

**INDIAN COUNCIL OF MEDICAL RESEARCH
DIVISION OF EPIDEMIOLOGY & COMMUNICABLE DISEASES**

I. Zika Virus preparedness and response

Introduction: ZIKA, a mosquito –borne virus was first identified in monkeys in Zika forest in Uganda in the year 1947. Thereafter, sporadic human cases have been reported in various parts of the world. Outbreaks due to Zika virus have been reported from various countries since 2007. However, in October 2015, Brazil reported association of Microcephaly (an abnormally small head often with consequent brain damage) and later Guillain Barre Syndrome associated with Zika virus infection. As per information available from WHO, till date, 84 countries, territories or subnational areas have shown evidence of Zika virus transmission. A total of 31 countries and territories have reported Zika associated microcephaly. India’s neighbouring countries: Thailand, Singapore, Philippines, Vietnam, Bangladesh etc. have reported Zika transmission.

Mode of transmission & incubation period: Incubation period lasts for a few days (exact duration unknown). Zika virus is transmitted by the bite of infected *Aedes* mosquitoes, which is also the vector of dengue and Chikungunya. Human to human transmission of Zika virus through infected blood and semen is also established. In India, *Aedes* mosquito is widely prevalent.

Clinical picture & Complications: A majority of those infected with Zika virus either remain asymptomatic (up to 80%) or show mild symptoms of fever, rash, conjunctivitis, body ache, joint pains which usually subside between 2-7 days of onset of illness. Based on the available information of previous outbreaks, severe forms of disease requiring hospitalization are uncommon and fatalities are rare.

Treatment: There is no available drug or vaccine effective against Zika virus. Treatment is mainly supportive. Bharat Biotech International Ltd., an Indian company has a Zika vaccine candidate entering shortly into Phase I clinical trials.

Prevention and Control of Zika Virus disease: Vector control is the key to prevention and control of Zika virus infection.

Laboratory Diagnosis: Zika virus can be detected through molecular techniques within the acute phase of illness (3 – 5 days). There is no reliable serological test for diagnosis of Zika. CDC is currently providing Triplex RT-PCR kits which has WHO EUAL (Emergency Use Authorization Licensure).

Zika vaccines: Currently no vaccine is licensed for use. Globally, 45-50 Zika vaccine candidates are in pipeline. India has one inactivated vaccine candidate, being developed by Bharat Biotech International Ltd.

Summary of actions taken by ICMR for enhancing preparedness to tackle Zika virus in the country:

i. Capacity building for Zika diagnosis:

ICMR's NIV, Pune has conducted three training programmes wherein they have trained a total of 25 laboratories (including IDSP labs) for Zika virus diagnosis. The trainings were done from 15th to 19th February 2016, 18th to 22nd October 2016 and 27th – 28th January 2017.

All these labs are routinely testing for Zika virus in dengue and chikungunya negative patients with history of fever or travel. The results are being compiled at NIV, Pune on fortnightly basis.

The list of labs having capacity for testing Zika virus are as follows:

1. King Institute of Preventive Medicine, Chennai, Tamil Nadu
2. NIV Field Unit, Allapuzha, Kerala
3. Manipal Centre for Virus Research, KMC, Manipal
4. BJ Medical College, Ahmedabad
5. Regional Medical Research Centre, Bhubaneswar
6. National Institute for Cholera & Enteric Diseases, Kolkata
7. King George Medical University, Lucknow
8. Regional Medical Research Centre, Dibrugarh
9. Regional Medical Research Centre, Jabalpur
10. Jawaharlal Institute of Post Graduate Education & Research, Puducherry
11. Post Graduate Institute of Medical Education & Research, Chandigarh, (VRDL)
12. Desert Medical Research Centre, Jodhpur (ICMR)
13. Amrita Institute of Medical Sciences, Kochi, Kerala
14. Sri Venkateswara Institute of Medical Science, Tirupati Andhra Pradesh (VRDL)
15. Gandhi medical College, Secunderabad, Telangana (VRDL)
16. Rajendra Memorial Research Institute of medical Sciences, Agamkuan, Patna (VRDL)
17. All India Institute of Medical Sciences, Bhopal Madhya Pradesh (VRDL)
18. The Tamil Nadu Dr. M. G. R Medical University, Chennai Tamil Nadu,
19. All India Institute of Medical Sciences, Raipur Chhattisgarh
20. All India Institute of Medical Sciences, New Delhi
21. Institute of Preventive Management, Hyderabad
22. Haffkine Institute, Mumbai
23. NIMHANS, Bengaluru
24. Central Research Institute, Kasauli
25. NEIGRIHMS, Shillong

