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GKP1001: **Diagnostic services for suspected Japanese encephalitis (JE) cases from eastern Uttar Pradesh.**

Investigators: VP Bondre, Hirawati Deval, Kamran Zaman, Gajanan Patil, Kamlesh Sah, Niraj Kumar & Mahima Mittal.

Funding: Intramural Duration: 2010-Ongoing

NIV Gorakhpur unit undertake the routine investigation of clinically suspected acute encephalitis syndrome (AES) cases admitted to BRD Medical College (BRDMC), Gorakhpur and provides diagnosis that guide the management of cases. Although Japanese encephalitis (JE) is the known cause of AES endemic in the region, our investigations during 2016 confirmed higher positivity for anti- *Orientia tsutsugamushi* (OTs - cause of scrub typhus) IgM antibodies in AES cases. In addition to it, to rule out the antigenic cross reactivity between JE and Dengue (DEN), all the AES cases hospitalized during 2017 were investigated for detection of anti - JE IgM, anti - OTs IgM and Dengue NS-1 antigen by ELISA assays as per the ICMR recommendations. The findings were communicated within 24-36 hrs to BRDMC and concerned State Health authorities. A total of 3662 clinical specimens (CSF and serum) were collected from 2131 AES cases. Anti-JE IgM, anti-OTs IgM and dengue NS-1 antigen positivity was documented in 299 (14.0%), 992 (47%) and 97 (5.5%) AES cases respectively. Among the OT positive cases, anti-OTs IgM antibodies were also detected in 33.5% (108/322) CSF specimens from cases in which either the serum was not available or inadequate for testing.

1. AES Affected region of Uttar Pradesh.

2. Distribution of AES cases in endemic region.

3. Distribution of Japanese encephalitis cases (2017).

4. Distribution of Scrub typhus cases (2017).

Fig. 1. Grogaphic distribution of AES, JE and Scrub typhus cases in affected area of UP state.

Maximum AES cases were reported from Gorakhpur district (576) followed by Kushinagar (450), Deoria (321) followed by scanty cases from other 4 districts (Fig. 1). The AES cases began to rise from the month of July, peaked during the months of August to October and decline in incidence of cases was noted in the month of November (Fig. 2). The most affected population was in the range of 1-5 years (877) followed by 5-10 years (n=655) of age group (Fig. 2). Of these cases, JE was reported maximum from Kushinagar (57) followed by Gorakhpur (47), of whom the children of age group 5-10 year were affected maximum during the months of September and October. Similarly Scrub typhus (ST) was reported maximum from Gorakhpur (283) followed by Kushinagar and Deoria (172 in each district). Children of age group 1-5 year (405) were affected maximum by ST and cases peaked during the months of August to November. In AES cases, Dengue positivity was mostly documented during the months of September (41) and October (25) while a few cases were documented during August and November months (Fig. 2). These findings suggest that scrub typhus is associated as one of the important etiological agents amongst AES cases in this region. However, increase in the incidence of JE cases is also alarming, despite of good vaccination coverage in the endemic area. Appropriate intervention to control mites and mosquitoes with the focus on increasing the coverage of JE vaccination is the need of the hour.

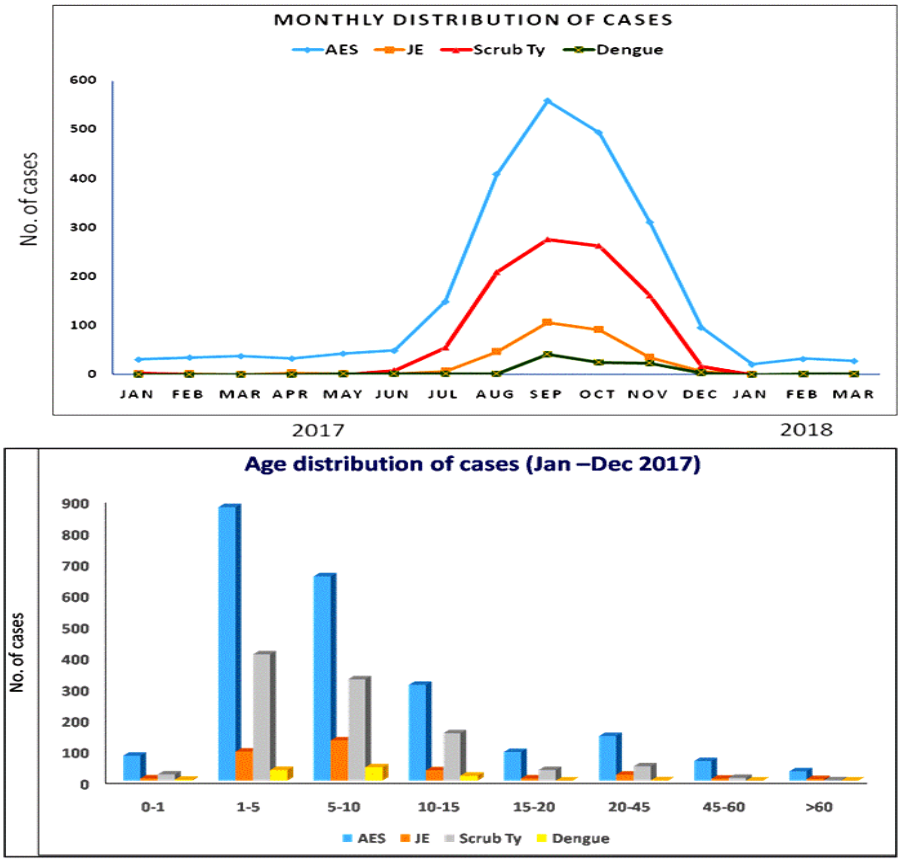

Fig. 2: Seasonal and age group wise distribution of AES cases during 2017.

GKP1501: Etiologic investigations in clinical specimens collected from acute encephalitis syndrome (AES) cases from Eastern Uttar Pradesh.

Investigators: VP Bondre, Hirawati Deval, Kamran Zaman, Rajeev Singh, AK Pandey, SP Behera, BR Misra, NM Rao, Niraj Kumar & Sanjeev Kumar.

