Investigations of seasonal outbreaks of acute encephalitis syndrome due to *Orientia tsutsugamushi* in Gorakhpur region, India: A One Health case study

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**Gorakhpur division consisting of Gorakhpur and neighboring districts Deoria, Kushinagar and Maharajganj in Uttar Pradesh, India, have been witnessing seasonal outbreaks of acute encephalitis syndrome (AES) among children for the last three decades. Investigations conducted during 2005 identified Japanese encephalitis (JE) virus as an aetiology of AES. With the introduction of JE vaccination and other control strategies, the incidence of JE in the region declined, however, outbreaks of acute febrile illness with neurological manifestations continued to occur. Subsequent investigations identified *Orientia tsutsugamushi*, as the major aetiology of AES outbreaks in the region. This review details clinical, epidemiological, animal and entomological investigations conducted for AES due to *O. tsutsugamushi* during 2015 and 2017 in Gorakhpur region. Surveillance of acute febrile illness among children attending peripheral health facilities identified scrub typhus as an important aetiology of febrile illness during monsoon and post-monsoon months. Population-based serosurveys indicated high endemicity of scrub typhus. Entomological studies demonstrated natural infection of *O. tsutsugamushi* in small animal hosts and vector mites. Children acquired this infection through recent exposure to outdoor environment, while playing, or visiting fields or defecating in open fields. A few of the children with scrub typhus progress to develop CNS manifestations. Hence, early administration of appropriate antibiotics is crucial in preventing progression of AFI due to scrub typhus to AES. The investigations conducted by the multi-disciplinary team helped understand the transmission dynamics of scrub typhus in Gorakhpur division and recommend strategies for its control.**

**Key words** Acute encephalitis syndrome - acute febrile illness - One Health - scrub typhus - vector
For the last three decades, Gorakhpur and its neighbouring districts Deoria, Kushinagar and Maharajganj have been witnessing seasonal outbreaks of acute febrile illness (AFI) with neurological manifestations such as altered sensorium and new onset of seizures, among children\(^1\,^2\). Between 2004-2013, around 1500 - 2000 acute encephalitis syndrome (AES) patients get admitted every year to BRD Medical College (BRDMC), Gorakhpur - the tertiary care hospital in the region\(^3\). These outbreaks occurred in monsoon and post-monsoon (June–October) months and were associated with high case fatality, in excess of 20 per cent\(^3\,^4\). The region experienced one of the worst outbreaks in 2005, where more than 5,737 cases of AES were reported from Gorakhpur and neighbouring districts, with 23 per cent deaths\(^5\). etiological investigations indicated that this outbreak was due to Japanese encephalitis virus (JEV)\(^6\). Following these investigations, the Government of India introduced live attenuated JE vaccine (SA-14-14-2) in the area, initially as mass vaccination campaigns targeting children aged 1-14 years and subsequently introduced the vaccine in the Universal Immunization Programme\(^7\). In 2013, a two-dose vaccination schedule was introduced, with first dose given at the age of 9-12 months and second dose at the age of 16-24 months\(^8\).

With vaccination and Information Education and Communication (IEC) campaigns, the incidence of JE declined\(^4\,^5\), however, outbreaks of AFI with neurological manifestations continued to occur in the region. Investigations conducted by a team of researchers from different ICMR institutes; Manipal Centre for Virus Research, Manipal; Christian Medical College (CMC), Vellore and Jawaharlal Institute of Postgraduate Medical Education & Research (JIPMER), Puducherry identified *Orientia tsutsugamushi*, the causative agent of scrub typhus, as the major aetiology of AES outbreaks in the region\(^9\). It was observed that nearly half of the AES cases had serological or molecular evidence of *O. tsutsugamushi* infection, while JEV and dengue virus accounted for about 10 and 7 per cent AES cases, respectively\(^9\). The presence of higher levels of *O. tsutsugamushi* IgM and IgG antibodies among AES patients than among controls further confirmed the role for scrub typhus in the aetiology of AES in Gorakhpur\(^10\).

