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Case Report

Outbreak of Kyasanur Forest disease in Thirthahalli, Karnataka, India, 2014

Pragya D. Yadav [a], Anita M. Shete [a], Deepak Y. Patil [a], V.K. Sandhya [b], K.S. Prakash [c], Rajesh Surgihalli [b], Devendra T. Mourya [a,*]

[a] National Institute of Virology, Microbial Containment Complex, 130/1 Sus Road, Pashan, Pune 411001, India
[b] Virus Diagnostic Laboratory, Shimoga, Karnataka, India
[c] District Health Authority, Karnataka, India

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SUMMARY

Kyasanur Forest disease virus (KFDV) was first identified in 1957, when it was isolated from a sick monkey from the Kyasanur Forest in Karnataka State, India. Since then it has been reported to be enzootic in five districts of Karnataka State, India. Recent reports of human infections have reached an alarming level, in spite of the availability of a vaccine. This disease has also been reported from new areas, such as Tamil Nadu and Kerala State. During January–March 2014, KFDV-positive cases were detected in Thirthahalli taluk, Shimoga District, Karnataka State, India. Here, we report an outbreak of Kyasanur Forest disease occurring in the Kannangi and Konandur area, Thirthahalli taluk in Karnataka State, India, with sporadic cases from eight other areas.

1. Introduction

Kyasanur Forest disease virus (KFDV) is a member of the genus *Flavivirus* and family *Flaviviridae*. It is highly pathogenic, causing a haemorrhagic disease in humans. In nature it is maintained in ticks, mammals, and birds.[1] Kyasanur Forest disease (KFD) is characterized by an incubation period of approximately 3–8 days, followed by chills, a frontal headache, body aches, and a high fever for 5–12 days; the case-fatality rate is 3–5%.[2] It has a unique existence in five districts of Karnataka State: Shimoga, Chikmagalur, Uttara Kannada, Dakshina Kannada, and Udupi.[2] During the period December 2011 to March 2012, 215 suspected cases were identified in 80 villages of Shimoga District, Karnataka State.[3] Subsequently, the spread of KFD was noticed in Mudumalai Tiger Reserve (MTR), Tamil Nadu State and there was a human case in Kerala.[4] More recently there has been an increase in KFD cases in Karnataka State. Here, we describe an outbreak of this disease that occurred in Kannangi and Konandur, Thirthahalli taluk, Karnataka, India during January–March 2014.

2. Case report

During January–March 2014, a total of 166 human blood samples were collected by the district health authorities from patients attending 10 primary health centres in Thirthahalli taluk of Shimoga and Chikmagalur District. Four monkey necropsy samples were also collected from Thirthahalli and Hosanagara taluk of Shimoga District. Subsequently, 180 tick pools were collected from Shimoga, Chikmagalur, Chamrajnagar, Uttar Kannada, and Mysore districts of Karnataka State. These specimens were transported in cold chain to the National Institute of Virology (NIV), Pune for confirmation of aetiology.

The human serum samples were tested for anti-KFDV IgM antibodies by IgM ELISA and for KFDV viral RNA by real-time RT-PCR, as described previously.[5] The monkey necropsy samples

* Corresponding author. Tel.: +91 20 26006201; fax: +91 20 26122669.
E-mail address: directorniv@gmail.com (D.T. Mourya).

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and tick pools were homogenized using a Geno-grinder; supernatant collections were subjected to real-time RT-PCR. Two human serum samples that showed low cycle threshold (Ct) values in the real-time RT-PCR were inoculated into *Swiss albino* mice by intracerebral route for virus isolation, as described previously.[2] All the work on suspected samples was carried out in a biosafety level 4 laboratory (NIV, Pune).

Of the 166 suspected human samples tested for KFDV by real-time RT-PCR, 73 were found to be positive for KFDV viral RNA; IgM antibodies were detected in 35 samples (Table 1). Altogether, from the results of both assays, the number of KFDV-positive cases was 92; thus the use of both tests (ELISA and real-time RT-PCR) is recommended for diagnosis. Out of four monkey necropsy samples tested by real-time RT-PCR, one was found to be positive. Out of 180 tick pools tested using real-time RT-PCR, eight tick pool samples were confirmed positive for the presence of KFDV (Figure 1, Table 1). The mice inoculated with KFDV-positive human serum samples (Ct = 21) died within 5–6 days. The sick mice showed typical signs of paralysis, ruffled coat, and hunched posture.

The highest KFDV positivity was recorded from Kannangi (32/63) and Konandur (23/48) area of Thirthahalli taluk of Shimoga District. One positive human case was also confirmed from Chikamagalur District. Consequently, sporadic KFDV-positive cases were also reported from different primary health centres of Thirthahalli taluk.