Funding: Intramural Duration: 2015- Ongoing

Globally etiological identification of AES is ascertained to the maximum of 50% cases. JE is historically known cause of AES in this region. In addition to it, anti-OTs IgM antibodies and genome was detected in about 50% cases investigated during 2016. Hence, to streamline the utility of minimum amounts of available clinical specimens and identification of the cause associated with of AES, comprehensive efforts were made. Investigation of cases through the best use of epidemiological, clinical and biochemical parameters collected from each case, a diagnostic algorithm for investigation of JE negative cases hospitalized in BRDMC was developed to investigate the viral as well as bacterial infections (Fig. 3). As per clinical presentation, virological diagnosis in CSF samples were done by PCR assay for Herpes simplex virus (HSV 1/2/7), Cytomegalovirus (CMV), Varicella Zoster virus (VZV), Epstein Bar virus (EBV), Enterovirus (human, bovine, porcine), Parvovirus P4, Parvovirus B19 and Flaviviruses (including majority of the human infectious viruses including JEV, West Nile virus (WNV), Dengue, Tick borne encephalitis (TBE) virus, ZIKA virus, etc.). Depending on the CSF and serum biochemical characteristics, the JE negative CSF samples were also tested to detect bacterial infections including *Streptococcus pneumoniae*, *Neisseria meningitidis* & *Haemophilus influenzae* by multiplex PCR.

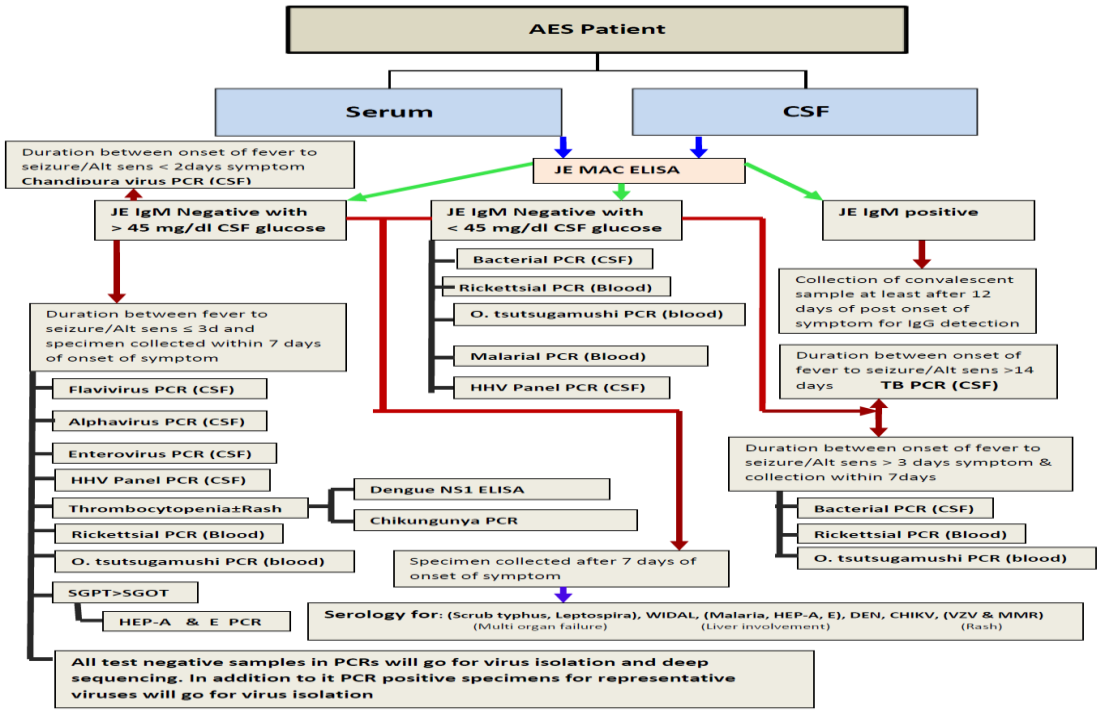

Fig. 3. Laboratory diagnostic algorithm developed for investigation of AES cases hospitalized in BRDMC.

The clinically suspected viral encephalitis cases with abnormal brain functions [electroencephalogram (EEG)] and brain pathology [Magnetic resonance imaging (MRI)] found positive by PCR for HSV -1 (4/314: 1.2%), VZV (3/294: 1%), EBV (1/294, 0.3%) while HSV-2, HSV -7, CMV and flaviviruses were not detected in any of the case (Table 1). Cases selected on the basis of rash and anemia tested positive for Enteroviruses (6/241: 2.4%) by RT-PCR, Parvovirus P4 (2/39: 5.1%) while Parvovirus B19 was not detected in any of the case investigated. CSF samples from the selected JE negative cases suspected of bacterial infection (meningeal symptoms) also were investigated for bacterial infection including *Streptococcus pneumoniae*, *Neisseria meningitidis* and *Haemophilus influenzae* by multiplex PCR, but none of them were detected. In 7.5% (41/542) of the whole blood specimens from JE and OTs negative AES cases with rash and thrombocytopenia, infection with rickettsia of spotted fever group was detected by PCR. Genome amplification of OTs in 21.2% (504/1170) cases positive by anti-OTs IgM ELISA further strengthen the finding on high OTs positivity by sero-diagnosis. Even in 43% (14/66) cases OTs PCR was positive in CSF tested where whole blood was not available for laboratory confirmation. These findings suggest major contribution of OTs and other rickettsia in the non-JE AES occurring in the region (Table 1).