Scrub typhus is a vector-borne zoonotic disease with the potential of causing life-threatening febrile infection in humans. It is transmitted by the bite of trombiculid mite infected with Gram negative *O. tsutsugamushi*. Mites are the vectors and the primary reservoir for scrub typhus. The infected mite population is maintained usually by a number of small rodents and shrews. The disease is endemic in several countries in Southeast Asia and Western Pacific region\(^11\). Scrub typhus patients with multi-system involvement may have high case fatality and hence early treatment with appropriate antibiotic is important\(^12\). Following the diagnosis of *O. tsutsugamushi* as the major aetiology of AES outbreaks in Gorakhpur region, the ICMR recommended administration of intravenous azithromycin to all hospitalized AES cases at BRD medical college, in September 2014 (unpublished data). Considering the zoonotic nature of the illness, a transdisciplinary team was constituted consisting of human and animal health experts and a number of studies were initiated to describe the clinical spectrum of children with AES due to *O. tsutsugamushi* infection, estimate disease burden, understand the transmission dynamics, and identify risk factors to develop control strategies. The results of these findings characterized the scrub typhus transmission dynamics in the affected region and recommended appropriate interventions.

Several of these efforts have been documented as individual research findings and warrant a composite presentation to describe the in-depth and multi-faceted studies done by different research teams. This review details the clinical, epidemiological, animal and entomological investigations conducted for AES due to *O. tsutsugamushi* during 2015 and 2017 in Gorakhpur region.

**Clinical spectrum of AES patients with *O. tsutsugamushi* infection (2016)**

To strengthen the AES surveillance, the ICMR established an AES Cell in the department of Paediatrics in BRD Medical College, Gorakhpur in 2016. This Cell coordinated collection of clinical details from AES cases on a standard case report form, collection and aliquoting of blood and CSF samples and ensured biochemical, haematological as well as aetiological investigations of all samples collected. In a case series of 230 AES cases with *O. tsutsugamushi* infection (based on PCR/IgM positivity in serum and/or CSF), the median age of patients was 61 (IQR: 36-120) months. All cases were from rural areas and males and females were equally affected. AES patients developed CNS manifestations such as seizures (88.7%) and altered sensorium (69.6%) after a median interval of six (IQR: 4-9) days after fever onset. Other presenting symptoms among AES patients included vomiting.
(46.5%), headache (13.9%), abdominal pain (15.2%) and diarrhoea (4.8%). The median Glasgow Coma Scale (GCS) score at admission was eight (IQR: 8-10).

Important findings on physical examination included hepatomegaly (43.5%), peri-orbital oedema (33.0%), splenomegaly (10.4%) and rash (5.2%). None of these patients had eschar. Thrombocytopenia, abnormal liver and kidney functions were the commonest haematological and biochemical abnormalities. CSF was clear, with pleocytosis and mildly raised proteins. Most patients received intravenous azithromycin. The case fatality ratio (CFR) was lower among AES patients positive for *O. tsutsugamushi* infection (35/230=15.2%) as compared to those negative for OT infection (51/141=36.2%, *P*=0.001).

Aetiology of AFI among children (2016)

All the studies about the aetiology of AES were conducted among patients hospitalized at the BRD Medical College, Gorakhpur. About one fourth of these patients gave a history of febrile illness for at least a week before developing neurological manifestations.

A qualitative study conducted to understand the health seeking behaviour for febrile illness revealed that most parents took their children to traditional healers first, and parents took the child to BRD Medical College or district hospital if the child developed seizures or altered sensorium (unpublished data). It was therefore, considered worthwhile to find out the aetiology of children presenting with AFI attending peripheral health facilities during monsoon/post-monsoon months. A facility-based surveillance for AFI was established in three peripheral health facilities and all children presenting with fever of four days or more were enrolled in the surveillance. Analysis of serum specimen from 224 children with AFI during August to October, indicated that about one-fourth had IgM antibodies against *O. tsutsugamushi*. Three children also had eschar, which is pathognomonic of scrub typhus. The other common aetiologies of AFI were dengue fever (8%), spotted fever group rickettsiae (SFGR) infection (6%) and leptospirosis (3%). These findings indicated that *O. tsutsugamushi* infection was the commonest aetiology of AFI among children during monsoon/post-monsoon months.

