

Research Article

An Integrated Approach for Effective Containment of Japanese Encephalitis: First JE Case of Pune

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A B S T R A C T

Japanese Encephalitis (JE) is a viral vector-borne disease. Natural hosts of the JE virus are water birds and pigs these amplifying hosts play an important role in disease transmission; man is an accidental host.

Pune district of Maharashtra state is non-endemic for JE; nevertheless, a sporadic case of JE was reported in November 2022 in an urban area of Pune. A six-year-old male child was found severely infected with JEV when tested by IgM ELISA technique using a CSF sample at the National Institute of Virology (NIV), Pune. In sero-surveillance, no other confirmed JE-positive case was observed.

In a veterinary survey carried out in the Vadgaonsheri area, 57 pig serum samples were collected and 40 pigs were found positive for JEV-neutralising antibodies, which confirmed JE virus infection in the locality. Necessary entomological survey and control measures were also implemented. Though the Pune district is not endemic for JE and routine vaccination is not suggested, a thoughtful decision to vaccinate the patient's two siblings aged below 15 years was taken as a precaution.

An integrated approach of the public health department, Maharashtra state, Pune municipal corporation and its Sassoon general hospital, ICMR-NIV- Pune, and NCVBDC, GOI on various fronts has resulted in early detection of JE case, effective and successful containment of JE using entomological, epidemiological, serological and veterinary surveillance.

Keywords: Japanese Encephalitis, JEV, Pigs, Pune, Non-endemic Region, Surveillance

Introduction

JE is a zoonotic infection and is a major vector-borne disease in Asia. JE is caused by the Japanese encephalitis virus (JEV).^{1,2} JE viral infection is caused by an RNA virus belonging to the Flavivirus genus. It belongs to the same genus as dengue, yellow fever and West Nile viruses.^{2,3} JE is a major zoonotic disease i.e. both animals and humans get infected with JE virus. The first case of JE was documented in 1871 in Japan and the Japanese encephalitis virus was first isolated from the brain of a patient with fatal encephalitis in Tokyo in 1935.^{4,5} It is transmitted by mosquitoes in humans primarily by *Culex* type, which feed on infected birds, pigs and other mammals including humans. People especially those who are living and working in rural areas around rice paddies and irrigation systems are more prone to getting infected as these are mosquitoes' preferred habitats.^{6,7}

JE is mostly considered a rural disease because of available and favourable conditions for *Culex* mosquitoes, rice paddies, slow current streams with vegetation in the farms and nearby fields, presence of water birds and pigs, and poor management of sanitation management practices and system.^{2,9} Besides, underdeveloped medical facilities with many of them lacking pediatric intensive care units (PICUs) etc. also add to critical situation in treatment. However, the risk of JE is changing from rural to semi-urban or urban areas. In an urban area, especially economically weak localities unhygienic and distorted sanitation system is also seen. Nullahs and rivers are also not in a clean state and the presence of slums on the banks of rivers and nullahs is very common. Piggeries are comparatively few in number but mostly located in slums and in crowded localities. These all factors act as input factors for the development of diseases like JE in both rural and urban areas.^{10,11} This relative risk of JE shift from rural to urban areas is also reported in other countries of southeastern Asia namely South Korea, China, Taiwan and Singapore.¹²⁻¹⁶

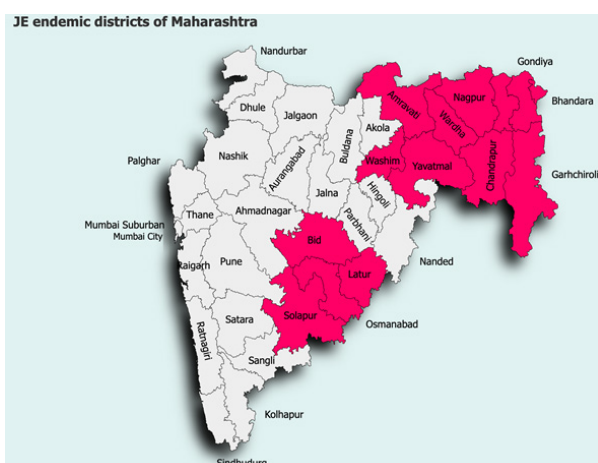


Figure 1. JE endemic districts of Maharashtra where regular immunisation is implemented are highlighted¹⁷

In Maharashtra, Vidarbha and Marathwada regions are mostly affected by JE. This region comprises around 19 sensitive districts for JE. These districts are namely, Nagpur, Chandrapur, Gondia, Gadchiroli, Washim, Akola, Latur, Parbhani, Wardha, Amravati, Yavatmal, Buldhana, etc.

