Annual Report

2018-19

ICMR-Regional Medical Research Centre,
Gorakhpur.

ICMR-Regional Medical Research Centre, Gorakhpur

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SECTION-1: MAJOR PROJECTS UNDERTAKEN DURING THE YEAR

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

Investigators: Kamran Z, Hirawati D, Niraj K, Kamlesh S, Vijay K, Gajanan P & Mahima M.

Funding: Intramural Duration: 2010 – Ongoing

ICMR-RMRC Gorakhpur, undertake the routine investigation of clinically suspected Acute Encephalitis Syndrome (AES) cases admitted to the BRD Medical College (BRDMC), Gorakhpur and provides diagnostic services that guide the management of cases. All the AES cases hospitalized during (1st April 2018 to 31st March 2019) were investigated for detection of anti-*Japanese encephalitis (JE)* virus specific IgM (anti-JE IgM), anti- *Orientia tsutsugamushi* IgM (anti - OTs IgM) and Dengue NS-1 antigen (DEN NS-1 Ag) by ELISA assays as per the ICMR recommendations.

A total of 1884 clinical specimens (CSF and Serum) was collected from 1009 AES cases. Anti-JE IgM, anti-OTs IgM and DEN NS-1 Ag positivity were documented in 159 (15%), 474 (47%) and 55 (5.6%) AES cases respectively. In 11 AES cases, neither serum nor CSF was available for testing. (**Table 1**)

In addition, we received specimens of suspected cases of viral encephalitis (VE) (n= 1830), which were tested as per AES diagnostic protocol. Anti-JE IgM positivity was found to be 12% of the cases, anti-OTs IgM (29.6%) and DEN NS-1 Ag positivity was documented in 6.9% of cases. (**Table 1**) Of the 1830 cases, 227 suspected cases converted to AES during their hospital course.

Table 1: Specimens tested with anti-JE IgM and anti-OTs IgM and Dengue NS-1 Ag ELISA

2018- 2019 (No cases)	Specimens Type	Total samples received and tested	Anti-JE IgM Positivity (%)	Anti-OTs IgM Positivity (%)	DEN NS-1 Ag Positivity (%)
AES (1009)	CSF	910	72 (7.91%)	379 (41.6%)	--
	SERUM	974	150 (15.4%)	447 (45.9%)	55 (5.6%)
Suspected VE (1830)	CSF	477	31 (6.5%)	133 (27.9%)	--
	SERUM	1799	220 (12.2%)	521 (29.0%)	127 (6.9%)

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

Investigators: Kamran Z, AK Pandey, Hirawati D, Rajeev S, SP Behera, BR Misra, Niraj K, Sanjeev K, Asif K, Kamlesh S, Ravishankar S

Funding: Intramural Duration: 2015- Ongoing

ICMR recommended algorithm entitled "Laboratory testing algorithm for AES in India" was followed to investigate the other viral /bacterial causes of AES. As per clinical presentation, laboratory finding and algorithm, virological diagnosis in CSF samples were done by PCR assay and found positive for Herpes simplex virus (HSV)-1 (0.86%), Varicella Zoster virus (VZV) (0.86%), Parvovirus P4 (2.43%), while EBV, CMV, HSV-2/7, Parvovirus B19 were not detected in any of the cases. Results of the cases with rash, tested positive for Enteroviruses (0.81%) and flaviviruses (0%) (**Table 2**). Whole blood sample of OTs IgM seropositive cases was tested by PCR and found positive in 27.5%. Rickettsia (spotted fever group) was detected by PCR in 12.72% of the whole blood specimens of JE/ OTs/ Dengue negative AES cases with rash and thrombocytopenia. CSF samples from the selected JE negative cases suspected of bacterial infection (meningeal symptoms) also were investigated for *Streptococcus pneumoniae, Neisseria meningitidis* & *Haemophilus influenzae* by multiplex PCR, one case found positive for *S.*

pneumoniae. These findings suggest a major contribution of OTs and other rickettsial agents in the non-JE AES cases occurring in the region.

