

## Trend of Japanese encephalitis in Uttar Pradesh, India from 2011 to 2013

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### SUMMARY

As indicated by the sporadic Japanese encephalitis (JE) cases reported from the districts of Uttar Pradesh (UP), India, the disease is endemic in the state despite the fact that a JE vaccination programme has been ongoing in the state since 2006. Hence, the present study was undertaken to study the annual trend of JE in UP during January 2011 to December 2013. CSF and/or serum samples collected from acute encephalitis syndrome (AES) cases were referred to the virology laboratory at King George's Medical University, Lucknow and were tested for anti-JEV IgM antibodies by JEV MAC-ELISA kit. The study reveals that 26·9%, 9·9% and 14·8% of AES cases were positive for anti-JEV IgM in the years 2011, 2012 and 2013, respectively. Of the total JE confirmed cases, 30% were adults. Males were more commonly affected than females. A distinct peak of JE was seen in the monsoon and post-monsoon season, although sporadic cases were also reported in other months. JE vaccination by district in UP is discussed. This study reports that the proportion of JE positives in AES cases is decreasing in UP although the number of AES cases has not decreased. The study also discusses the probable causes of this decrease, including JE vaccination and natural periodicity due to herd immunity.

**Key words:** Epidemiology, general virology.

### INTRODUCTION

Japanese encephalitis virus (JEV) is a single-stranded RNA virus belonging to the family Flaviviridae, genus *Flavivirus*. The first major epidemic of Japanese encephalitis (JE) was described in 1924 in Japan, in which about 6000 cases were reported [1]. Since then, JE has been increasingly recognized from most countries of East and South-East Asia, causing an estimated 67 900 JE cases annually in 24 JE-endemic

countries [2]. JE has a high case-fatality rate of 20–30% and is known to cause residual neurological or psychiatric sequelae in 30–50% of survivors [2].

In India, the preliminary evidence of the presence of JEV was found in 1952, and the primary human case was reported from Tamil Nadu in 1955 [1]. The disease was confined to the southerly parts of India with a low prevalence until 1973, when the first major epidemic occurred in the north-eastern state of West Bengal [3]. Currently, in India, JE is the principal cause of vaccine-preventable encephalitis. The annual incidence of JE varies from 1714 to 6594 and mortality varies from 367 to 1665 individuals [4]. JE is now endemic in several states in India, including

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Bihar, Uttar Pradesh (UP), Assam, Manipur, Andhra Pradesh, Karnataka, Madhya Pradesh, Tamil Nadu, Haryana, Kerala, West Bengal, Orissa, Union territories of Goa and Pondicherry [5], with epidemic activity in northern and central parts of India [6].

Since the occurrence of the first epidemic of JE in UP in 1978, annual outbreaks of the disease have been recorded from the northern and eastern districts of the state [1]. Factors conducive to JEV exist in these areas and include: the vector (*Culex* spp. mosquitoes); environmental conditions suitable for the vector; ample mosquito habitats such as irrigated paddy fields, swamps, marshes; and presence of amplifying hosts, including pigs and birds. Therefore, in 2006 the Government of India launched a one-time mass JE vaccination programme in highly endemic districts of UP under which a single dose of SA14-14-2 vaccine was given to children aged 1–15 years [7]. This was followed by incorporation of the vaccine in the Universal Immunization Programme in the same districts. Seven of 11 districts (Gorakhpur, Deoria, Kushinagar, Maharajganj, Lakhimpur-Khiri, Sant Kabir Nagar, Siddharthnagar) reported more than 95% coverage of the estimated target. After this success, the programme was extended further in a phased manner to other endemic districts. In 2007, seven more districts (Bahraich, Balrampur, Basti, Gonda, Raebareilly, Saharanpur, Shravasti) were covered. In 2008 and 2009, nine (Azamgarh, Ballia, Bareilly, Faizabad, Hardoi, Lucknow, Muzzafarnagar, Sultanpur, Unnao) and seven (Allahabad, Pratapgarh, Kanpur Nagar, Shahjahanpur, Fatehpur, Jaunpur, Ghazipur) districts, respectively, were included [1].

Based on the laboratory surveillance data from January 2011 to December 2013, the annual trend of JE in UP, India is reported here, considering Lucknow as the epicentre for reporting purposes. The clinical records of the cases were analysed for demographic details.

