections in these areas may have been missed previously because of the lack of an organized surveillance system.

During the first week of December 2012, immediately after the KFD outbreak was confirmed, the Karnataka public health department vaccinated 322 persons, including villagers, forest officials, health workers, and members of local tribes in the Maddur Forest Range of Bandipur Tiger Reserve. Hot-spot areas caused by monkey deaths were dusted with malathion insecticide to kill ticks. In addition, to prevent additional human infections, epidemiologists recommended establishment of a health education campaign and the use of protective clothing and tick repellents, especially by persons frequently visiting forested areas.

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References

1. Work TH, Roderiguez FM, Bhatt PN. Virological epidemiology of the 1958 epidemic of Kyasanur Forest disease. Am J Public Health Nations Health. 1959;49:869–74. http://dx.doi.org/10.2105/AJPH.49.7.869
2. Pattnaik P. Kyasanur Forest disease: an epidemiological view in India. Rev Med Virol. 2006;16:151–65. http://dx.doi.org/10.1002/rmv.495
3. Dobler G. Zoonotic tick-borne flaviviruses. Vet Microbiol. 2010;140:221–8. http://dx.doi.org/10.1016/j.vetmic.2009.08.024
4. Bhatt PN, Work TH, Varma MGR, Trapido H, Murthy NDP, Rodrigues FM. Isolation of Kyasanur Forest disease from infected humans and monkeys of Shimoga District, Mysore State. Indian J Med Sci. 1966;20:316–20.
5. Kasabi GS, Murhekar MV, Yadav PD, Raghunandan R, Kiran SK, Sandhya VK, et al. Kyasanur Forest disease, India, 2011–2012. Emerg Infect Dis. 2013;19:278–81 http://dx.doi.org/10.3201/eid1902.120544
6. Mourya DT, Yadav PD, Mehla R, Barde PV, Yergolkar PN, Thakare JP, et al. Diagnosis of Kyasanur Forest disease by nested RT-PCR, real-time RT-PCR and IgM capture ELISA. J Virol Methods. 2012;186:49–54. http://dx.doi.org/10.1016/j.jviromet.2012.07.019
7. Mehla R, Kumar SR, Yadav P, Barde PV, Yergolkar PN, Erickson BR. Recent ancestry of Kyasanur Forest disease virus. Emerg Infect Dis. 2009;15:1431–7. http://dx.doi.org/10.3201/eid1509.080759
8. Padbidri VS, Wairagkar NS, Joshi GD, Umarani UB, Risbud AR, Gaikwad DL, et al. A serological survey of arboviral diseases among the human population of the Andaman and Nicobar Islands, India. Southeast Asian J Trop Med Public Health. 2002;33:794–800.
9. Pavri KM, Anderson CR. Serological response of man to Kyasanur Forest disease. Indian J Med Res. 1970;58:1587–607.

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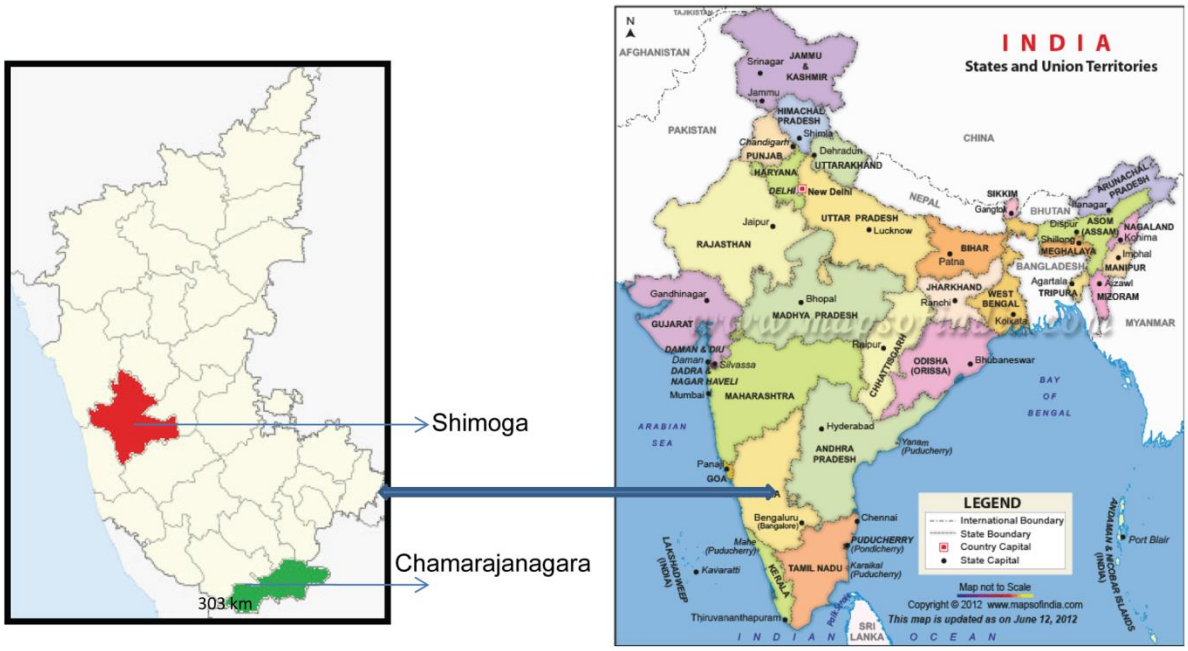
Spread of Kyasanur Forest Disease, Bandipur Tiger Reserve, India, 2012–2013