Etiology	Samples	Test	Positive /total samples	% Positivity
Flaviviruses (JEV, DENV, WNV, Zika, etc.)	CSF	RT-PCR	0/33	0%
Parvo P4	CSF	PCR	1/41	2.43%
Parvo B19	CSF	PCR	0/41	0%
HSV 1	CSF	PCR	1/116	0.86%
HSV 2	CSF	PCR	0/116	0%
CMV	CSF	PCR	0/116	0%
VZV	CSF	PCR	1/116	0.86%
EBV	CSF	PCR	0/116	0%
Enterovirus	CSF	RT-PCR	1/122	0.81%
O. tsutsugamushi	Blood	PCR	121/439	27.56%
Rickettsial	Blood	PCR	14/110	12.72%
N. meningitidis	CSF	PCR	0/43	0%
S. pneumoniae	CSF	PCR	1/43	2.85%
H. influenzae	CSF	PCR	0/43	0%
MTB	CSF	PCR	0/12	0%

Table 2: Summary of the diagnostic finding of AES cases during 2018

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: Kamran Z, Avinash D, Hirawati D, Niraj K, Asif K, Vishal N.

Funding: Intramural Duration: 2015- Ongoing

JE, OTs and other Rickettsia and Dengue have emerged as the associated causes with about 60% AES cases investigated during 2018. Maximum AES cases were reported from Gorakhpur district (274) followed by Kushinagar (166), and Deoria (158). AES cases began to rise from the month of July, peaked during the months of August to October and decline in the incidence of cases was noted in the month of November as the similar pattern shown in previous years. The most affected population was in the range of 1-5 years (337) followed by 5-10 years (261) of the age group. Of these cases, JE was reported maximum from Gorakhpur (34) followed by Kushinagar (18). Similarly, Scrub typhus (ST) was reported maximum from Gorakhpur (135) followed by Deoria (82) and Kushinagar (69). Children of age group 1-5 year (156) 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 (23) and October (15). Azithromycin was given to 899 out of 1017 AES patients. Children who received azithromycin were less likely to die of AES (Yates Corrected Chi squared value: 5.66; p- 0.017).

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

Investigator: Rajeev S, Hirawati D, Kamran Z, V Janardhan, Ravishankar S, Manoj K & Sanjeev K

Funding: Intramural Duration: 2015-Ongoing

Virus isolation is regarded as the ‘gold standard’ in the investigation of viral etiologies as well as genetic characterization of viruses. Fifty four serum specimens collected from Dengue fever cases admitted to Gorakhnath hospital, Gorakhpur, all were positive for Dengue NS1 antigen. These

serum samples were attempted for virus isolation in Porcine Stable kidney (PS) cells. Moreover, 11 serum specimens collected from suspected viral encephalitis cases from BDR medical college, Gorakhpur and positive for anti-Dengue IgM were also attempted for virus isolation. None of the cultures showed any cytopathic effect till the fourth passage.

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

Investigators: Hirawati D, Kamran Z, SP Behera, Niraj K, Asif K, Kamlesh S, Sanjeev K, Vijay K, Manoj K

Funding: Intramural Duration: 2015-Ongoing

Apart from diagnostic services to referred AES cases, we also provide diagnostic services to non-AES referred samples from BRD Medical College and other hospitals from Gorakhpur and nearby districts. Based on the clinician recommendation, 140 clinical specimens (79 CSF, 46 serum, 4 blood, 5 throat swab, 4 skin swab and 2 urine) were investigated for JE, OTs, DEN, HSV, VZV, CMV. Anti-JE IgM antibodies were detected in 5.26 % (2/38) of Serum and 1.49% of CSF (1/67), anti-OTs IgM in 7.89% of sera (3/38) and 4.47 % of CSF (3/67) specimens, while Dengue NS1 was diagnosed in one serum (1/38). The CSF of 67 patients was subjected for molecular diagnosis for HSV-1/2, VZV and CMV. Blood, serum and urine specimens were detected positive for CMV in one patient. In addition, VZV was detected in skin swab (n=2), throat swab (n=2), and serum (n=1) samples tested.