## MATERIAL AND METHODS

The King George's Medical University (KGMU), Lucknow, is an apex hospital in the state of UP, that attracts patients from across the state especially from central and eastern parts of the state. These cases use the institutional laboratory services, including that of the Virology laboratory for diagnosis. This study analysed the clinical and laboratory data sheets of these cases in a cross-sectional observational study pattern.

## Geography of UP

UP is located between latitude 24–31° N and longitude 77–84 °E. By area, it is the fourth largest state of India and is made up of 76 administrative districts, grouped into 17 divisions. The entire state, except for the northern region, has a tropical monsoon climate. There are three seasons in UP: (i) summer season (March–May), when the daytime temperature remains very high and usually touches 45 °C, (ii) winter season (October–February), when the night time, temperature dips to freezing point and (iii) monsoon season (June–September), with 85% of the average annual rainfall of 99 cm and a temperature of 30–45 °C. The topography of the region makes it prone to annual flooding; the most affected regions being five districts in eastern UP (Gorakhpur, Kushinagar, Maharajganj, Sant Kabir Nagar, Siddharthnagar), traversed by major rivers originating in the Nepali hills.

## Study population

Data analysed are of cases referred to the Virology laboratory with clinical diagnoses of acute encephalitis syndrome (AES) during January 2011 to December 2013 for aetiological diagnosis. A case of AES was defined as a person of any age, at any time of a year with the acute onset of fever and a change in mental status (including symptoms such as confusion, disorientation, comatose, or inability to talk) and/or new onset of seizures (excluding simple febrile seizures) according to WHO guidelines [8]. Cerebrospinal fluid (CSF) and/or serum samples from the patients were tested for anti-JEV IgM antibodies. For diagnosing JEV infection, CSF was the preferred sample. When CSF was not available, anti-JEV IgM testing was done on the serum sample.

Patients' age, sex, and residential address were collected from the laboratory request forms and clinical data sheets. Written informed self or parental consent was taken for each case for laboratory test at the time of enrolment. The study was approved by the institutional (KGMU) ethics committee.

## Laboratory diagnosis

Anti-JEV IgM antibodies were tested by IgM antibody capture (MAC) enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions. Commercially available kits (Panbio

Inverness Medical, Australia) were used until December 2011. Anti-JEV MAC ELISA kit supplied by the National Institute of Virology (NIV), Pune, India was used thereafter. These reagents are an integral part of National Vector Borne Disease Control Programme (NVBDCP), India. The anti-JEV IgM kit from NIV contains all ready-to-use reagents, and its test performance was evaluated by the Christian Medical College, Vellore, India [9] and the Centers for Disease Control (CDC), USA [10]. According to US CDC performance evaluation, the NIV kit has a sensitivity of 75% in CSF, and 71% in serum and a specificity of 96% in CSF and 77% in serum. By contrast, the Panbio kit has a sensitivity of 75% in CSF, and 32% in serum and a specificity of 100% in CSF and 98% in serum [10]. All anti-JEV IgM positive cases were tested for anti-dengue (DENV) IgM antibodies by ELISA (MAC ELISA kit, NIV Pune, India).

#### Collection of JEV vaccination data

Details of JEV vaccination were obtained from the Health Management Information System (HMIS) of National Rural Health Mission (NRHM) [11].

#### Statistical analysis

The statistical test adopted for this study was the test of significance. Data were analysed using GraphPad Prism version 5 (<http://graphpad-prism.software.informer.com/5.0/>).

## RESULTS

During the study period, total 2692 AES cases were enrolled, of which 460 (17.1%) tested positive for anti-JEV IgM antibodies and negative for anti-DENV IgM antibodies. The number of JEV positives was high in 2011 (26.9%), but decreased markedly in the years 2012 (9.9%) and 2013 (14.8%) (Table 1). In 2011, the majority of cases were diagnosed using serum samples. Between 2012 and 2013, JE diagnosis was predominantly based on CSF testing, as obtaining a CSF sample from each case of AES was insisted upon. However, the year-wise percent positives of anti-JEV antibodies in CSF and serum samples were comparable (Table 1).