Technical Appendix

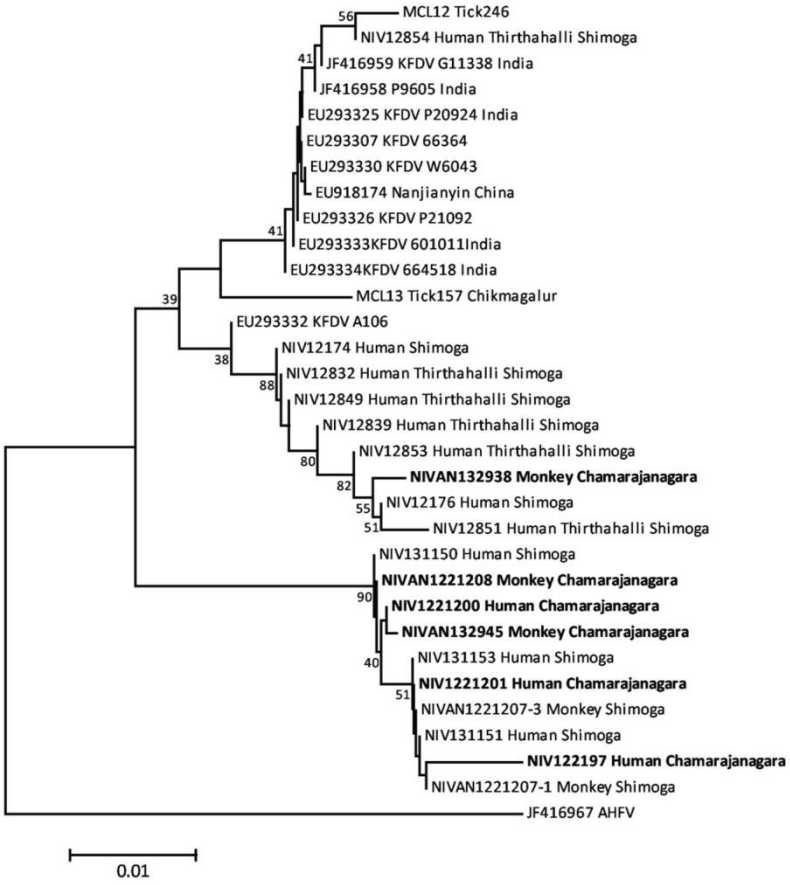
Technical Appendix Table. Results of screening for Kyasanur Forest disease virus in human specimens, monkey specimens, and tick pools from Karnataka, Kerala, and Tamil Nadu States, India, December 2012–May 2013*

Specimen type, ID no.	Date sample collected	Specimen type	Age of human or monkey	Location‡	rRT-PCR result, C_t	RT-PCR result	Final result
Human†							
1221197	Nov2012	Serum	22 y	Madduru Forest Range, Chamarajanagara	38.5	+	+
1221199	Nov 2012	Serum	44 y	Madduru Forest Range, Chamarajanagara	38.7	–	+
1221200	Nov 2012	Serum	46 y	Madduru Forest Range, Chamarajanagara	34.7	+	+
1221201	Nov 2012	Serum	55 y	Madduru Forest Range, Chamarajanagara	27.5	+	+
13192	Jan 2013	Serum	28 y	Chamarajanagara	33.5	+	+
131149	Jan 2013	Blood	60 y	Chamarajanagara	33.5	–	+
131150	Jan 2013	Blood	35 y	Chamarajanagara	30.5	–	+
131151	Jan 2013	Blood	45 y	Chamarajanagara	26.5	+	+
131152	Jan2013	Blood	60 y	Chamarajanagara	28.0	+	+
131153	Jan 2013	Blood	43 y	Chamarajanagara	28.5	+	+
131154	Jan 2013	Blood	19 y	Chamarajanagara	33.5	–	+
135724	May 2013	Blood	18 y	Wayanad District, Kerala State	30.0	+	+
Monkey†							
AN1221204-1	Nov 2012	Brain, liver	Adult	Halegoudana camp, Chamarajanagara	36.5 38.0	–	+
AN1221207-3	Nov 2012	Brain, liver	Adult	Halegoudana camp, Chamarajanagara	22.20 22.80	+	+
AN1221208	Nov 2012	Brain	Adult	Halegoudana camp, Chamarajanagara	36.40	+	+
AN131058	Nov 2012	Brain	Adult	Nilgiri, Tamil Nadu State	26.80	+	+
Tick 114	Feb 2013	Hyalomma mixed tick pool		Chamarajanagara	22.00	+	+

*Screening was performed by the National Institute of Virology in Pune, India. rRT, real-time reverse transcription; ID, identification; C_t, threshold cycle.
†All humans and monkeys were male.
‡Chamarajanagara is a district in Karnataka State.



Technical Appendix Figure 1. Kyasanur Forest disease outbreak in new area of Karnataka State, India, 2012–2013. Map source: www.mapsofindia.com.



Technical Appendix Figure 2. Phylogenetic analysis of Kyasanur Forest disease virus sequences from recent outbreak and earlier Kyasanur Forest disease virus sequences from Karnataka State, India.