GKP 1601: **Setting up of AES Cell at Baba Raghav Das Medical College, Gorakhpur.**

Investigators: M Mittal, Kamran Z, Hirawati D & AES cell study group

Funding: Extramural Duration: 2016-2019

'AES cell' was established on recommendations of ICMR to streamline the process of clinical specimen collection, distribution for different investigations and storage for future research on AES cases. Genetic characterization of ST and rickettsia was carried out to define the prevalence and circulation of different strains and to define their genetic relationship. OTs IgM positive whole blood samples were processed for PCR targeting the 56 kDa gene specific primers of OTs, and PCR positive samples were further reconfirmed by nucleotide sequencing. The percentage positivity was 27.56% (121/439) in whole blood specimens. Further the PCR positive samples were selected for genetic analysis to know the prevalent genotype of OTs in the Gorakhpur region. The phylogenetic analysis was carried out using sequences retrieved in our study along with global reference sequences downloaded from NCBI GenBank database. The sequence analysis confirmed Gilliam genotype is the most prevalent genotype in this area comprising about 95.1% (79/83) and the karp genotype is about 4.81% (4/83) of OTs (Fig 1). The percentage nucleotide identity is >99.95% in-between the sequences of the Gilliam genotype isolated in this study. (**Figure 1**).

In addition to it, 110 selected cases of OTs IgM /PCR negative with the sign of a rash and multi organ involvement were investigated for Spotted Fever Group (SFG) of rickettsia with primers targeting the 23s-5s Inter genomic region of rickettsia. The PCR result found that 12.72% (14/110) cases were positive and sequence analysis followed by preliminary blast result showed the prevalence of *R. conorii* in most of the cases.