Effective vaccines are available for the prevention of JE. In the year 2006, India launched the JE vaccination campaign in the 11 most sensitive states including Maharashtra for children falling under the age group of 1–15 years old. A regular vaccination program is implemented in these districts so as to cover maximum immunisation in the community.¹⁸ The details of JE vaccination accomplished in the year 2022 are given in Table 1.

Table 1. Coverage of JE Immunisation across Endemic Districts in Maharashtra State

District	JE 1st Dose Achievement (%)	JE 2nd Dose Achievement (%)
Amravati	99	89
Washim	100	100
Yavatmal	98	96
Beed	105	100
Latur	100	94
Osmanabad	85	46
Bhandara	93	92
Chandrapur	104	66
Gadchiroli	98	94
Gondia	108	93
Nagpur	103	98
Wardha	97	49
Solapur	93	66
State	98	83

In endemic districts, a single dose of live attenuated SA-14-14-2 JE vaccine was given to 1–15 years of age group. Since 2013, along with routine immunisation two doses of the JE vaccine, one at the age of 9 months and a second at the age of 16-24 months have been implemented.^{19,20} Many new vaccines including the JENVAC vaccine have been developed and introduced in India.^{21,22}

If considering the JE case and death data of Maharashtra from the year 2017 to the present, years 2017 and 2019 were observed to be mostly affected. Briefly, in the year 2017 a total of 26 JE positive cases with five deaths were reported, whereas in 2019 year, 39 JE cases with 110 deaths had been reported. The Western Ghats and the entire western belt of Maharashtra are non-endemic for JE. So, the JE immunisation program is not recommended here.

In areas endemic for JE, viral load is endogenous in

the locality. Sometimes it continuously gets expressed sometimes hibernates for a while and reappears. However, in non-endemic regions, it is a big challenge to identify the reservoir or the source of infection, and based on its mode of viral transmission can be further focused. Climate change can be a crucial factor playing a role in the infestation of JE in non-endogenous regions. An increase in temperature and Carbon dioxide (CO₂) levels may develop and strengthen the capacity of JEV and mosquito survival.^{23,24} Additionally, extended monsoons, deluges and stagnant water can also make the conditions favourable for the emergence of JE outbreaks in non-endemic regions, consequently, a threat of deadly zoonotic disease spread may loom.²⁵ Considering Maharashtra, Pune district is non-endemic for JE diseases; a report of the first JE case was itself an alert to the health system of Maharashtra state. However, a strong and integrated multi-disciplinary approach proved effective in the containment of the outbreak.

In addition to the treatment of the patient, identification of reservoirs, containment of the outbreak using multiple approaches of outbreak management and application of the preventive control measures were key challenges. This article focuses on the management of the first JE outbreak that occurred in Pune during October–November 2022.

Timeline of Clinical Management in the Hospital

Treatment of the JE-positive patient was carried out at Government-run tertiary care Sassoon General Hospital attached to BJ Government Medical College, Pune. It falls in the jurisdiction of Pune Municipal Corporation (PMC). On admission of the patient to Sassoon General Hospital on November 3, 2022, the patient required intensive care in the Pediatric Intensive Care Unit (PICU) for 18 days. The patient was on a mechanical ventilator for nine days followed by oxygen support for the next 9 days. Feeding was initiated via a nasogastric tube from the 10th day in PICU followed by being afebrile. Since then, the patient was given physiotherapy and antiepileptic medications. Details of the implemented clinical management of the JE case are discussed below.

Initial Evaluation

Initial workup from the emergency department of Sassoon Hospital revealed that the child was febrile with a headache for two days. Two episodes of convulsions and weakness on the left side of the body since day one were noted.