During this outbreak, KFDV positivity was recorded in a monkey necropsy sample ($n = 1$), tick pools ($n = 3$), and human serum samples ($n = 32$) from Kannangi area by real-time RT-PCR. These results confirmed the outbreak of KFD in Kannangi area of Thirthahalli taluk. The suspected cases had high fever, cough, myalgia, and arthralgia. There was no viral hemorrhagic fever case or death reported during this period. Out of 35 IgM-positive samples, 19 were found to be negative for viral RNA by real-time RT-PCR (Table 1). This again suggests that the information of post onset days of illness along with other patient clinical details should always be provided when referring samples for laboratory investigation. This helps the laboratory investigator to decide on the best diagnostic tool for screening of the suspected samples depending on the post onset days of illness.

Analysis of the real-time RT-PCR- and IgM ELISA-positive cases revealed that the viral RNA could be detected from the first day of illness until day 10 post onset of illness, and IgM antibodies could be detected from day 4 post onset of illness. Hence, molecular diagnostic tools like real-time RT-PCR can be used for a quick diagnosis and confirmation of such an outbreak. This will help in patient management and in applying preventive measures to control the outbreak. However, these diagnostic facilities are available only at national referral laboratories; other laboratories are not equipped with the higher level of biosafety. There are only a few biosafety level 3 laboratories in India, and some of them working in the biomedical field have the capacity to deal with the diagnosis of such viral agents. The limited number of such laboratory facilities in Karnataka State is one of the major constraints in dealing with the diagnosis of KFD.

3. Discussion

The present study documented a KFD outbreak in Kannangi and Konandur and sporadic cases in eight other areas of Thirthahalli taluk (Table 1). In Kannangi area, human cases have been reported for a long time, in Yetagnabailu village in 1978 and then in Garga village in 1992 and most recently in 2002. A continuous vaccination programme was followed in this area until 2006. However, since 2006, vaccination has been carried out only in outbreak areas. Soon after the confirmation of the current KFD outbreak, the Karnataka State Health authorities took the necessary preventive measures to control the outbreak. They obtained stocks of medicines for supportive treatment, which were immediately made available at primary health centres in the affected areas. A separate ward was set up to treat KFD cases at

Table 1
Data obtained from the screening of suspected Kyasanur Forest disease samples from humans, monkeys, and ticks in different areas of Karnataka; 2014

Affected area	Taluk	District	Human samples		Tick pools	Monkey samples
			Real time RT-PCR	IgM ELISA	Real time RT-PCR	Real time RT-PCR
Kannangi	Thirthahalli	Shimoga	32/63	19/63	3/12	1/2
Konandur	Thirthahalli	Shimoga	23/48	9/48	1/9	-
Mandagadde	Thirthahalli	Shimoga	4/9	0/9	-	-
Bejavali	Thirthahalli	Shimoga	1/1	0/1	-	-
Hanagari	Thirthahalli	Shimoga	1/1	0/1	-	-
Horogulige	Thirthahalli	Shimoga	2/3	0/3	-	-
Malur	Thirthahalli	Shimoga	5/10	1/10	-	-
-	Thirthahalli	Shimoga	3/16	0/16	0/98	-
Guddekoppa	Thirthahalli	Shimoga	0/2	0/2	-	-
Gunddepal	Bhavikaisaru	Shimoga	0/1	0/1	-	-
Ravae	Hosanagara	Shimoga	0/1	0/1	-	-
Belur	Belur	Hasan	0/1	0/1	-	-
Beluvagi	Mangalore	Mangalore	0/1	1/1	0/3	-
Doorvasapura	Thirthahalli	Shimoga	0/1	1/1	-	-
Hosanagara	Hosanagara	Shimoga	0/1	1/1	-	-
Humcha	Hosanagara	Shimoga	0/2	1/2	-	-
Humchadakatte	Thirthahalli	Shimoga	1/2	0/2	2/4	-
Yogimalali	Thirthahalli	Shimoga	0/1	0/1	1/8	-
Kadur	Hosanagara	Shimoga	-	-	1/2	-
Shimoga	Shimoga	Shimoga	0/1	1/1	-	-
-	Hosanagara	Shimoga	-	-	-	0/2
Kattinamane	N. R. Pura	Chikmagalur	1/1	1/1	-	-
-	-	Chikmagalur	-	-	0/28	-
-	-	Uttara Kannada	-	-	0/10	-
-	-	Chamrajnagar	-	-	0/3	-
-	-	Mysore	-	-	0/3	-
Total number			73/166	35/166	8/180	1/4