Besides, NIV has also provided primers, SoPs for testing etc to the following laboratories:

1. National centre for Disease Control, Delhi
2. Sher-i-Kashmir Institute of Medical Sciences, Srinagar
3. SMS Medical College, Jaipur

ii. Human surveillance network for Zika virus disease (ZVD) set up by ICMR:

a. Testing of human serum/blood and urine samples for Zika virus is being done by the above 25 trained laboratories. Till date, a total of 59,849 samples have been tested for Zika virus through this surveillance mechanism.

b. From Jan to June 2017, ICMR had set up AFI surveillance at BJ Medical College, Ahmedabad wherein samples from febrile patients attending BJMC OPD/IPD (including gynecology OP) referred to VRDL for dengue and chikungunya testing were being aliquoted and stored at the VRDL. These samples were transported to NIV for RTPCR. On an average, the VRDL received 40-50 samples daily. From Jan to June 2017, a total of 953 samples had been tested for Zika virus through this surveillance mechanism.

c. From Jan to June 2017, ICMR had set up ANC screening of pregnant women – An aliquot of samples collected from pregnant women attending ANC clinic of BJMC for routine testing (HIV/HBsAg etc) were collected and stored. These samples were sent to NIV for ZV serology. A total of 1180 samples had been tested for Zika virus through this surveillance mechanism.

Till date, a total of 61,982 samples have been tested for Zika virus through the above mechanisms. Samples from four different individuals have been found to be positive for Zika virus.

Details of the four positive cases are as follows:

1. Details of the first case reported from BJ Medical College, Ahmedabad:

A 34 year –old female of low socio-economic status, residing at Soneriya Block, Bapunagar, Ahmedabad came to BJ Medical College Hospital for delivery on 9/11/2016. A clinically well baby of 3.7 kg weight was delivered by Caesarean section on the same day. Patient was discharged after one week (on16/11/2016). No history of fever during pregnancy and no history of travel for the past three months. During hospital stay, she developed low grade fever, after delivery. Sample was collected on 14/11/2016, to test for dengue virus. Department of Microbiology at BJMC tested patient’s dengue and chikungunya negative serum sample for Zika and found it positive. Patient subsequently had difficulty in wound healing and visited the hospital twice.

Sample from the patient was referred to the Viral Research & Diagnostic Laboratory (VRDL) at BJ Medical College, Ahmedabad for dengue testing. Sample was thereafter found to be positive for Zika virus.

Sample was then referred to NIV, Pune. Further confirmation of etiology by RT-PCR and sequencing has been done. The sample was confirmed as Zika virus positive by Rt-PCR and sequencing at NIV, Pune.

2. Details of the second case reported from Ahmedabad, Gujarat through active ANC surveillance:

A total of 111 samples for ANC surveillance were collected from BJ Medical College, Ahmedabad between 6th to 12th January 2017. One sample from a 22 year old pregnant female in 37th week of pregnancy has tested positive.

3. Details of the third case reported from Ahmedabad, Gujarat through active AFI surveillance:

A total of 93 blood samples were collected during the Acute Febrile Illness (AFI) surveillance established at BJ Medical College, Ahmedabad, Gujarat. All samples had been collected between 10th to 16th February 2017 and were tested for Zika virus. One sample from a 64-year-old male presenting with febrile illness of 8 days' duration tested negative for dengue infection at BJMC, Ahmedabad. The sample turned out to be positive for Zika virus. This is the first Zika positive case of AFI surveillance at BJMC, Ahmedabad, Gujarat State.