Immunisation History

The patient and his elder siblings all are under the age of 10 years and are not vaccinated for JE, as it is not recommended for residents of JE non-endemic districts including Pune.

Laboratory Investigations

After the admission of the patient to Sassoon General

Hospital a series of diagnostic tests were performed to get an exact diagnosis. The events reported by the hospital are as follows:

- On November 3, 2022, the child's Cerebrospinal fluid (CSF) was collected for diagnostic investigation. A clear and colourless CSF sample was noted with 120 lymphocytes, 60 macrophages and 70 polymorphs. Total sugar was 75mg/dl whereas total proteins were 16.68%.
- On November 11, 2022, a Computed Tomography (CT) scan of the brain was performed. No significant abnormality was detected. Further Magnetic Resonance Imaging (MRI) scan was advised.
- On November 17, 2022, the second time CSF sample was collected and cultured. Even the second time a clear and colorless CSF sample was identified. This time only two lymphocytes were detected in the CSF sample with a total sugar of 79 mg/dl, whereas total proteins were 59.52%. No growth was observed in CSF culture. To rule out the possibility of Tuberculosis meningitis, a CSF CBNAAT test was also performed which resulted negative. Then CSF and serum samples of the patient were sent to the Indian Council of Medical Research (ICMR)-NIV, Pune for proper diagnosis of encephalitic syndrome.

Diagnosis

On November 9, 2022, CSF and serum collected from the patient were referred for diagnosis to ICMR-NIV, Pune where these specimens were investigated by IgM capture ELISA to detect JE, West Nile and Dengue virus infections as described earlier by Sapkal et al.²⁶ Simultaneously, the CSF was investigated to detect JE infection using JEV specific RT-PCR and Herpes Simplex virus infection using HSV PCR as reported earlier by Sapkal et al. and Bondre et al.^{26,27} Both the CSF and sera collected during the acute phase were tested positive by IgM ELISA while JEV RT-PCR and HSV PCR yielded negative results. The second CSF specimen investigated at ICMR-NIV by JE IgM ELISA also tested positive confirming the patient as a JE case.

As soon as a positive JE case was detected, another major part of the outbreak management was the implementation of immediate and strong control-preventive measures for JE outbreak containment in the Vadgaon Sheri area of Pune.

Micro-planning for JE Containment

On a report of the first case of JE in Pune, combined meetings and visits by officials of the Public Health Department, Government of Maharashtra along with ICMR-NIV and Pune Municipal Corporation (PMC) and its Sassoon Hospital were carried out. Technical guidance from NCVBDC, GoI was obtained. A strong and detailed action plan covering multiple aspects was prepared for early case detection,

clinical as well as epidemiological investigations, appropriate and complete treatment and containment measures to save the patient and avoid further transmission of JE in the community.

The details of multi-disciplinary-approach micro action plans are discussed below:

JE Case Background

The JE case was reported from Vadgaonsheri (at geographical coordinates 18.54984° N, 73.92109° E) which falls under the Pune Municipal Corporation administration area. Multidisciplinary teams including the Public Health

Department, Government of Maharashtra along with PMC worked together as a government authority for the overall management of the outbreak, ICMR-NIV, Pune contributed to surveys and laboratory investigations whereas Sassoon General Hospital was involved in the clinical management and treatment of the patient. The teams worked together to investigate the outbreak collaboratively for case detection, early initiation of appropriate and treatment, epidemiological investigations, and suggestions for containment measures to avoid further transmission of JE in the community.

