ZIKV outbreak in Thiruvananthapuram, Kerala, India, 2021 – a primary report

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Short Report
Abstract

The recent global outbreaks of the ZIKA Virus (ZIKV) reported in 85 countries and territories caused severe complications such as microcephaly among neonates and Guillain-Barre Syndrome among the older population. Recently, an outbreak of ZIKV was reported from Thiruvananthapuram, the capital city of Kerala, India with 66 cases being reported so far. We conducted an outbreak investigation and the primary findings are described here. A cluster of ZIKV cases from the Kadakampalli / Anamugham administrative wards of the Thiruvananthapuram Municipal Corporation area was reported where Kerala Institute of Medical Sciences (KIMS) is located. Later many ZIKV cases were reported from other wards of the city. The density of known Aedes vectors was high in this region of the metropolitan city. Aedes albopictus, Aedes aegypti and Aedes vittatus collected from the focal area of the outbreak were found to be naturally infected with ZIKV. Male specimens of Ae. albopictus were naturally infected, indicating trans-ovarian transmission of the virus. This is first report of incrimination of Ae. albopictus and Ae. vittatus in ZIKV transmission from India. The virus was characterized and the partial sequences clustered with the Asian strain of ZIKV reported from India. The NS5 sequences of human and Ae. albopictus pools from Thiruvananthapuram were 100% similar indicating an ongoing active ZIKV transmission.

The state health authorities were sensitized and appropriate containment and vector control measures have been initiated to contain the outbreak. This report underscores the importance of continued human and vector surveillance as well as genomic sequencing to understand the virus evolution and implications on public health.

Main Text

WHO declared the Zika virus (ZIKV) outbreak as a Public Health Emergency of International Concern (PHEIC) on 1st February 2016, owing to high incidence of microcephaly caused by it in Brazil [1]. The benign viral disease hitherto, emerged highly neuro-virulence, causing microcephaly in neonates and Guillain-Barre syndrome in older people during the 2015 outbreak. The ZIKV outbreak reported initially in Brazil, affected about 85000 people in 85 Countries and territories across the Globe [2]. Three distinct ZIKV lineages, African, Asian and American exist globally [3,4]. The 2016 PHEIC of ZIKV was attributed to the American lineage. The S139N mutation, detected first in ZIKV outbreak of French Polynesia in 2013 was linked with microcephaly and enhanced virulence remained as a major genetic characteristic of this American lineage [5,6]. Primary transmission of ZIKV is through Aedes vectors, mainly Aedes aegypti and Aedes albopictus [7]. However, other transmission routes such as sexual, congenital, blood transfusion, and through body fluids have also been reported [8].

India reported sporadic ZIKV infections during 2016-2018 from Gujarat, Tamil Nadu, Rajasthan, and Madhya Pradesh. The maximum number of cases were recorded from Jaipur, Rajasthan (n=159) [9]. Genetic analysis of ZIKV strains at different time points established that the infections from India were due to Asian lineage of the virus [10,11]. ZIKV was detected in Ae. aegypti specimens collected from Jaipur, Rajasthan [12] and also belonged to the Asian lineage. None of the strains detected in India had
the crucial non synonymous mutations viz., S139N in the pre-Membrane gene, A188V in the NS1 region and M114V in the NS5 gene, the genetic signatures of the American Lineage of the Virus [5,6]. In November 2016, Zika was removed from the list of PHEIC, as the reported transmission of the disease declined.

Here we report a recent outbreak of the ZIKV from Thiruvananthapuram, the capital city of Kerala state, India. The area of the city is 310 Sq. Km, and the population is 1.69 million (National Census, 2011). Administratively the city have 100 wards. The population density is high, with 5400 persons/ Sq. Km. The city enjoys a tropical climate. Frequent outbreaks of dengue are being reported as the Aedes vector population is very high [13]. The first case of ZIKV was reported from a 24 year old pregnant female, a nearby resident who moved to the city about six days before developing symptoms of fever and erythematous rash. She was admitted to the Kerala Institute of Medical Sciences (KIMS), a multi-specialty hospital in Thiruvananthapuram city. As many cases of ZIKV were being reported, on the request of State Health authorities, we investigated the outbreak situation, to contain it. The primary outcome of the early investigation is reported here.

We visited the reported index case to track the primary source of infection. The duration of stay of the patient in the designated area, incubation period of the disease and vector density suggested that the first confirmed case would have contracted the disease in Thiruvananthapuram city (in Ward 25), where she resided, before developing the initial symptoms. In addition, it was ascertained that the index case was not her, as 14 out of 19 archived dengue and chikungunya negative samples, collected from symptomatic individuals in the same hospital since 15th May 2021 were re-tested positive for ZIKV. They were mostly hospital staff, who were residents of different wards of the city corporation viz., Kadakampally (Ward 92), Anamugham (Ward 95), Kannammoola (Ward 94), Nanthancode (Ward 25), Attukal (Ward 70), Pangappara (Ward 4), etc. while some resided in the hostel facility near the hospital. This led to clustering of cases in Kadakampalli / Anamugham (Wards 92/95) where the KIMS hospital is also located as well as in other areas such as Nanthancode (Ward 25), etc. It is suspected that the active transmission could have been due to high vector density while the contact with the body fluids of the patients cannot be ruled out.

As of 12th August 2021, 66 ZIKV cases had been reported, and their distribution showed that the cases occurred as a major cluster in the central region of the city (Figure 1). Subsequently, the cases have been reported from other parts of the city. A few cases (n=6) were reported from the surrounding regions of the metropolitan city and the neighbouring districts. On investigation, it appeared that most of these ZIKV infected persons from outside had frequently travelled for work to Thiruvananthapuram corporation areas. So far, six pregnant women have been diagnosed with ZIKV infection who are being actively followed up by the District Health authorities.