S. N.	Etiologies	Specimens	Assay(s)	Outcome	% Positivity	Positive in tested	Cumulative Positivity (%)
1.	**JEV**	CSF	IgM ELISA	179/1849	9.7	299/2131	14.0
		Serum	IgM ELISA	241/1810	13.3		
2.	**DENGUE**	Serum	NS1 ELISA	93/1169	8	97/1771	5.5
		Serum	IgM ELISA	4/602	0.6		
3.	***O. tsutsugamushi***	CSF	IgM ELISA	108/322	33.5	992/2102	47.2
		Serum	IgM ELISA	884/1780	49.6		
4.	**VZV**	CSF	PCR	3/294	1.02	8/314	2.54
5.	**HSV-1**	CSF	PCR	4/314	1.27		
6.	**HSV-2**	CSF	PCR	0/314	0		
7.	**HSV-7**	CSF	PCR	0/294	0		
8.	**EBV**	CSF	PCR	1/294	0.34		
9.	**CMV**	CSF	PCR	0/294	0		
10.	**Parvovirus P4**	CSF	PCR	2/39	5.12	2/39	5.12
11.	**Parvovirus B19**	CSF	PCR	0/39	0		
12.	**Flavivirus Genus (JE/DEN/WNV/Zika)**	CSF	RT-PCR Generic	0/148	0	-	-
13.	**Enterovirus Generic**	CSF	RT-PCR Generic	6/241	2.49	6/241	2.49
14.	***H. influenzae***	CSF	PCR	0/32	0	-	-

15.	***N. meningitides***	CSF	PCR	0/32	0	-	-
16.	***S. pneumonia***	CSF	PCR	0/32	0	-	-
17.	***O. tsutsugamushi***	Blood	PCR	504/1170	21.2	511/1178	43.4
18.		CSF	PCR	14/66	43.0		
19.	**Other Rickettsia**	Blood	PCR	41/542	7.56	41/542	7.56

Table 1: Summary of diagnostic finding of AES cases of 2017.

GKP 1502: Epidemiological and clinical correlation of acute encephalitis syndrome cases with JE, non-JE viral and other AES associated etiologies from eastern Uttar Pradesh.

Investigators: VP Bondre, Kamran Zaman, Avinash Deoshatwar, Hirawati Deval, Rajeev Singh, Niraj Kumar, Asif Kavathekar & Vishal Nagose

Funding: Intramural Duration: 2015- 2018

JE, OTs, other Rickettsia and Dengue have emerged as the associated causes with about 70% AES cases investigated during 2017 as these infections were documented in 14, 47.2, 7.56 and 5.5% cases. However, clinical outcome in lab confirmed JE, OTs, Rickettsia and Dengue cases was largely similar independent of the associated etiology. Thus, to define any clinical, biochemical and / or pathologic features that might be helpful in differentiating these infections, clinical biochemical, treatment and physiological parameters were collected from all cases hospitalized during 2017 from the day of hospitalization to recovery / death. A total of 2247 AES cases were hospitalized in BRDMC during the year. Mean age of JE cases was higher (10.49 years) than the OTs infected cases (8.39 years) indicating relatively younger group is affected by JE. During 2017, overall AES case mortality was 22.8%, which is 4.2% lesser than previous season (26.4% CFR during 2016). In all AES cases, fever appeared to be the first clinical symptom followed by headache (96.7%), vomiting (99.2%) and abdominal pain (96.8%). Up rolling of the eyeball and frothing from mouth was recorded as predominant neurological features in almost all AES cases (>99%) (Table 2). Neurological examination marked severe brain injury (GCS=3-8) in a large number of patients (AES 46.8%; JE-AES 54.6% and OTs-AES 43.4%). The SGOT, SGPT and CSF-protein levels were significantly higher in OTs-AES patients as compared to JE-AES patients (for SGOT mean±SEM 177±5.6 vs.131.8±9.4, $p<0.001$; for SGPT mean±SEM 116.53±4.05 vs. 89.3±10.4, $p<0.01$; for CSF-protein mean±SEM 116.02±2.6 vs. 99.6±4.49, $p<0.01$). However, the CSF-glucose level was significantly lower in OTs-AES patients as compared to JE-AES patients (mean±SEM 61.4±0.94 vs. 72.4±2.44, $p<0.001$) as shown in Fig. 4. In addition to it, the JE, Dengue and OTs negative 541 AES cases were investigated for infection with other rickettsial (Rick) Spp. Rick DNA was detected in whole blood collected from 41 AES cases. The comparison of clinical and biochemical parameters between Rick positive and negative cases showed that duration of onset of fever, total leukocyte counts (TLC) and serum urea were significantly higher in Rick positive cases than Rick negative cases (for onset of fever median 6 day [range 3-15day] vs median 5day [range 1-

61day], p<0.05; for TLC median 19500 cells/mm3, range 6200-37800 vs median 12900cells/mm3, range 600-49900, p<0.01; for serum urea median 55.3 mg/dl, range 19.2-424.6 vs median 35.3mg/dl, range 13.6-316.4, p<0.01). However, the platelet count (PC) was significantly less in Rick positive cases than the negative cases (median 41000 cells/mm3, range 11000-413000vs median 245000 cells/mm3, range 10000-891000, p<0.001). Further, comparison between Rick positive and OTs positive cases showed that TLC, polymorphonuclear leukocytes (PML) and serum urea were significantly higher in Rick positive cases (for TLC median 19500 cells/mm3, range 6200-37800 vs median 13350cells/mm3, range 1400-81000, p<0.001; for PML median 72.7%, range 36.2%-89% vs median 56%, range 3.7%-92.6%, p<0.001, for serum urea median 55.3 mg/dl, range 19.2-424.6 vs median 36.5mg/dl, range 2.4-282.5, p<0.05). However, the duration of fever onset, lymphocytes count (LC) and PC were significantly lesser in Rick positive cases than OTs positive cases (for onset of duration of fever median 6 day, range 3-15 days vs median 7 days, range 1-31 days, p<0.05; for LC median 20.8%, range 7.9%-56.7% vs median 35.6%, range 4.7%-80.3%, p<0.001; for PC median 41000 cells/mm3, range 11000-413000 vs median 95000cells/mm3, range 4000-975000, p<0.001). These findings clearly indicate that the critical demographic, age group, clinical and physiological analysis of the cases can be helpful in differentiating the AES associated with different etiologies. The age group, mortality, duration of illness, thrombocytopenia, CSF protein and glucose levels along with serum urea levels emerged as differentiating factors in different infections.