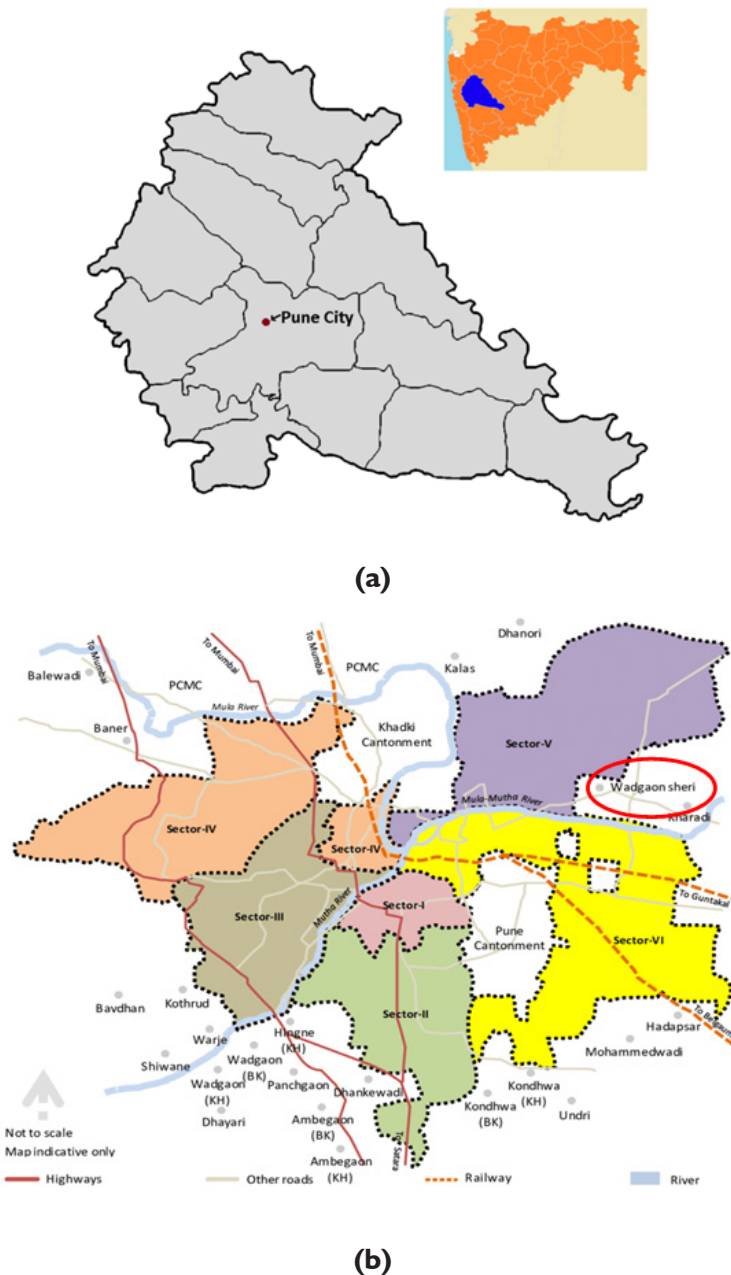


Figure 2(a).Map of Pune City (b)Vadgaonsheri Area in a Pune City Map is Circled with Red Colour^{28,29}

Surveillance

In this JE outbreak, four major surveillances were implemented. The five major surveillances planned were namely epidemiological, fever, serological, veterinary and entomological. These surveillances were carried out in coordination with different field workers and experts to strengthen the effectiveness of the control and prevention measures for the JE outbreak.

Epidemiological Surveillance

The expert's team visited Vadgaonsheri ward of Pune Municipal Corporation. The reported JE-positive patient resides in a crowded and below-poverty area. The house of the patient was marked by the presence of piggeries around and a nullah passing through the nearby area. Another major notation was the presence of a school (Inamdar School) in the surrounding of the patient's residence.

Anthropological Details of the JE Case

The JE-positive case of Pune is a male child of age four years. By birth, the child was reported to be healthy. The development and growth of the child were normal, without previous seizures or any medical deformities.

Social History

The identified JE patient lives with his parents and two elder siblings who are under the age of 10 years. The mother of the child works as a housemaid whereas the father is a bus driver for a private company and drives in and around Pune.

Migration History

The patient had no travel history in the last four months.

Capacity Building

The Pune Municipal Corporation health department staffs viz, multi-purpose workers (MPWs), and ASHAs were first trained by experts to conduct a JE special survey, what and to whom questions to ask, etc. These trained human resources along with supervisors divided into teams. These teams were allotted particular areas to carry out the survey.