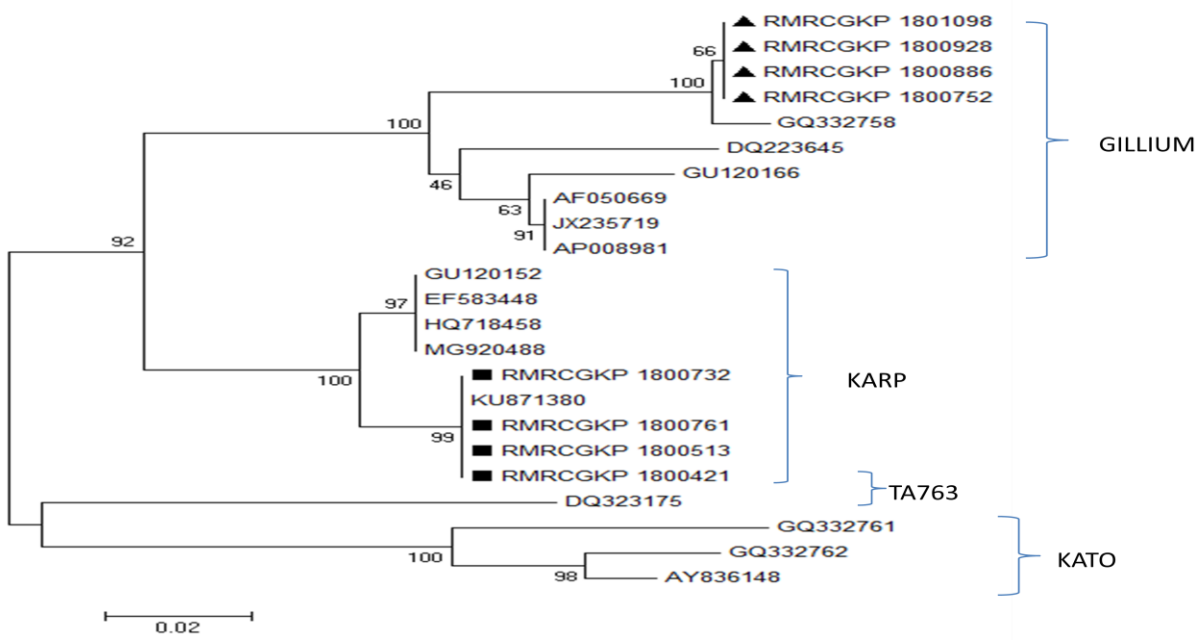

Figure: 1 Phylogenetic tree of *Orientia tsutsugmaushi* based on the nucleotide sequences of the 56-kDa cell surface antigen gene.

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

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

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

The clinical outcome of the disease is influenced by factors involving host, virus and environment. Polymorphisms in the genes coding for various molecules involved in the immune response against JEV might affect their expression and functioning and may be associated with susceptibility to encephalitis in JEV infection. To find out polymorphisms in genes coding for pattern recognition receptors and inflammatory mediators and receptors, a total number of 312 healthy controls without any history of encephalitis and 148 encephalitis cases positive for JEV were recruited from villages in blocks of Gorakhpur, Deoria, Maharajganj, Kushinagar and Sant Kabir Nagar. Patients were followed up for assessing post JE disability as well as a large spectrum of the behavioral disturbance. The frequency of TNFA -308 G/A [Odds Ratio (95%CI); 2.14 (1.10-4.16)], CCL2 -2518 G/G [1.89 (1.07-3.33)] genotype were significantly higher in JE cases as compared to controls. CCL2 -2518 G/G genotype associated with JE was of recessive mode (GG/G vs. A/A + A/G). A higher frequency of heterozygous genotype of CCR5 ▲32 mutation was observed in JE cases but the difference was not significant. The results suggest that G/A genotype of *TNFA -308* and G/G genotype of *CCL2 -2518* might be associated with susceptibility to Japanese encephalitis.

GKP1801: Establishment of a Health and Demographic Surveillance System [HDSS], Gorakhpur, Uttar Pradesh.

Investigators: Kamran Z, Avinash D, Nivedita G, M Murekhar, Kaushik K, Raja Ram Y & HDSS Gorakhpur study group

Funding: Extramural Duration: 2018-2021

Establishment of HDSS helps to monitor demographics of the population and provides a well characterized denominator for complex observational and interventional epidemiological

studies. Findings of HDSS will generate evidence for making holistic policies for this region. Such data collected over the years will be valuable because of its longitudinal nature.

The study area has been identified which includes 28 villages having about one lakh population. Recruitment of two project scientists, two data entry operators and seven technical staffs was completed. The basic household survey schedule was prepared after thoroughly studying the existing survey schedules. We performed a pilot survey on 23 households (123 individuals) in one of the 28 villages, to check the appropriateness of the questions to the target population. Training of the core team at NIE Chennai helped us in understanding the techniques and modalities for carrying out and resolving the technical issues during the field survey. The open data kit (ODK) application for tablet based data entry and data transfer will be used. GPS based mapping of the study area will be carried. The finalized questionnaire along with the subject information sheet and consent forms were submitted to the Institutional Human Ethical Committee (IHEC) NIV Pune for approval prior to using it in the field area.

Till date, the household listing (which include numbering of each residential structure) and manual mapping of 10 villages has been completed and a total of 3800 house numbered. Training plan for training field staffs and technical staffs and manual for mapping and household listing have been prepared.

GKP1802: **Reducing the burden of AES due to scrub typhus through the empiric treatment of acute febrile illness with doxycycline/ azithromycin: An implementation research study**

Investigators: M Murhekar, Jerome T, Kamran Z, AK Pandey, Avinash D.