Parameters	**Findings in AES cases [n=2247]**	**Findings in JE cases [n= 299]**	**Findings in OTs cases [n=992]**
Sex Ratio [M/F]	1173/1074	151/148	495/505
Age Mean [Years]	10.49	10.54	8.39
Mortality [%]	22.9 [516/2247]	25.4 [76/299]	13 [129/992]
High Grade Fever [%]	75.8 [1401/1846]	71.4 [185/259]	77.4 [664/857]
Fever [in days] before hospitalization	7.55 [n=2106]	7 [n=284]	8.28 [n=924]
Headache	96.7 [381/394]	95.3 [61/64]	96.3 [187/194]
Vomiting	99.2 [1277/1287]	99.3 [160/161]	99.1 [639/645]
Abdominal Pain	96.9 [279/288]	89.6 [26/29]	97.2 [177/182]
Altered level of consciousness	67.2 [1511/2247]	71.9 [215/299]	63.7 [632/992]
Up rolling of Eye Boll	99.6 [1397/1402]	100 [201/201]	99.5 [648/651]
Frothing from mouth	97.9 [573/585]	98.7 [78/79]	97.1 [204/210]

Glasgow COMA scale = 7	92.2 [1602/1737]	91.8 [225/245]	95 [781/822]
Neck rigidity	9.95 [184/1848]	8.3 [22/265]	10.4 [86/825]
Kerning's sign	8.13 [150/1843]	6.8 [18/265]	8.86 [73/823]
Hepatomegaly	15.8 [328/2073]	13.6 [38/279]	23 [209/907]
Splenomegaly	2.6 [53/2047]	2.17 [6/276]	4.05 [36/888]
Hemoglobin >10gm/dl	40.4 [774/1913]	45.9 [118/257]	28.2 [238/844]
Total leukocyte count >13000 cells/mm^3	49.3 [946/1915]	48.4 [124/256]	51.4 [435/845]
Total leukocyte count in CSF >5 cells/mm^3	80.7 [1376/1704]	88.2 [209/237]	92.2 [710/770]
Platelet count <1x10^5cells/mm^3	62.8 [1198/1907]	69.1 [177/256]	47.1 [397/842]
SGOT >45 IU/L	77.8 [1430/1838]	76.9 [187/243]	88.3 [713/807]
SGPT >45 IU/L	58.5 [1080/1835]	47.4 [116/243]	77.4 [624/806]
Urea >40gm/100ml	41.9 [766/1827]	41.1 [99/241]	42.2 [346/819]
Creatinine>1gm/100ml	16.2 [303/1869]	13.8 [34/246]	16.2 [133/822]
CSF protein [>45mg/dl]	73.7 [1278/1733]	83.6 [205/245]	91.3 [707/774]
CSF glucose [>75 mg/dl]	30.2 [523/1730]	36.7 [90/245]	23.1 [179/776]

Table 2: Comparison between JE-AES and OTs-AES patients with respect to the demographic, clinical and biochemical features.

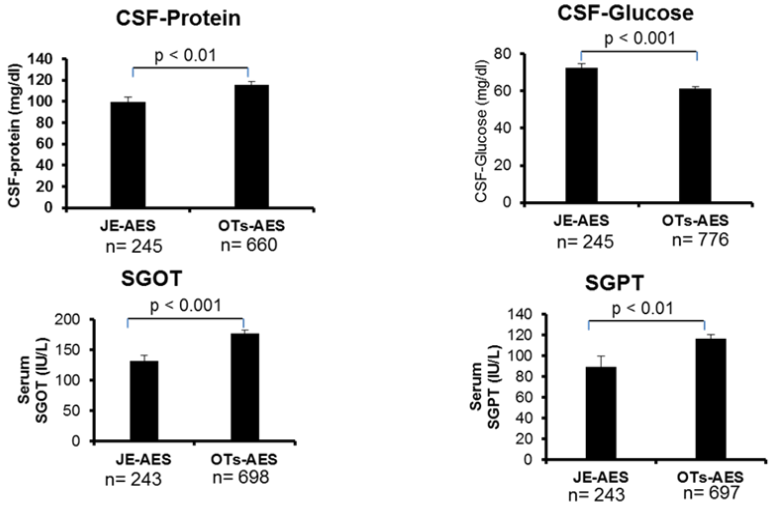

Fig. 4. Comparison of biochemical features between JE and OTs positive AES cases.

GKP 1503: Isolation, identification and genetic characterization of viruses isolated from acute encephalitis syndrome cases from eastern Uttar Pradesh.

Investigators: VP Bondre, Hirawati Deval, Rajeev Singh, V Janardhan, Ravishankar Singh & Sanjeev Kumar

Funding: Intramural Duration: 2015- ongoing

Virus isolation is regarded as the 'gold standard' in the investigation of viral etiologies. To improve upon the diagnosis of AES, 71 CSF specimens collected from suspected viral encephalitis cases and 10 Dengue RT-PCR positive serum specimens were attempted for virus isolation in multiple cell lines including Porcine Stable kidney (PS), Baby Hamster Kidney (BHK) 21, and Vero-E6 cells. None of the cultures showed any cytopathic effect (CPE) till 4 passages. Of the addition to above, 6 Enterovirus PCR positive samples attempted for cell culture isolation in RD cell line, only one sample showed CPE in 3rd passage after 48hr of incubation and it was confirmed by RT-PCR and sequencing (Fig. 5).

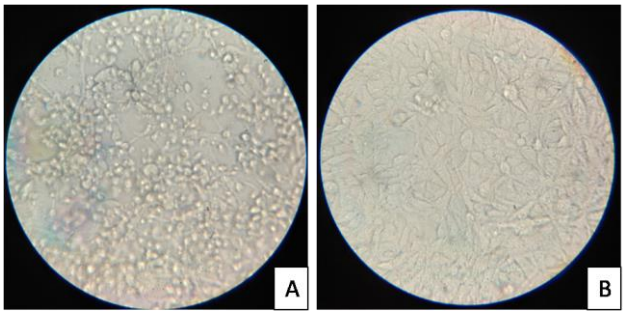

Fig. 5: Microscopic image of RD cell line after 48hrs of incubation with culture supernatant obtained from 3rd passage of CSF inoculated cell line (A) and control cells with supernatant (B).

GKP 1504: Etiological investigations of non-AES referred cases from Gorakhpur region.