Table 2 details the demography of anti-JEV IgM-positive cases. The data on all the AES cases are not shown here to avoid selection bias that may

Table 1. Year-wise JEV positivity in AES cases diagnosed by detection of anti-JEV IgM antibodies in CSF/serum samples

Samples tested	Positive/total tested (%) for anti-JEV IgM		
	2011	2012	2013
Total cases	225/836 (26.9)	81/812 (9.9)	154/1044 (14.8)
CSF	92/319 (28.8)	46/532 (8.6)	146/974 (14.9)
Serum	133/517 (25.7)	35/280 (12.5)	8/70 (11.4)
P value	0.33	0.08	0.48

JEV, Japanese encephalitis virus; AES, acute encephalitis syndrome; CSF, cerebrospinal fluid.

arise due to the hospital-based nature of the study. It is interesting to see that 30% of all JE cases were adults (>15 years) and 68% of all JE-positive cases were males. A similar pattern was seen throughout the study period as seen by year-wise analysis.

The majority of the AES cases referred to the Virology laboratory was from Lucknow and the surrounding districts (within 150 km radius of Lucknow) which included the districts Hardoi, Sitapur, Barabanki, Sultanpur, and Lakhimpur-Khiri (Table 3). All these districts lie in the traditional JE belt of eastern UP. The JE vaccination coverage in the districts referring cases to the Virology laboratory is shown in Figure 1. Districts with high JE vaccination coverage (>70% vaccination coverage) include Bahraich, Balrampur, Gonda, Lakhimpur-Khiri, Hardoi, Siddharthnagar, Basti, Shravasti, Gorakhpur, Fatehpur, Deoria and Kanpur. Details of JE vaccination coverage are shown in Figure 1.

JEV cases were plotted by month, using the recorded date of onset of symptoms (Fig. 2). In 2011, 2012 and 2013 a distinct peak of JE cases occurred during August to October. Sporadic cases of JE were observed throughout the year in 2011, with an additional peak in February. A small second peak was also observed in February in 2012 and 2013.

## DISCUSSION

JE is one of the most important causes of viral encephalitides in UP [12, 13]. In the current study, an attempt was made to analyse the trend of JE during January 2011 to December 2013. The present study as well as another study from West Bengal [3] showed that the percentage of anti-JEV IgM-positive cases declined sharply after 2011. This may be attributed

Table 2. Age and sex distribution of anti-JEV IgM antibody-positive acute encephalitis syndrome cases diagnosed at the Virology laboratory, King George's Medical University, Lucknow during 2011–2013

Age group (years)	2011 (N = 225)			2012 (N = 81)			2013 (N = 154)			Combined (N = 546)		
	M, n	F, n	Total n (%)	M, n	F, n	Total n (%)	M, n	F, n	Total n (%)	M, %	F, %	Total, %
<5	37	16	53 (23.5)	7	5	12 (14.8)	12	8	20 (12.9)	12	7	19
≥ 5–14	70	35	105 (46.6)	28	17	45 (55.5)	64	24	88 (57.1)	35	15	50
≥ 15	47	20	67 (29.7)	13	11	24 (29.6)	27	19	46 (29.8)	19	11	30
Total (%)	154 (68.5)	71 (31.5)	225	48 (59.3)	33 (40.7)	81	103 (66.9)	51 (33.1)	154	68	32	

JEV, Japanese encephalitis virus; M, Male; F, female.

to mass vaccination, better awareness programmes, or herd immunity. Additionally, cross-protection by other flavivirus infections, notably dengue, could have contributed to the decline in JE cases. Previous studies have demonstrated the neutralizing activity of anti-DENV envelope glycoprotein antibodies and anti-DENV NS1 antibodies against JEV [14]. A steady rise in the number of dengue cases has also been reported from India after 2011 [15].