Funding: Extramural(NIE, Chennai) Duration: 2018-Ongoing

Scrub typhus (ST) is the major etiology of AES outbreaks, accounting for >60% AES patients while Japanese encephalitis virus accounted for <10% cases. While most infections are asymptomatic, few children develop febrile illness and small proportion progress to AES. Hence early administration of appropriate antibiotics is crucial. Indian Council of Medical Research had recommended the state health authorities to provide empiric doxycycline/azithromycin (EDA) treatment to children with acute undifferentiated febrile illness (AUFIs) attending peripheral health facilities in Gorakhpur and Basti divisions during August–November, when AES cases peak. We estimated the effectiveness of EDA treatment in Gorakhpur district.

Medical Officers (MO) and pharmacists from all the PHCs/CHCs in the three blocks (Bhathat, Campiergunj and Jungle Kaudia) were sensitized about etiology of AES in the region, the rationale of EDA strategy, an algorithm for treatment of AUFIs including dosage of doxycycline/azithromycin by weight as well as age. All AUFI patients aged <=15 years, after their consultation with MOs were enrolled and followed up telephonically on the third and fifth day to collect information about clinical status including recovery, improvement or worsening of the clinical condition. At public health facilities, 801 (86%) were prescribed doxycycline/azithromycin. The compliance to prescribe EDA ranged between 76.4% to 92.7% across three health facilities. Of the 725 AUFI patients followed up, 621 (86%) had received EDA. Six of the 621 AUFI patients who received EDA and five of the 124 who did not receive EDA progressed to AES with cumulative incidences of 0.96% and 4.8% respectively. The effectiveness of EDA strategy was 79.9% (95% CI: 35.4–94).

EDA treatment to children with AUFI in peripheral health facilities between August and October could prevent progression to AES due to ST. For reducing the burden of ST-AES in the region, it is necessary to sensitize health professionals in public as well as private sector before the outbreak. It is also necessary to monitor the implementation of this strategy including the drug resistance.

SECTION-2: SIGNIFICANT HIGHLIGHTS/MAJOR ACHIEVEMENTS OF 2018-19 HAVING PUBLIC HEALTH IMPORTANCE

1. Stone foundation Ceremony on 2nd Sept 2018: Foundation stone of ICMR-Regional Medical Research Centre was laid today by Shri Yogi Adityanath, Chief Minister, Uttar Pradesh and Shri J P Nadda, Union Minister of Health and Family Welfare, Govt of India in the presence of Shri Ashutosh Tandon, Minster of Technical and Medical Education, UP Govt and Prof Balram Bhargava, Secretary to Govt of India, Department of Health Research and Director General, Indian Council of Medical Research (ICMR). The institute will be constructed in 3448 sq mt of land in BRD Medical College campus area with an estimated cost of Rs. 84 crore.


2. Biomedical communication workshop on 14th & 15th Feb 2019: RMRC, GKP oorganized a two day workshop on Biomedical communication for scientists and officers of the institute including medical professionals of BRD Medical College and Gorakhpur State health officials. The workshop was an initiative of Research Management Policy Planning and Coordination (RMPPC) Division of ICMR, New Delhi to strengthen the ability of scientists and academicians in the field of media management, academic writing, communication and converting research findings into policy briefs. A total of 47 participants (RMRC staff: 27, BRD Medical College Faculty: 14 and State Health official, Gorakhpur: 6) were benefited from the workshop.

3. **HDSS Project launch on 21st March 2019:** The project was launched will monitor the demography to deal with the public health problems in this area. The project will collect data on socio-demography, health and source of care, which will help to find out the factors associated with poor

health conditions and it will create a base population in which interventional studies could be conducted.

4. **Scrub typhus related research:**

- **Risk Factors for Acquiring Scrub Typhus among Children in Deoria and Gorakhpur Districts, Uttar Pradesh, India, 2017:** A case-control study indicated that children residing, playing, or visiting fields; living with firewood stored indoors; handling cattle fodder; and practicing open defecation were at increased risk for scrub typhus. Communication messages should focus on changing these behaviors.