Investigators: Hirawati Deval, Niraj Kumar, SP Behera, Kamlesh Sah, Gajanan Patil, Sanjeev Kumar, AK Agrawal & VP Bondre

Funding: Intramural Duration: 2015-2018

Apart from diagnostic services to referred AES cases, NIV Gorakhpur unit also provided diagnosis to non-AES cases referred from BRDMC and other tertiary care centers in the region. Depending on the clinical diagnosis, 147 clinical specimens (97 CSF and 50 blood / serum) were referred for investigations on JE, ST, DEN, HSV, VZV, EBV and Measles infections. JE was detected in 5.15% (5/97) of CSF and 20% of serum (10/50), ST in 39% of sera (14/36) while Dengue IgM was negative in 21 sera. Anti-Measles IgM antibodies detected in 1/21 sera. Molecular diagnosis of these specimens for HSV-1/2, VZV, CMV and EBV in CSF specimens detected HSV-2 in 2/92 (2.17%) while VZV and EBV were detected in one each of the 92 (1.08%) CSF tested. In addition to it, sera from clinically suspected dengue cases admitted in Gorakhnath Hospital were referred for identification of dengue serotypes associated with

outbreaks occurring in the region. Phylogenetic analysis of complete envelope gene sequence directly amplified from sera suggests circulation of three genetically distinct strains belonging to the cosmopolitan genotype of Dengue virus serotype 2 (Fig. 6). Further sequence analysis clearly suggests their close genetic relationship with Dengue virus 2 strains recently isolated in Singapore and Delhi and probably introduced through febrile travelers. In addition to it, studies on 33/48 (68.7%) RT-PCR positive Dengue fever cases are in progress.

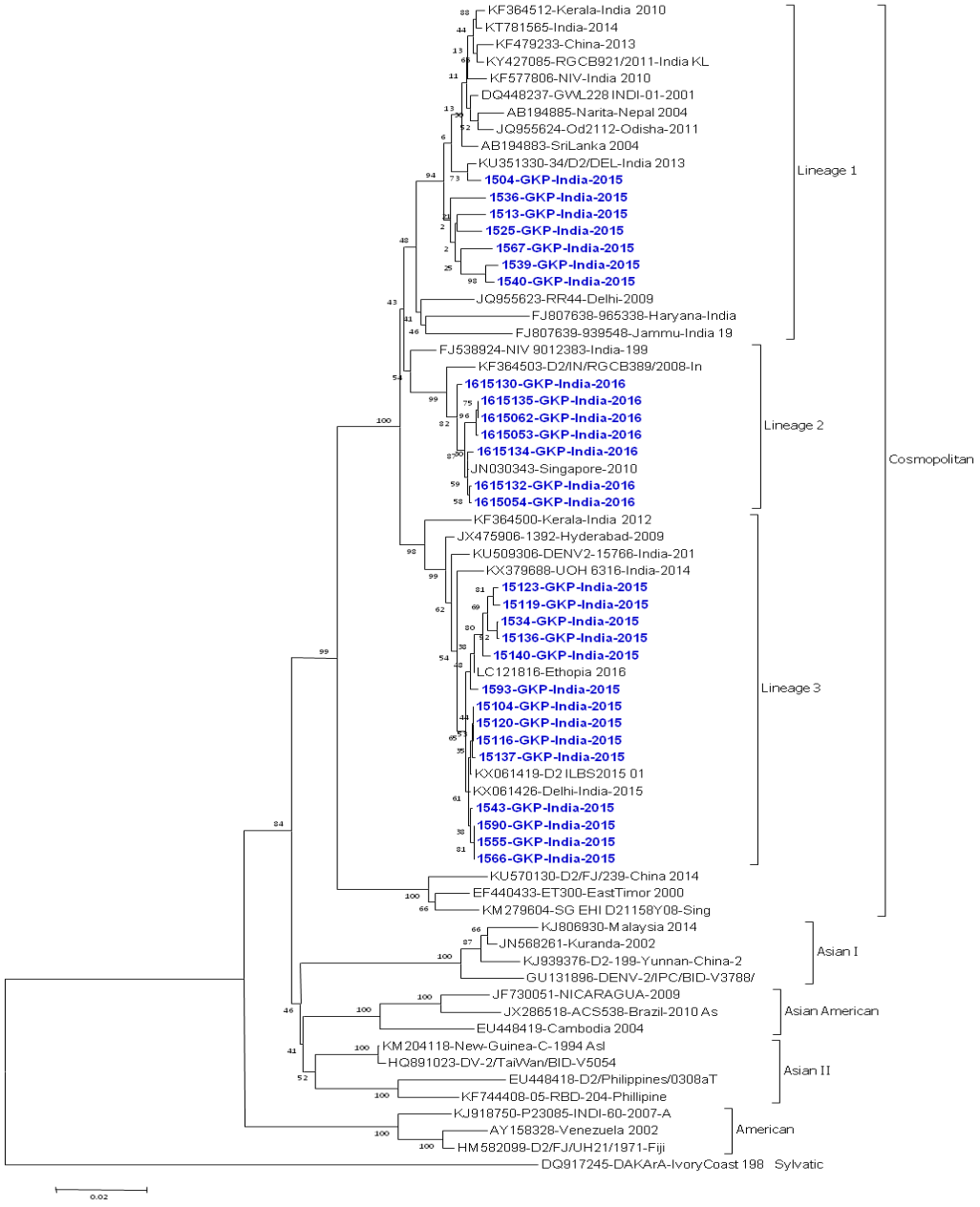

Fig. 6. Phylogenetic analysis of Dengue serotype 2 complete envelope gene sequences directly amplified from patient's sera and isolates.

GKP1702: Detection of anti-JEV IgM in urine samples of Japanese encephalitis-acute encephalitis syndrome (JE-AES) patients.

Investigators: Rajeev Singh, Niraj Kumar, Kamran Zaman, Hirawati Deval, Vishal Nagose &VP Bondre

Funding: Intramural Duration: 2017- ongoing

JE is endemic in eastern UP with outbreak occurring every year since 2005. JE diagnosis is mainly based on detection of virus or IgM antibodies primarily in CSF and / or serum collected during acute phase of illness. However, lumber puncture is difficult task and expertise is not available in peripheral health settings. In addition to it, mainly pediatric the age group is affected by JE and blood collection encounters number of difficulties. Therefore, in such cases, there is need for noninvasive specimens which can be explored in disease diagnosis. Number of laboratories worldwide explored use of non-invasive body fluids in disease diagnosis and urine has been successfully explored in molecular diagnosis of many viral infections including Zika, Dengue, Chikungunya, Tick borne encephalitis, West Nile, BK, JC, Mayaro, CMV, Hepatitis B and E. To determine the utility of urine specimens collected during the acute phase of illness, study was carried out on urine specimens collected from hospitalized, lab diagnosed JE IgM positive cases from BRDMC.