The preferred sample for establishing the diagnosis of AES is CSF. The presence of virus-specific antibodies in serum may indicate previous vaccination or asymptomatic infection [16]. In the present study, efforts were made constantly to insist on CSF collection and testing in each case of AES; therefore, the number of CSF samples increased steadily from 2011 to 2013. In 2011, since serum samples were more commonly referred than CSF samples, the Panbio kit with a high specificity in serum samples was used to preclude false positives, although the kit has a low sensitivity. After 2011, the NVBDCP declared a mandate, according to which all laboratories in Government settings in India were required to use the MAC ELISA kit manufactured by NIV, Pune to collect uniform countrywide data. Hence, the ELISA kit was changed in 2012. Low sensitivity of ELISA kits used in the present study might [10] have missed diagnosis of JEV in some cases, leading to an underestimation of the real burden of disease. Consequently, the data presented here may give a wrong assurance of successful vaccination campaigns. To exclude JE false positives, the samples were also tested for DENV infection (cross reactivity), and each CSF sample was examined macroscopically and microscopically prior to testing by JE ELISA to rule out blood contamination of CSF during a lumbar puncture.

The data presented in this study summarize the age and sex distribution of JE-positive cases and do not take into account all the AES cases enrolled. This was done to avoid selection bias that may arise due to the hospital-based nature of the study. It suggests that JE may affect all the age groups, but children aged between 5 and 15 years bear the brunt of the disease. Unlike previous studies on AES in UP [17], this study shows that of all the JE encephalitis cases, 30% are adults. About one-third of all JE cases in adults indicates a transient immune status of the community. Studies have indicated that in endemic areas in unvaccinated populations, JE is largely a paediatric disease [18], and most people have acquired immunity by

Table 3. Japanese encephalitis (JE) cases diagnosed at the Virology laboratory, King George's Medical University, Lucknow during 2011–2013

District	2011		2012		2013		Distance from Lucknow (km)
	No. tested for JE (N = 836)	JE positive by IgM (N = 225)	No. tested for JE (N = 812)	JE positive by IgM (N = 81)	No. tested for JE (N = 1044)	JE positive by IgM (N = 154)	
Lucknow	156	36 (23.1)	123	3 (2.4)	158	8 (5.1)	0
Hardoi	63	16 (25.4)	79	10 (12.7)	104	13 (12.5)	101
Sitapur	87	25 (28.7)	73	5 (6.9)	100	19 (19)	91
Barabanki	74	28 (37.8)	47	4 (8.5)	75	18 (24)	28
Sultanpur	60	12 (20)	54	8 (14.8)	73	13 (17.8)	135
Lakhimpur-Khiri	33	9 (27.3)	29	6 (20.7)	54	14 (25.9)	130
Faizabad	31	8 (25.8)	29	3 (10.3)	49	8 (16.3)	119
Gonda	43	14 (33)	42	5 (12)	46	9 (20)	119
Raebareili	27	8 (29.6)	25	6 (24)	42	6 (14.3)	77
Bahraich	43	18 (41.9)	36	4 (11.1)	41	7 (17.1)	128
Basti	32	8 (25)	31	3 (9.7)	38	4 (10.5)	206
Unnao	38	8 (21.1)	35	2 (5.7)	36	5 (13.9)	65
Ambedkarnagar	15	2 (13.3)	28	2 (7.1)	28	3 (10.7)	220
Balrampur	13	3 (23.1)	0	0	28	6 (21.4)	157
Amethi	3	0	11	4 (36.4)	25	5 (20)	138
Shravasti	3	0	0	0	18	2 (11.1)	163
Siddharthnagar	8	1 (12.5)	13	2 (15.4)	11	2 (18.2)	252
Pilibhit	1	0	0	0	10	2 (20)	261
Gorakhpur	8	2 (25)	16	0	9	0	270
Other districts of Uttar Pradesh	59	19 (32.2)	139	14 (10.1)	101	12 (11.9)	

Values in parentheses are percentages.