Seroprevalence in the community (2016)

Scrub typhus outbreaks have been reported in occupational groups with frequent exposure to outdoor environment, such as military personnel, agriculture workers, etc. Most AES cases seen at the tertiary care hospital in Gorakhpur were children aged ≤14 years. This age distribution of AES cases suggested exposure to *O. tsutsugamushi* infection during childhood. Population based serosurveys were conducted to estimate age-specific prevalence of *O. tsutsugamushi* infection in different villages in Gorakhpur district reporting AES cases

The first survey was conducted during April-May (n=1085), and second during October–November (n=906), corresponding respectively with the lean and epidemic period. The overall seroprevalence of IgG antibodies during AES epidemic period was higher (70.8%) as compared to that of lean AES period (50.6%, *P*<0.001).

In both survey periods, seroprevalence increased with age, with higher prevalence among females. The serial nature of the serosurveys also provided opportunity to estimate sero-incidence of *O. tsutsugamushi* infection. Of the 254 seronegative cases for serological markers of *O. tsutsugamushi* infection during April-May, 19.7 per cent seroconverted; more than half of whom had no history of febrile illness during the intervening period suggesting subclinical nature of infection. The sero-incidence of new infection was not different by age-group and sex.

*O. tsutsugamushi* infection in small animals and mites (2015)

Although the clinical and laboratory findings among AES and AFI patients indicated the role of *O. tsutsugamushi* infection, evidence of the presence of the pathogen in animal hosts and vector mites was considered confirmatory to its transmission to humans.

A cross-sectional survey of trombiculid mites was carried out during July and October 2015, in randomly selected villages in Gorakhpur with recent report of AES cases. In the selected villages Sherman live traps were set in peri-domestic areas to catch rodents/shrews. Blood sample was collected from the trapped rodents/shrews and serum samples were tested using Weil–Felix test for antibodies against three antigens: OX-19 (*Rickettsia typhi*), OX-2 (*Rickettsia conorii*), and OX-K (*O. tsutsugamushi*). The trapped rodents were euthanized and ectoparasites, including chigger (larval) mites, were collected by combing the animals. Also, the ears, limbs, and axillary regions of individual rodents were examined for mite attachment. Based on the exoskeleton structure, mites were identified to species level following standard taxonomical keys, and the tissue samples were pooled and subjected to PCR assays. Molecular diagnosis of the scrub typhus was targeted for two different gene fragments, *groEL* and
Seasonal abundance of *Leptotrombidium deliense* (2016-17)

A year-round study was conducted in 2016-2017 to examine the seasonal abundance of *L. deliense* in rural areas of Gorakhpur district. During the monthly collections, a total of 903 animals (rodents/shrews) were collected using 6484 Sherman traps. *S. murinus* was predominantly (67%) trapped. A total of 5526 mites belonging 12 species under nine genera of trombiculids were collected from the trapped rodents/shrews. *Leptotrombidium* (L) *deliense* was the predominant species (64.7%) followed by Schoengastiella ligula (17.4%), the suspected vector of scrub typhus. The overall chigger index was 5.3 per animal. The *L. deliense* index was relatively higher during July to November with a peak in October (Fig. 1). The index of *S. ligula* was very low particularly during this season. The natural infection of *O. tsutsugamushi* was detected only in *L. deliense* specimens, collected during rainy months (July - October). Among the 5526 mite samples tested as 352 pools in nested PCR, four pools were positive for 56 kDa gene. The peak abundance of *L. deliense* coincided with peak incidence of AES cases in the area (Unpublished data).

Risk factors for scrub typhus infection (2018)

The next step in the investigation was to find out household characteristics and behavioural risk factors associated with scrub typhus infection among children. In this case control study, 155 febrile children positive for IgM antibodies against *O. tsutsugamushi* were compared with 409 febrile children seronegative for IgM and IgG antibodies\(^\text{19}\). Cases, controls and their parents or guardians were interviewed to collect information on socio-demographics, household characteristics, behaviours, and environmental exposures during the preceding two weeks before fever onset. The findings of this study revealed that children residing in houses within or adjoining agriculture fields and that stored firewood indoors had higher odds of acquiring scrub typhus. Children who had a recent exposure to outdoor environment while defecating in open, playing in or visiting agricultural fields were also at higher risk of scrub typhus infection\(^\text{19}\).