Urine samples from 136 JE cases were simultaneously processed by RT-PCR / nested PCR (140µl) and IgM ELISA (10µl). Anti-JE IgM antibodies were detected in 22/136 (16.17%) cases while viral RNA was not detected in any of the case. To further explore the utility of urine in diagnosis, it was concentrated (10 and 20 – fold) by ultra filtration using vivaspin2 (Sartorius, Inc.) with 10kDa cutoff. Use of different concentrations for detection of IgM antibodies by ELISA yielded positive results in 34 JE cases (18 at 10X and additional 16 at 20X concentration). Out of 136 JE patients, 56 (40%) tested positive for anti-JEV IgM in urine samples after concentration. These experiments were repeated twice which confirmed IgM positivity in 22, 18 and 16 cases at neat, 10X and 20X concentrations respectively. The level of anti-JEV IgM antibodies in serum didn't correlate with its presence in urine. However, the concentration of anti-JEV IgM in urine showed significant correlation with its level in CSF samples ($r = 0.345$, $p<0.05$). Among the 136 patients investigated, anti-JEV IgM was detected in CSF of 82 cases and in serum of 119 cases. Further clinical data analysis of these cases did not correlated the present of IgM antibodies in urine with disease severity. However, the cases tested positive for IgM antibodies in urine showed higher concentration of IgM antibodies in both CSF and sera than the cases tested negative for IgM antibodies in urine (for serum mean P/N ratio± SEM12.55±0.85 vs. 8.27±0.61, $p<0.001$; for CSF mean P/N ratio± SEM 15.49±1.28 vs 8.82±1.09, $p<0.001$). Further studies on duration of IgM detection post infection, any correlation with CNS pathology or damage to non-CNS organs is in progress. Standardization of this assay based on use of non-invasive body fluids needs more cases to be incorporated and investigated for different parameters.

GKP1703: Case based entomological investigation in AES affected area of Gorakhpur region.

Investigators: Brij Ranjan Misra, Vijay Kumar, S P Behera &Vijay Bondre

Funding: Intramural Duration: 2016-2017

Our investigations during 2016 AES season established association of OT and Rickettsia infection with AES cases occurring in the endemic region. Considering the primary role of different arthropods ectoparasites (ticks, mites, fleas and louse) in transmission of these bacterial infections along with a variety of human infectious viruses and protozoa, case based entomological survey was carried out to substantiate their role in natural cycle. Upon diagnosis, mites were collected from rats in the vicinity along with ticks from different domestic animals during the months of September and October, 2017. The pilot study was performed on 50 AES cases from villages located in the Chargawan, Bhathat and Pipraich blocks of Gorakhpur district. Engorged arthropods were separated from the body parts of animals. Arthropods specimens were identified according to their morphological keys and the identified pools were subjected to molecular diagnosis for Flaviviruses, *Orientia tsutsugamushi*, Rickettsia genus and *Ehrlichia/Anaplasma* genus using standard reagents. Out of 307 pools tested, 4 pools were positive for *O. tsutsugamushi*, 18 pools were positive for rickettsia and majority (39 pools) were positive for Anaplasma/Ehrlichia genus (Table 3). The positivity of *O. tsutsugamushi* in chigger mite was 16.6% as this region is endemic for scrub typhus. One pool of *H. suis* and *X. cheopis* were positive for OTs. Among the 18 rickettsia positive pools, 3 pools were identified as *Rickettsia felis* by sequencing.

Arthropod Species (no of pools tested)	**Flavivirus**	***O. tsutsugamushi***	**Rickettsia genus**	***Ehrlichia/ Anaplasma***
***Rhipicephalus (Boophilus) microplus* (84)**	0	0	3	4
***Rhipicephalus sanguineus* (1)**	0	0	0	1
***Dermacentor auratus* (1)**	0	0	0	0
***Hyalomma Kumari* (5)**	0	0	0	0
***Haematopinus suis* (26)**	0	1	3	6
***Pediculus humanus capitis* (70)**	0	0	1	5
***Polyplax spinulosa* (6)**	0	0	0	1
***Echinolaelaps echidninus* (1)**	0	0	0	0
***Leptotrombidiumdeliense* (12)**	0	2	0	1
***Ornithonyssus bocoti* (49)**	0	0	5	13
***Xenopsylla cheopis* (52)**	0	1	6	8
Total (307)	0	4	18	39

Table 3: Table showing arthropods and their pathogens detected by PCR assays.

GKP1601: Setting up of AES cell at Baba Raghav Das Medical College, Gorakhpur.

Investigators: Mahima Mittal, VP Bondre, Hirawati Deval, Manoj Murhekar, Kamran Zaman & AES cell group

Funding: Extramural (ICMR) Duration: 2016 - 2018

'AES cell' was established on recommendations of ICMR to streamlining the process of clinical specimen collection, distribution for different investigations and storage for future research on AES cases. The clinical and epidemiological data set along with different lab investigations and findings was created for each case. Apart from investigating all the hospitalized AES cases primarily for JE, ST, and Dengue infections, the negative cases were also investigated for other known encephalitic etiologies including rickettsia and neurotropic viruses by different serological and molecular techniques. Genetic characterization of ST and rickettsia was carried out to define prevalence and circulation of different strains and to define their genetic relationship with worldwide identified strains. All the OTs-IgM positive whole blood specimens and CSF (if whole blood was unavailable) were processed by standard universal PCR followed by nested PCR to detect 456 bp product from 56kDa protein (outer membrane) coding gene of all OTs serotypes. The percentage positivity was 21.2% (14/66) in CSF (tested in cases where adequate sera were not available for diagnosis) and 43% (504/1170) in whole blood specimens. The phylogenetic study was carried out using reference sequences (GenBank) and 19 representative sequences from OTs positive AES patients. The genetic analysis suggests that most of the Gorakhpur sequences clustered in JG related serotype and 3 in Karp serotype while 2 sequences grouped with the Kato serotype of OTs (Fig. 7). In addition to it, the OTs IgM / PCR negative 541 cases with signs of rash and multi-organ involvement were also investigated for infection with Spotted Fever Group (SFG) of Rickettsia. Genetic analysis of SFG specific genome sequence amplified from 41 cases suggests prevalence of multiple strains of Rickettsia including *R. conorii*, *R. felis* and *R. parkeri* and their association with AES cases (Fig. 8).