Fever Survey

First of all, areas were marked in such a way that the entire population of the designated area would be covered. To these marked areas, teams were allotted accordingly and a fever survey was carried out. House to house fever survey was carried out. Teams were provided with a JE-related questionnaire to ask in the survey. These questions were as follows:

- Does anybody had/ have fever in the house?
- If yes, then when did you get fever?
- Have you visited anywhere in the month?
- Do you have any neurological symptoms (Headache, seizures?)

- Are there any piggeries nearby?

Teams were asked to focus mainly on all the children below 15 years of age. They were asked to be screened thoroughly and serum sample collection was a must for them. Teams were advised to collect serum samples for testing from all the fever patients. In the fever survey, 100 houses in proximity to the patient's residence were screened. A total of 23 individuals were identified with fever or history of fever.

In the surveillance, it was noted that a school is located in the proximity of the JE-positive patient's residence. So as a precaution and preventive measure, an extended fever survey for nearby Inamdar School children was carried out to detect if any other JE incidence has occurred and circumvent further transmission in the community. However, no child was observed with fever and other supporting symptoms for JE.

Sero-surveillance

In the fever survey performed on November 31, 2022, in close proximity to the patient's residence, a total of 23 fever cases were detected. These suspected cases were kept under observation at a sub-district hospital in Pune. Serum samples of these suspected cases were collected by the PMC health department and NIV staff. At NIV, Pune, the sera from fever cases were investigated for JE infection using JE IgM ELISA in which three cases tested positive for JE IgM antibodies however second sample as well as the CSF test of these three patients were detected negative, hence not considered as confirmed JE positive case. All remaining 20 patients tested negative for JE IgM antibodies.

Entomological Surveillance

Dusk collection of mosquito larvae was performed in the nearby waterbodies, ditches and drains and collected using the dipping method. Resting adult mosquitoes were collected using mechanical aspirators. A CO₂-based trap was set up in the premises of the positive case to trap flying adult mosquitoes during the nighttime. The collected larvae and adults were brought to the laboratory for further processing. After digestion of blood meal, these mosquitoes were identified up to species level and separated into different pools (pool size varying from 10 to 100 mosquitoes). The pools were homogenised and tested for the JE virus using the RT-PCR technique.

Entomological surveillance was a major section as mosquito species namely *Culex* group species *tritaeniorhynchus* and *vishnui* are primary vectors for JE transmission. These mosquitos favour dirty slop water with vegetation. *Culex* group is zoophilic which mostly feeds on pigs and cattle.³⁰ The public health department along with ICMR- NIV, Pune carried out adult and larval surveillance. A total of 1144 *Culex* mosquitoes were processed in 99 pools. All the pools

tested negative for JE virus in RT-PCR. The identified species of mosquitoes were *C. gelidus*, *C. tritaeniorhynchus*, *C. quinquefasciatus*, *C. vishnui*, *C. fuscocephalus* and *Anopheles subpictus*. The details of mosquito species collected and identified are mentioned in Table 2.

Table 2. Species of Mosquitos and Numbers Collected in the JE-affected Vadgaonsheri Area of PMC

Mosquito Species	Number
<i>C. gelidus</i>	680
<i>C. tritaeniorhynchus</i>	394
<i>C. quinquefasciatus</i>	60
<i>Anopheles subpictus</i>	8
<i>C. fuscocephalus</i>	1
<i>C. vishnui</i>	1

Veterinary Surveillance

After the JE case identification, a major step was to investigate the reservoir of the disease in the affected area. The veterinary department of Pune Municipal Corporation was also informed about the JE case. The veterinary department collected 57 serum samples of the pigs from the surrounding area of the patient's residence. These samples were investigated at NIV, Pune to determine the presence of JE virus RNA by RT-PCR and anti-JEV neutralising antibodies by plaque reduction neutralisation test. All the 57 pig sera tested negative by RT-PCR whereas the presence of anti-JEV neutralising antibodies in 40 (71%) pig sera was detected.

This investigation was another strong piece of evidence confirming the presence of JE in Pune. This was also a key point in identifying the source of infection and further control measures for planning and avoiding transmission.

In coordination with these surveillance activities following were implemented in the JE-affected area:

Integrated Vector Management

Apart from surveillance activities in the hotspot area, vector control and preventive activities under integrated vector control management were carried out. This involved activities like space spray using Ultra Low Volume (ULV) fogging with pyrethrum/ malathion indoors and outdoors. Anti-larval activities involved chemical larvicides- abating and identification of breeding sites and introduction of larvivorous Guppy fishes to breeding sites.