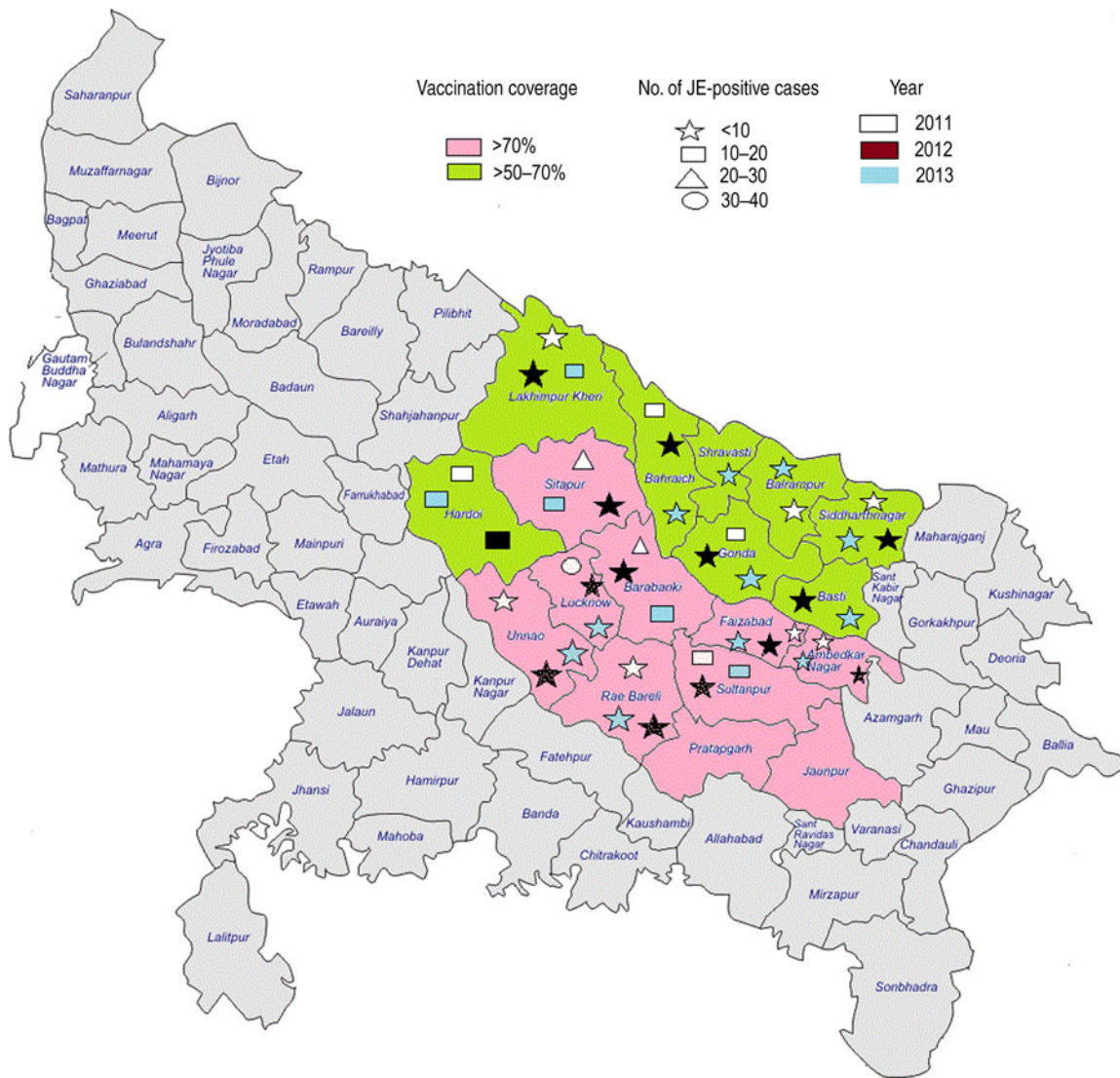
adulthood owing to subclinical infections [19]. A different pattern has been observed in areas with long-standing, high-quality childhood vaccination programmes, where JE is usually a disease of non-immune adults [20]. Males were more commonly affected than females as has also been reported previously [17]. This may be attributed to more outdoor exposure of males during the peak biting time of the exophilic mosquito vector due to nature of their work [17].

In the present study, the JEV positives also belonged to the vaccine-covered districts. The possible explanations include (1) a moderate JE vaccination coverage in the majority of the districts during 2011–2013, thereby leaving a considerable proportion of the population still at risk; (2) recent vaccination or asymptomatic infection may lead to anti-JEV IgM positivity in serum. The SA14-14-2 live attenuated JEV vaccine used in India; derived from GIII strain cause significant cross-reactivity with the heterologous strains of JEV representing genotypes, I–IV [21], and (3) ineffective vaccination.

History of JE vaccination was not available in many cases and therefore, was not used for analysis in the present study.