Fig. 7. Phylogenetic tree of *Orientia tsutsugmaushi* based on the nucleotide sequences of 56-kDa cell surface antigen gene.

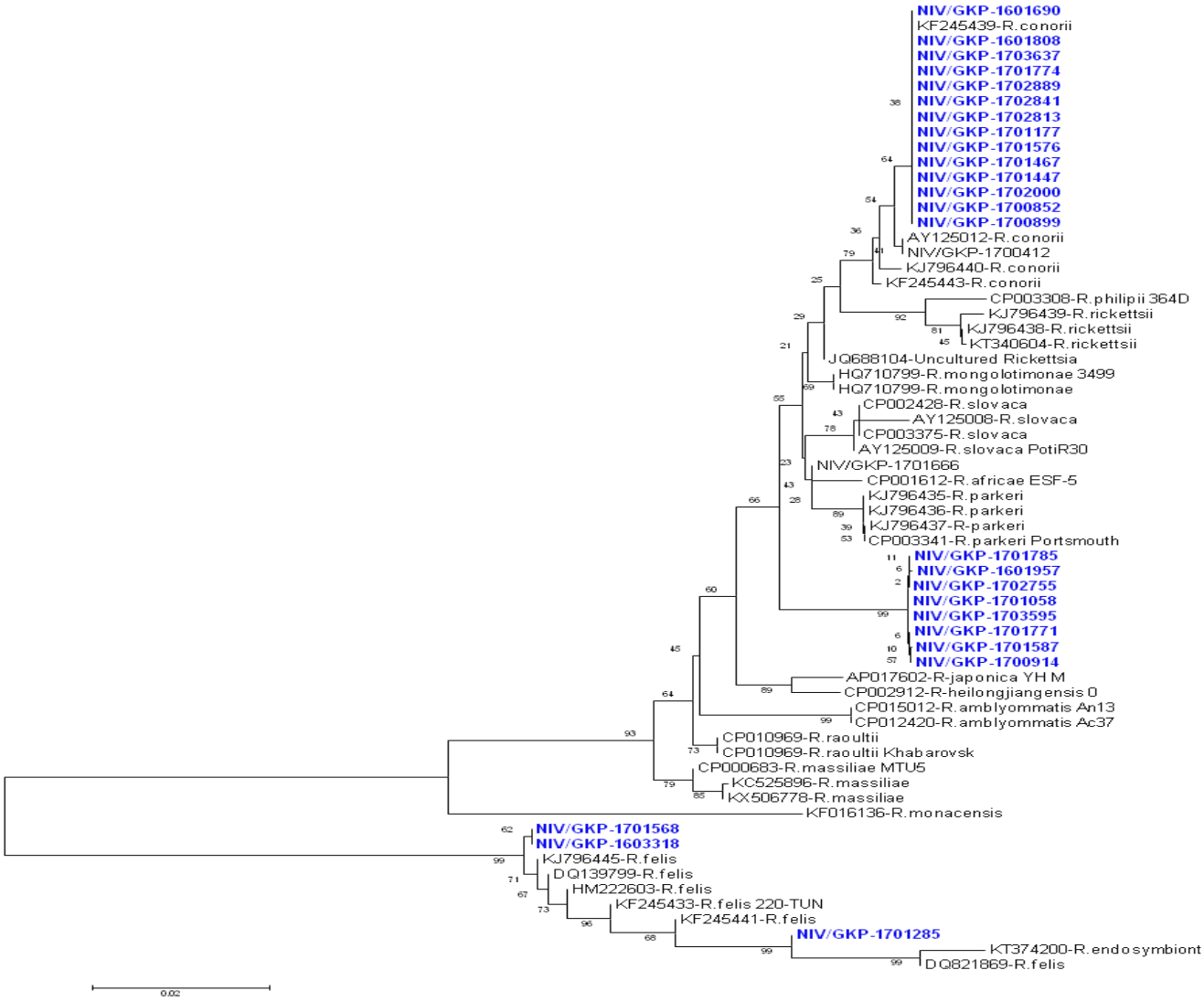

Fig. 8. Phylogenetic tree of SFG Rickettsia directly amplified from whole blood of AES cases.

GKP 1701: Genetics of susceptibility to encephalitis in Japanese encephalitis virus infected children from Uttar Pradesh.

Investigators: Hirawati Deval, Alagarsu K, VP Bondre & Mittal M.

Funding: Extramural-ICMR Neuroscience Task Force Project Duration**:** 2017-2020

About 8-10% of AES cases in UP are due to JEV. The asymptomatic to symptomatic disease ratio is reported to be in the range of 25-1000:1. About 70% of the symptomatic infections manifest as encephalitis and 30% of the symptomatic infections are fatal. Clinical outcome of JE is influenced by factors involving host, virus and environment. There are only a few reports on the role of host genetic factors in the development of encephalitis in JEV infected children. Present work was carried out to study single nucleotide polymorphisms in genes coding for pattern recognition receptors, inflammatory mediators and receptors and matrix metalloproteinase and its association with AES caused by JEV in children from UP.

During the study period, a total of 197 apparently healthy controls without any history of encephalitis and 97 JE cases were recruited and genomic DNA was extracted from blood.