Surveillance at Latur

Fever surveillance was carried out at the Chakur block of Latur district, which is a native place of patients. All the required instructions were provided to the district from the

state. A house-to-house fever survey was carried out. No JE-positive patient was identified in this survey.

Information, Education and Communication (IEC)

Strong IEC activity for community sensitisation and awareness by health department staff through visits and personal communication, using banners, and meetings at schools, public places, and health facility campuses were carried out.

Immunisation

Another major issue was precautions to be taken to prevent JE infection in the patient's siblings. The patient's two siblings were also under the age group of 10 years, which is a sensitive age group for JE infection. Hence JE vaccination to these siblings was given as a precautionary measure towards their chances of acquiring JE from patients or surroundings. Safe and effective JE vaccines are available to prevent disease.¹⁹⁻²²

Discussion

Japanese encephalitis is a serious vector-borne disease threat as the case fatality rate of JE can be around 30%. Intensive care support can help reduce mortality. Besides, permanent neurologic or psychiatric sequelae can occur in 30% to 50% of those JE cases.³¹⁻³⁴

The major vectors responsible for JE transmission are *Culex sp.* of mosquitoes. Water birds and pigs are the main sources of infection. Pigs are amplifying vectors and have the capacity to multiply the virus many-fold without suffering seriously from disease and maintaining it for a prolonged period. In India, around 12-44% pig population has been reported to be positive for JE antibodies. Unorganised domestic piggeries are a major factor contributing towards increased risk of JE transmission among humans.^{18,35,36}

In the present scenario, pigs were identified as the chief amplifying reservoir of the JE virus. The presence of neutralising antibodies against JEV in 40 pig sera samples suggests active transmission of JEV in the affected region. The piggeries were in close proximity to the patient's house. According to literature if a healthy adult pig contracts JE viral infection, it develops symptoms such as fever and nasal secretions for 4-5 days. Further, they develop antibodies against the virus.^{37,38} Mother-to-foetus transmission of antibodies has been documented in pigs. The newborn may have antibodies against the JE virus for the first few months after birth.³⁸

Besides, JE transmission among pigs in the absence of an arthropod vector is also reported. Tonsils are the major site of JEV replication in the pigs. In spite of a large number of neutralising antibodies present in the tonsils, JEV persists in

tonsils for 25 days. Pigs shed JEV in their oronasal secretions and this becomes a critical source of infection among pigs. This transmission mechanism is a serious threat to JE infection in humans.^{19,37,38}

In the current JE outbreak in Pune, among the 57 collected serum samples of the pigs, 40 samples were found positive for antibodies. Viral transmission through nasal secretions could be a major reason for JE virus infection among these pigs.

The density of *Culex* mosquitoes in this area is also very high. All *Culex* spp which are known transmitters of JE infection were found to be present in the area. Conducive environment like unclean drains is abundantly present in the area. It could also be postulated that the pig population along with high vector density and unclean environmental conditions could have played major roles in the transmission of the virus to the human population.

However strong and integrated multi-disciplinary approaches of vaccinating the sibilings, integrated vector control, isolation of suspected pigs and cleaning of surroundings proved effective in disease containment in a single JE case. Strengthening surveillance for fever and other neurological symptoms among school children and in the affected locality was an important preventive measure for early case detection if any exists. Also, the availability of medical facilities especially pediatric intensive care units (PICUs) is an essential factor in the clinical management of JE cases.

Conclusion

We report the successful multi-disciplinary control efforts involving the community, health authorities and local government that have resulted in early control of a potential JE outbreak. The investigation helped to identify the possible risk factors that were widely prevalent in the area. Acute Encephalitic Syndrome (AES) surveillance along with intermittent sero-surveillance in symptomatic cases as well as in pigs must be carried out even in the JE non-endemic regions for JE to get an early warning signal of a possible JE outbreak. It is also advisable to carry out JE vaccination among pigs to avoid JE virus transmission to humans.

Conflict of Interest: None

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