Maximum cases of JE were concentrated during August–October, corresponding to the monsoon and the post-monsoon seasons, when the transmission of vector-borne diseases rises due to an upsurge in vector density. Previous studies conducted to identify favourable ecological conditions of mosquitoes have indicated that temperature between 22.8 °C and 34.5 °C is optimal for JEV vectors [22]. A desirable temperature, combined with the flooded habitats during the wet season provides an ideal niche for larval development and adult survival. The importance of temperature has been highlighted by the fact that fewer mosquitoes are present at locations where temperatures do not fall into the favourable range during monsoons [23]. At a higher temperature due to a rise in frequency of viral replication and increased human–mosquito contacts, a decrease in virus transmission time is seen [23]. With the onset of the winter season, temperature decreases and JEV subsides. A second





**Fig. 1.** Map of Uttar Pradesh (UP). Number of JE-positive acute encephalitis syndrome cases, year-wise, in various districts of UP is shown. The number of cases testing positive for anti-JEV IgM is shown by various shapes and the year is represented by a colour, e.g. a blue star indicates that <10 cases were positive in 2013. Grey areas usually do not refer cases to the Virology laboratory; hence data from these districts were not available. JE vaccine coverage over 2011–2013 is also shown. Colour coding of percent vaccination coverage in districts of UP is given in the legend (source: HMIS under NRHM) [11].

small cluster of JE cases was observed in February, when the weather conditions and the vector mosquito populations are generally moderate in UP. Studies from Japan [24] and Gorakhpur [25] have observed clustering of JE cases during low vector population periods and suggest that difference in the total and infected populations of vector mosquito species is probably responsible for this pattern. Another study indicated that the vertical transmission of JEV in the vector *Culex tritaeniorhynchus* mosquito species occurs in both hot and cool seasons, and the virus is thus regularly maintained in nature even during the

non-transmission season [24]. Taking both the above points into account, annual surveillance of JE cases is essential in the state of UP to confirm further this observed change in JE seasonality.

The present study reports that the percentage of JE positivity is decreasing in UP probably due to JE vaccination, although the number of AES cases has not declined. Systematic studies are required to access the impact of vaccination on the incidence of JE encephalitis. Involvement of a significant proportion of the adult population and predominantly male involvement is the highlight of the study. Continuous year-round

Climate variables	Summer (Mar.–May)	Winter (Oct.–Feb.)	Monsoon (Jun.–Sept.)
Average high temperature in °C	36.66	30.44	29.23
Average low temperature in °C	19.36	13.5	21.87
Average precipitation in mm	17.6	21	146.25

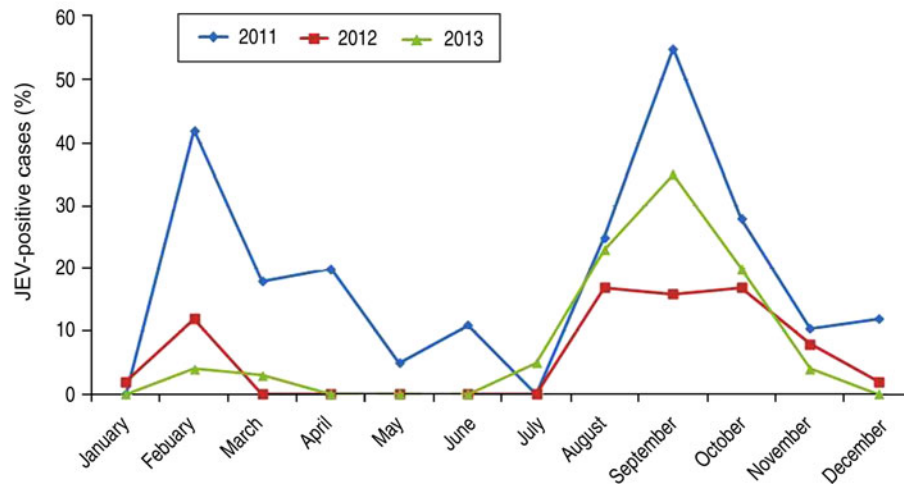


Fig. 2. Seasonal distribution of Japanese encephalitis virus (JEV)-positive acute encephalitis syndrome cases (percentage).

surveillance is essential in UP to diagnose JE cases out of season and to validate the changing seasonality of JE.

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## DECLARATION OF INTEREST

None.

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