Four blood samples were also collected from suspected ZIKV cases in the area, who reported symptoms, after informed consents. After serum separation, RNA was extracted and subjected to Real-time RT-PCR using the Realstar ZIKV Virus RT-PCR kit, Altona Diagnostics, GMBH, Germany approved for EUAL by the
WHO for ZIKV diagnosis. The reactions were performed in Roche Light cycler 96. Two samples tested positive for ZIKV by RT-PCR.

The vector surveillance in the affected areas, Kadakampalli (Ward 92), Nanthancode (Ward 25), and Kumarapuram (Anamugham - Ward 95) of the city was launched, and *Aedes* mosquitoes were sampled from indoors and outdoors using standard tools. Entomological surveys showed a large population density of both *Ae. albopictus* and *Ae. aegypti*. The mean *Aedes* indices recorded were, Breteau Index (BI) = 58.49 and Pupal Index (PI) = 80.39. Other species collected were *Aedes vittatus*, *Culex quinquefasciatus*, *Armigeres subalbatus*, *Heizmannia discrepans*, *Mansonia annulifera* and *Mansonia uniformis*. All the *Aedes* species were processed for ZIKV infection after viral RNA extraction from the specimens. For extraction, the specimens were sorted as pools (maximum pool size of five specimens), based on sex and feeding status (abdominal condition) of *Aedes* females. Among mosquito samples processed (*Ae. aegypti*: n = 12; *Ae. albopictus*: n = 57; and *Ae. vittatus* n = 4) in 33 species-wise and sex-wise pools, six pools were found positive for ZIKV infection, which included one pool of *Ae. aegypti* (1 unfed female); 4 pools of *Ae. albopictus* (1 pool of male and three unfed (UF) female specimens) and one pool of *Ae. vittatus* (4 UF females).

This is the first report of *Ae. albopictus* and *Ae. vittatus* incrimination in ZIKV transmission from India. The Cq values of the eight positive samples (2 human isolates & 6 *Aedes* isolates) ranged from 12.04 to 35.05, with the positive control having a value of 30.48. The cut-off value of Cq for a positive reaction was set at 37.0, as per the kit protocol. The viral load of one *Ae. albopictus* was very high, with a Cq value of 12.04.

All the positives pools were subjected to conventional RT-PCR for ZIKV diagnostics [14], and ~180bp fragment of the NS5 gene sequences was amplified from two human samples as well as from two pools of *Ae. albopictus* specimens (one male and one female pool). One human ZIKV positive sample was from Kadakampalli (Ward 92), while the other was from Nanthancode (Ward 25) (Figure 1). Both the positive mosquito pools of *Ae. albopictus* were from the Nanthancode ward. The partial NS5 sequences confirmed that the infections were due to ZIKV (GenBank Acc. Nos: MZ670001, MZ686204, MZ686203 & MZ686202). One (from Kadakampalli ward) was 99.47% similar to the Asian strain of virus reported from India during the current pandemic season, and the other three pools recorded from Nanthancode were 100% identical to the Rajasthan strain of ZIKV (GenBank Acc. No. MK238037). The NS5 sequences of human and *Ae. albopictus* pools from Thiruvananthapuram were 100% similar. These initial findings indicated a primary role played by *Ae. albopictus* as a vector species in this outbreak. ZIKV infection in male specimens of *Ae. albopictus* evinced transovarial transmission of the ZIKV as described elsewhere [15].

Two synonymous SNPs were recorded among Thiruvananthapuram isolates and the Brazilian isolates of the virus in the partial NS5 gene sequences analyzed. The conventional PCR did not amplify NS5 sequences of ZIKV from *Ae. aegypti* and *Ae. vittatus*, which could be due to the low sensitivity or the low
viral load. Further processing of samples by sequencing of larger regions of the pre-Membrane, NS1, Envelope, and NS5 genes of the virus is being undertaken to detect the genetic lineage of the virus.

Overall, the investigation revealed active ZIKV transmission in Thiruvananthapuram city by at least three different species of *Aedes* mosquitoes. Based on these investigations, the district and state health officials were alerted and sensitized on the aetiology of the outbreak as well as on the immediate need to undertake intensive disease control measures. Targeted vector control interventions to reduce the adult *Aedes* population by Indoor Residual spraying and ULV fogging in addition to the intensive reduction of the vector breeding sources, had been initiated by the Health Department of Kerala.

This report highlights the need for enhanced case detection and containment measures in the areas experiencing high ZIKV transmission in Kerala. Besides, it is imperative to strengthen human and vector surveillance for ZIKV in various parts of India. Continuous genomic sequencing of emergent strains of ZIKV is also critical to understand the molecular evolution of the virus and its impact on human health.

**Declarations**

**Conflict of Interest Statement:**

Authors declare no conflict of interest in respect of this article

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This investigation was carried out as a rapid public health response on request of the Department of Health Services, Government of Kerala, India, to contain the disease outbreak. Most of the samples processed were from Aedes mosquitoes, as a part of vector surveillance. For four human samples collected from symptomatic cases during the investigation, informed consents were duly obtained from the patients as per institutional ethical guidelines for outbreak investigations.

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**Figures**
Figure 1

Distribution map of ZIKV cases (indicated as stars) in Thiruvananthapuram Corporation, Kerala, India. The red-coloured circle indicates the clustering of cases.