4. Details of the fourth case reported from Anchetty, Tamil Nadu:

A 27 year old male admitted at Primary Health Centre, Anchetty, with acute febrile illness since last three days. He was admitted on 29th June 2017 wherein blood and urine samples as well as throat swabs were collected. Repeat samples were also collected on 2nd July 2017. All samples were tested at Manipal Centre for Virus Research, Manipal. The blood and urine samples collected on 29th June turned out to be positive for Zika virus RNA by Trioplex Real Time PCR (CDC Atlanta, USA). Further it tested positive for Zika virus RNA in blood and urine samples by WHO Zika virus generic and Asian lineage Real Time PCR. From the samples collected on 2nd July 2017, only urine tested positive for Zika whereas blood was negative.

Conventional PCR for Zika virus was done alongwith sequencing on 3rd July 2017 and 98% sequence similarity with Zika virus Asian lineage was detected. Further confirmation of Zika virus positivity was also carried out at NIV, Pune.

iii. Vector surveillance:

Three ICMR Institutes had initiated limited vector surveillance in different parts of the country since July 2016. List of labs conducting entomological surveillance for Zika virus:

1. Vector Control Research Centre (VCRC), Puducherry,
2. Centre for Research in Medical Entomology (CRME), Madurai,
3. National Institute of Malaria Research (NIMR), Delhi

RESULTS OF XENODIAGNOSIS FOR ZIKA VIRUS:

S. No.	Name of the Institute	Name of the mosquito collection site	No. of Mosquito pools tested	Results
1.	VCRC, Puducherry	Kerala state (8 Districts - Trivandrum, Ernakulam, Malappuram, Kottayam, Alappuzha, Idukki, Pathanamthitta and Thrissur) and Puducherry	Altogether, 21331 Aedes specimens (<i>Ae. aegypti</i> , <i>Ae. albopictus</i> and <i>Ae. vittatus</i>) were processed in 2930 PCR pools.	Negative for Zika

2.	CRME, Madurai	Tamil Nadu Nilgiris, Kanyaumari, Tirunelveli, Ramanathapuram and Madurai 3 districts	422 pools (9020 <i>aedes</i> mosquitoes tested)	Negative for Zika
3.	NIMR, Delhi	i. Delhi – 157 different localities - South Delhi & West Delhi and ii. different sites of Ahmedabad city: Bapunagar, B.J. Medical College, Sonariya Block etc.	566 pools (4256mosquitoes) 12 pools (129 mosquitoes)	Negative for Zika

Total mosquitoes tested = 34736. Technique used: conventional RT-PCR

Future course of action:

- The Reproductive & Child Health Div. of Ministry of Health & Family Welfare, under the Rashtriya Bal Suraksha Karyakram has initiated programmes on New Born Birth Defect (NBBB) Screening and still birth surveillance. The current NBBB registry focuses on reporting of 8 externally visible birth defects including microcephaly from 55 Medical Colleges of the country. The MoH&FW is keen to collaborate with ICMR for linking Zika testing in microcephaly cases. Out of the 55 Medical Colleges in the NBBB programme, DHR/ICMR VRDLs are located in 21 Medical Colleges. Directives have been issued to all 69 VRDLs of DHR and ICMR to initiate tracking and sample collection from all cases of Zika. Centres with capacity may test on their own, whereas others may collect appropriate samples and send them to NIV, Pune for testing. ICMR has disseminated SoPs for sample collection, transport and testing, to all 55 Medical colleges through the RBSK, MoH&FW.
- Vector mosquito surveillance for Zika virus is also being scaled up by training six more labs located in different regions of the country and thus increasing the geographic areas as well as numbers for testing. This will give an indication about mosquito carriage of the virus.
- Review of alternate strategies for vector control in India: ICMR in collaboration with Monash University, Australia is soon initiating work on Wolbachia based vector control for *Aedes* mosquitoes. MoU between ICMR and Monash University has been signed in February 2017. Further work is in progress.