SNPs and Genotypes	Japanese Encephalitis cases N (%)	Controls N (%)	Odds ratio with 95% confidence intervals	P value
TNFA -308				
G/G	76 (87.4%)	35 (94.6%)	1.00	
G/A	**11 (12.6%)**	2 (5.4%)	2.53 (0.53-12.04)	0.20
IFNG +874				
A/A	22 (25%)	9 (24.3%)	1.00	
A/T	62 (70.5%)	25 (67.6%)	1.01 (0.41-2.51)	0.75
T/T	4 (4.5%)	3 (8.1%)	0.55 (0.10-2.94)	
IL10 -592				
C/C	**28 (32.2%)**	6 (16.7%)	1.00	
A/C	44 (50.6%)	20 (55.6%)	0.47 (0.17-1.32)	0.14
A/A	15 (17.2%)	**10 (27.8%)**	0.32 (0.10-1.06)	
CCL2 -2518				
A/A	**38 (43.7%)**	12 (32.4%)	1.00	
A/G	33 (37.9%)	**21 (56.8%)**	0.50 (0.21-1.16)	0.15
G/G	**16 (18.4%)**	4 (10.8%)	1.26 (0.35-4.51)	
TLR3 rs3775290				
G/G	**44 (50.0%)**	16 (43.2%)	1.00	
G/A	38 (43.2%)	**20 (54%)**	0.69 (0.31-1.52)	0.44
A/A	6 (6.8%)	1 (2.7%)	2.18 (0.24-19.55)	
OAS1 rs1077467				
A/A	41 (47.1%)	17 (47.2%)	1.00	
A/G	36 (41.4%)	**19 (52.8%)**	0.79 (0.36-1.74)	**0.02**
G/G	**10 (11.5%)**	0 (0%)	NA	
OAS1 rs1131454				
A/A	28 (42.4%)	13 (46.4%)	1.00	
A/G	27 (40.9%)	13 (46.4%)	0.96 (0.38-2.45)	0.43
G/G	**11 (16.7%)**	2 (7.1%)	2.55 (0.49-13.22)	

Table 4: Genotype frequencies *TNFA*, *IL10*, *CCL2*, *TLR3* and *OAS1* gene polymorphisms in JE cases and apparently healthy controls.

Genotyping of *TNFA* -308 was performed by allele specific PCR while genotyping of *IL10* -592, *IFNG* +874, *CCL2* -2518, *TLR3* rs3775290 and *OAS1* rs10776471 was performed by PCR-RFLP based methods in 87 encephalitis cases and 37 healthy control DNA samples. Genotyping of *IL10* -1082 has been performed by allele specific PCR in 38 samples and *OAS1* rs1131454 genotyping was done by PCR-RFLP in 95 samples.

Genotype frequencies of *TNFA* -308, *IL10* -592, *IFNG* +874, *CCL2* -2518, *TLR3* rs3775290, *OAS1* rs10776471 and *OAS1* rs1131454 in encephalitis cases and healthy controls were provided in Table3. The frequencies of *TNFA* -308 G/A genotype, *IL10* -592 C/C genotype, *CCL2* -2518 A/A and G/G genotype, *TLR3* rs3775290 G/G genotype, *OAS1* rs10776471 G/G genotype and *OAS1* rs1131454 G/G genotype were higher in encephalitis cases while the frequencies of *IL10* -592 A/A genotype, *CCL2* -2518 A/G genotype, *TLR3* rs3775290 G/A

genotype and *OAS1* rs10776471 A/G genotypes were higher among healthy controls. [Table 2] The frequency of OAS1 rs10776471 G/G genotype was significantly higher in encephalitis cases and was not observed in healthy controls (P = 0.022). Further sample collection and studies are in progress.

Number of samples tested (virus-wise details from each Group)

- Conferences attended as table:

1. Dr Niraj Kumar attended the workshop on "Zika virus diagnosis" at Diagnostic Virology Group, MCC Pashan, Pune from 7- 9th June, 2017
2. Dr Rajeev Singh attended "17th Foundation training programme for Scientists and Technologist" at Indian Institute of Public Administration, New Delhi from 22nd January to 16th March 2018.
3. Dr Kamran Zaman attended workshop on "Research Ethics and Good Clinical Practice – Current Scenario" at ICMR-National JALMA Institute for Leprosy and other Mycobacterial Diseases (NJIL& OMD), Agra from 15-16th January 2018.
4. Dr Niraj Kumar attended "12th Capacity Building Programme for Technical Personnelof Science and Technology Departments, Government of India" at Indian Institute of Public Administartion ,New Delh from 5-16th March, 2018.

Conference details with Venue, date	Presentations	Name of participant/List of Authors with presenting author underlined
	Title of Invited Talk, Oral or Poster presentation	

- Workshops / Training programs conducted by Group / Individual scientist belonging to the group. **Not Applicable**

- Product development, if any. *For Epidemiology Group: List of outbreaks investigated.* **Not Applicable**

- **List of Publications** (Published and accepted between 1/04/2017 – 31/03/2018).

1. Scrub Typhus as a Cause of Acute Encephalitis Syndrome, Gorakhpur, Uttar Pradesh, India.Mittal M, Thangaraj JWV, Rose W, Verghese VP, Kumar CPG, Mittal M, Sabarinathan R, **Bondre V**, Gupta N, Murhekar MV. Emerg Infect Dis. 2017 Aug;23(8):1414-1416.

2. Chickenpox and measles clusters among college students in Pune, Maharashtra. Deoshatwar AR, Bondre VP**,** Tandale BV. Virus disease. 2017 Sep;28(3):337-340.
3. Development of a novel rapid micro-neutralization ELISA for the detection of neutralizing antibodies against Chandipura virus. Damle RG, Patil AA, Bhide VS, Pawar SD, Sapkal GN, Bondre VP. J Virol Methods. 2017 Feb;240:1-6
4. Vivian Thangraj JW, Mittal M, Verghese VP, Kumar CPG, Rose W, Sabarinathan R, **Pandey AK**, Gupta N, Murhekar M. Scrub Typhus as an Etiology of Acute Febrile Illness in Gorakhpur, Uttar Pradesh, India, 2016.Am J Trop Med Hyg. 2017 Nov;97(5):1313-1315.
5. Murhekar MV, Oak C, Ranjan P, Kanagasabi K, Shinde S, **Pandey AK**, Mittal M, Gore MM, Mehendale SM. Coverage and missed opportunity for Japanese encephalitis (JE) vaccine, Gorakhpur division, Uttar Pradesh, India, 2015: Implications for JE control. Indian J Med Res. 2017; 145(1):63-69.

- Awards and recognition received by members of the group. **Not Applicable.**