- **Effectiveness of presumptive treatment of acute febrile illness with Doxycycline/ Azithromycin in preventing Acute Encephalitis Syndrome, Gorakhpur, India, 2018**: The pilot study showed the the effectiveness of EDA strategy was 79.9% (95% CI: 35.4–94). For reducing the burden of ST-AES in the region, it is necessary to sensitize health professionals in public as well as private sector before the outbreak.

SECTION-3: PATENTS IN YEAR 2018-19 OBTAINED/ APPLIED: NIL

SECTION-4: PUBLICATIONS INDEXED / NONE INDEXED WITH IMPACT FACTOR

1. Mittal M, **Bondre V**, Murhekar M, **Deval H**, Rose W, Verghese VP, Mittal M, Patil G, Sabarinathan R, Thangaraj JWV, Kanagasabai K, Prakash JAJ, Gupta N, Gupte M, Gupte D. Acute Encephalitis Syndrome in Gorakhpur, Uttar Pradesh, 2016: Clinical and Laboratory findings. **Pediatr Infect Dis J. 2018** May 9. doi:10.1097/INF.0000000000002099. **(Impact Factor: 2.305).**

2. Thangaraj JWV, Vasanthapuram R, Machado L, Arunkumar G, Sodha SV, **Zaman K**, Bhatnagar T, Hameed SKS, Kumar A, Abdulmajeed J, Velayudhan A, **Deoshatwar A**, Desai AS, Kumar KH, Gupta N, Laserson K, Murhekar M; Scrub Typhus Risk Factor Study Group. Risk Factors for Acquiring Scrub Typhus among Children in Deoria and Gorakhpur Districts, Uttar Pradesh, India, 2017. **Emerg Infect Dis. 2018** Dec; 24(12):2364-2367. doi: 10.3201/eid2412.180695**. (Impact Factor: 7.422).**

Section-5: FUTURE PLAN

A. The following proposals of RMRC-GKP were approved by Joint - Scientific Advisory Committee (Joint-SAC, 2018):
   - Identification of etiological agents of acute encephalitis syndrome by high-throughput sequencing in patients with AES
   - Clinical outcome and extent of disability (neurological sequelae) in children following acute encephalitis syndrome (AES) in Gorakhpur.
   - Detection of respiratory viruses in patients admitted to BRD Medical College, Gorakhpur

- Development of isothermal recombinase polymerase amplification and lateral flow analysis based rapid detection kit for *Orientia tsutsugamushi*. This proposal has recently been accepted by ICMR to fund as an extramural adhoc research project.

B. Moreover, the Chairman of Joint-SAC has also suggested to scientists of RMRC-GKP to propose work in following thrust areas:
   - To carry forward the ongoing work on encephalitis and to study JE vaccine coverage.
   - To study the prevalence, risk factors, develop a marker/screening system for oral and cervical cancer in eastern UP. Collaboration to be done with Cancer Department, BRD Medical College and ICMR- NICPR, Noida.
   - To study the association of dengue serotypes with disease severity and to develop a point of care test for Dengue infection
   - *Plasmodium vivax* associated encephalopathy, surveillance of malaria in eastern UP and to establish the microscopic set up for diagnosis in eastern U.P region.
   - To study on respiratory infection in less than 5 year children.
   - To carry out a survey of Diabetes, Hypertension and lipid profile abnormalities in local population especially in rural areas.
   - To estimate the burden of malnutrition and its associated morbidity.
   - To study the prevalence of Zoonotic/ rickettsial infections and Leptospirosis in animal/human population in Gorakhpur region.
   - Study on Mycobacterial infections in Gorakhpur region.
   - To carry the "Empirical Azithromycin/ Doxycycline for AFI" study at a larger level covering the whole Gorakhpur district in the upcoming season.
   - To propose the community based field study in HDSS site in collaboration with other scientists.