Summarizing the evidence

The findings of the studies described above provided data about the transmission dynamics of scrub typhus in the area. Entomological studies demonstrated natural infection of *O. tsutsugamushi* in small animal hosts and vector mites. *Leptotrombidium* mites were abundantly present on shrews during monsoon and post-monsoon months. High seroprevalence of IgG antibodies indicated that the population in rural areas of Gorakhpur division was frequently exposed to *O. tsutsugamushi* infection. Children acquired this infection through recent exposure to outdoor environment, while playing, or visiting fields or defecating in open fields. Although most infections were subclinical in nature, scrub typhus was an important aetiology of febrile illness among children, accounting for nearly one-fifth of febrile illness attending peripheral health facilities during monsoon and post-monsoon months. A few of the children with scrub typhus progressed to develop CNS manifestations. Hence, early administration of appropriate antibiotics is crucial in preventing progression of AFI due to scrub typhus to AES.
Presumptive treatment of AFI with doxycycline or azithromycin (2018)

There are limited laboratory facilities for diagnosis of scrub typhus infection at primary care facilities in Gorakhpur division. The use of rapid diagnostic tests for all fever patients attending these facilities could be cost-prohibitive. Moreover, serological tests become positive only after 5-7 days. In view of this, as well as considering the risk of progression of AFI patients to AES which is associated with high case fatality, and over-burdened public health facilities, treating children presenting with AFI at peripheral health facilities during monsoon and post-monsoon months, presumptively with doxycycline or azithromycin based on clinical suspicion was considered as a strategy for reducing incidence and mortality due to AES. The Government of Uttar Pradesh issued guidelines to the health facilities of districts in Gorakhpur division for this presumptive treatment in 2016\textsuperscript{20}. A pilot project conducted in three peripheral health facilities indicated that presumptive treatment of children with AFI with doxycycline/azithromycin had about 80 per cent effectiveness in preventing progression to AES\textsuperscript{21}.

Disability following AES caused by \textit{Orientia tsutsugamushi} (2018)

Survivors of infective encephalitis may have varying degrees of neurologic or neuropsychiatric sequelae. Little information was available about neurologic sequelae among scrub typhus patients with CNS manifestations. A study to estimate the proportion and spectrum of disability among 146 survivors of AES due to \textit{O. tsutsugamushi} indicated that 56 (38.4\%) had mild, whereas 19 (13\%) had moderate to severe degree of disability after median interval of five months of hospitalization. Most patients had impairment in the domain of cognition and behaviour, while <10 per cent had impairment in the domain of mobility and activity limitation in selfcare. The study indicated that disabilities were frequent among the survivors of AES caused by \textit{O. tsutsugamushi}\textsuperscript{22}.

Way forward

The investigations conducted by the multi-disciplinary team constituted by the ICMR helped to understand the transmission dynamics of scrub typhus in Gorakhpur division and recommend strategies for its control. The decline in the number of AES cases and deaths in the region (Fig. 2, unpublished data) observed in the last three years could be due to the presumptive use of doxycycline/azithromycin for febrile illness and administration of intravenous azithromycin for AES patients as well as awareness campaigns such as \textit{Dastak Abhiyan} to seek early treatment for AES and strengthening of peripheral health facilities to manage AES cases conducted by the Government of Uttar Pradesh. It is however, necessary to closely monitor the implementation of the presumptive treatment strategy during monsoon and post-monsoon season and ensure adequate supply of doxycycline and azithromycin at the public health facilities. Reduction in the AES disease burden would also require sensitizing clinicians in private sector about early treatment of suspected scrub typhus cases with appropriate antibiotics. Further, as part of long-term approach to facilitate early treatment of scrub typhus
at peripheral health facilities, introducing affordable point of care tests at the primary care level especially during AES season could help in early diagnosis of scrub typhus and initiate appropriate treatment 23. The environmental factors are conducive for transmission of *O. tsutsugamushi* in the area. Measures to control vector mites or rodent could be challenging. Hence, behaviour change communication about avoiding open defecation and exposure to outdoor environment, as well as providing household and community toilets through the Government flaghip programmes such as *Swachh Bharat Abhiyan* could also help reducing such infections in the region 23. Considering the wide range and magnitude of disability among survivors of AES patients, it is also necessary to screen all AES patients to early identify disabilities and initiate appropriate rehabilitative care in the nearest District Disability Rehabilitation Centres. Lastly, continued surveillance for AES in the region is necessary to monitor the trend as well as impact of different control strategies.

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