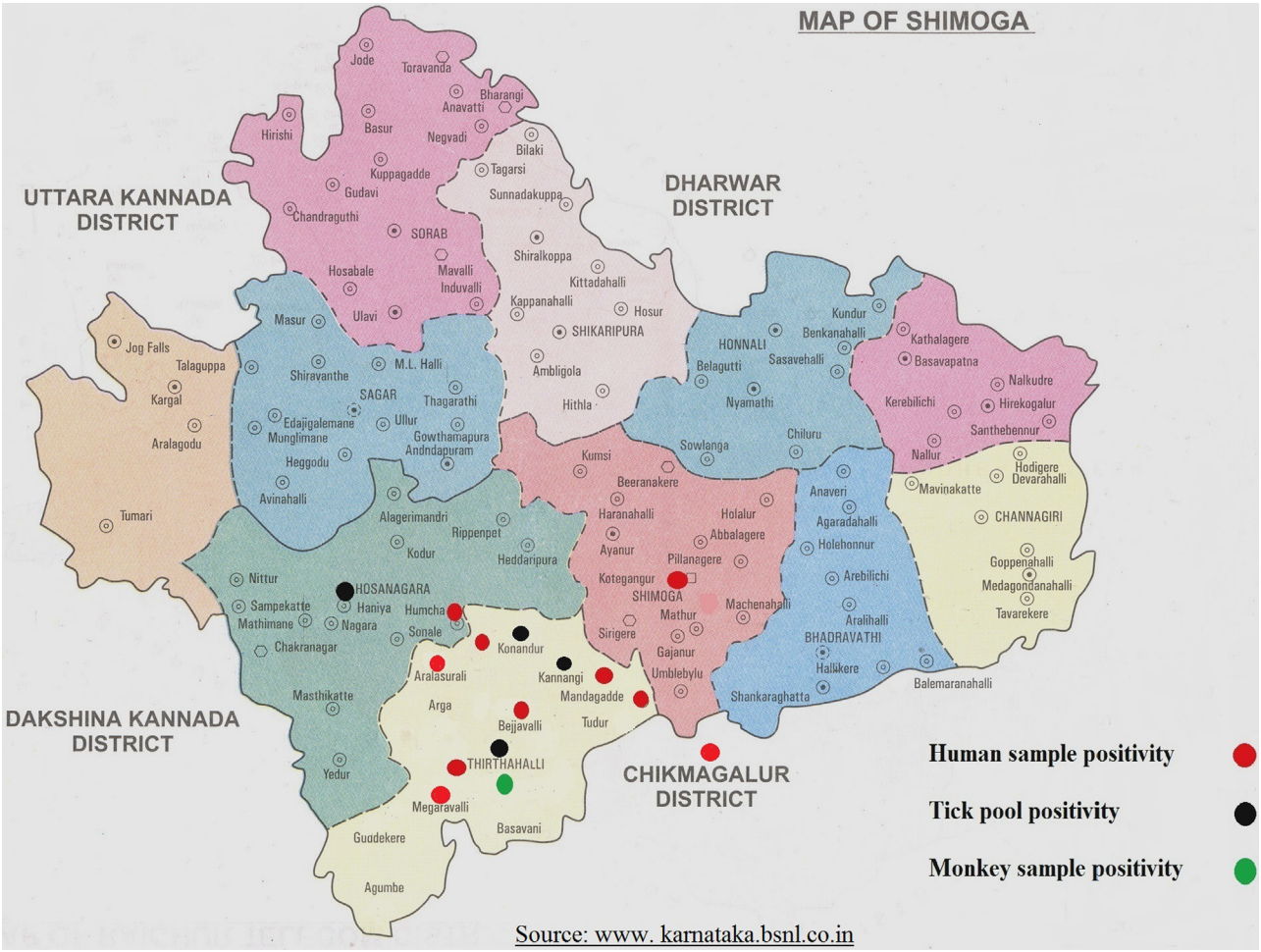

Figure 1. Human, monkey, and tick pool positivity for Kyasanur Forest disease virus in Shimoga District, Karnataka State, by area.

Jaya Chamarajendra (JC) taluk hospital in Thirthahalli. The present outbreak indicates the need for the implementation of vaccination among high-risk people who are residing in the affected areas and visit the forest for their livelihood in order to reduce infection with KFDV.

In some areas, although tick pools have been found positive, no human case has been reported. This means that no significant conclusions can be drawn from tick pool positivity; it only indicates the probability that the virus is enzootic or epizootic in the forest area but cannot be directly correlated with human cases. A focus on the collection and processing of tick pools for the presence of KFDV in affected areas will be helpful in assessing the existence of enzootic or epizootic disease in the forest area. This study also emphasizes the use of IgM ELISA for the diagnosis of KFD along with real-time RT-PCR, as there is chance of missing some positive cases if only one of the techniques is used.

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Ethical approval: Although this was an outbreak investigation, the institutional human ethics committee and institutional biosafety committee were informed. Confidentiality of the identity of cases and contact cases was maintained.

Conflict of interest: The authors do not have any conflict of interest. Financial support was provided by the National Institute of Virology, Pune, India.

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