Details of the research work duly signed by the applicant, for which the Sun Pharma Science Foundation Research Award is claimed, including references and illustrations (not to exceed 6000 words).

Population based seroepidemiological studies are very valuable in measuring the disease burden, understand the population level immunity and document the effectiveness of vaccination programs. In the last five years, I led a number of research studies on SARS-CoV-2, dengue, Chikungunya, diphtheria, hepatitis-B, Nipah, rubella and scrub typhus, which generated data about disease burden and supported key policy decisions.

Seroprevalence of SARS-CoV-2 in India, 2020-21

Surveillance for COVID-19 in ongoing in all Indian districts. This surveillance is likely to be influenced by the testing capacity and testing strategy as well as health seeking behavior of the population. Moreover, mild or asymptomatic cases, which account for majority of COVID-19 cases, hence might not undergo testing for laboratory confirmation and hence could be missed by surveillance system. In this context, seroepidemiological studies for COVID-19 are very valuable in measuring the extent of spread of infection and identify socio-demographic groups at higher risk. Serosurveys are also useful to in understanding the future trajectory of the COVID-19 pandemic.

During the COVID-19 pandemic, the Indian Council of Medical Research conducted four nationally representative serosurveys at different time-points in the pandemic to estimate the age-specific seroprevalence of IgG antibodies against SARS-CoV-2. These surveys were conducted in the same 70 districts, selected randomly from all the districts in India. From each district, 10 clusters (villages in rural and wards in urban areas) were selected by population proportional to size method and from each cluster 40 individuals were selected (10 from each random starting point). Each survey covered a sample size of more than 28,000. The first survey was conducted among adults, second and third among individuals aged >=10 years and fourth among individuals aged >=6 years. During the third and fourth surveys, healthcare workers working in district and sub-district level hospitals were also surveyed.

During May-June 2020, at the beginning of the pandemic in India, less than 1% (0.73%, 95% CI: 0.34%-1.13%) of India's adult population was exposed to SARS-CoV-2.[1] The prevalence among adults increased from 7.1% (95% CI: 6·2-8·2) by August – September 2020[2] to 24.1% (95% CI: 23.0-25.3%) during December 2020 – January 2021.[3] The fourth serosurvey conducted during June- July 2021 indicated that 67. 6% (66.4 - 68.7) of the surveyed population had antibodies against SARS-CoV-2 (Fig 1).[4] The seroprevalence among healthcare workers during the third and fourth surveys was 25.6% (95% CI 23.5-27.8%) and 85.2% (95% CI: 83.5 - 86.7) respectively.

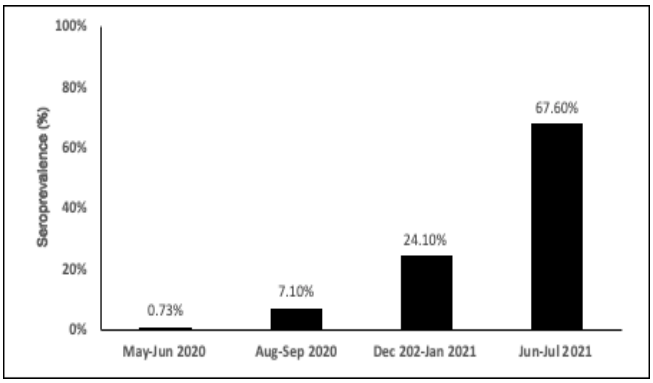

Fig 1: Trend of SARS-CoV-2 seroprevalence in India

Besides the rising trend of seroprevalence, these serosurveys also provided insights about the spread of infection by age group, sex and areas of residence. The serosurveys during the first wave of pandemic showed that the seroprevalence was highest among individuals residing in urban slums followed by urban non-slum areas and lowest in rural areas. After the second wave of COVID-19, this gradient in the seroprevalence seems to have faded, indicating that the infection was widespread in rural areas.

Public health implications: These serosurveys provided information about the adequacy of COVID-19 testing in India, by estimating the infection to case ratio (ICR, defined as number of SARS-CoV-2 infection per laboratory confirmed COVID-19 case) at different time-points in the pandemic. During May-June 2020, there were an estimated 81.6 (95% CI: 48.3-141.4) infections per reported COVID-19 case in India, indicating the need for increasing the testing capacity and expanding testing strategy. The ICR improved

[1] Murhekar MV et al. Prevalence of SARS-CoV-2 infection in India: Findings from the national serosurvey, May-June 2020. Indian J Med Res. 2020 Jul & Aug;152(1 & 2):48-60. doi: 10.4103/ijmr.IJMR_3290_20.
[2] Murhekar MV et al. SARS-CoV-2 antibody seroprevalence in India, August-September, 2020: findings from the second nationwide household serosurvey. Lancet Glob Health. 2021 Mar;9(3):e257-e266. doi: 10.1016/S2214-109X(20)30544-1
[3] Murhekar MV, et al. SARS-CoV-2 seroprevalence among the general population and healthcare workers in India, December 2020-January 2021. Int J Infect Dis. 2021 Jul;108:145-155. doi: 10.1016/j.ijid.2021.05.040
[4] Murhekar MV et al. Prevalence of Igg Antibodies Against SARS-CoV-2 Among the General Population and Healthcare Workers in India, June–July 2021. (Preprint) https://papers.ssrn.com/sol3/results.cfm

subsequently, from 26–32 during August – September 2020, to 26 – 27 during December 2020 – January 2021 to 22-27 during June – July 2021 on account of increased testing capacity as well as expansion of testing strategy in the country.

The findings of the recent serosurvey could help in understanding the future course of the pandemic and guide the response activities. About one third of the general population were not having detectable antibodies against SARS-CoV-2 by June 2021. It is therefore possible that more COVID-19 cases are likely to occur in coming months, especially in areas where the proportion of people without detectable antibodies is higher. It is also reasonable to expect that the future surge of cases in India would be lower than the second wave. Based on these findings, ICMR recommended acceleration of coverage of COVID-19 vaccination and ensuring adherence to non-pharmaceutical interventions, continuing surveillance for early detection of cluster of cases and monitor emergence of VOC for prevention of the transmission in the country and preparedness for the possible third wave. The importance of local level seroprevalence data for guiding response is equally important.

Burden of dengue virus infection in India, 2017-18

Dengue is the most rapidly spreading vector-borne disease globally. The dengue disease burden in India is poorly quantified. Existing public health surveillance systems are not sensitive; mild febrile illnesses are less likely to be diagnosed and reported. The data from the private sector, where most patients seek care, largely remains untapped. Moreover, surveillance systems are not designed to capture subclinical infections, which account for about 75% of dengue infections.

Dengue endemicity data was required for a policy decision for introduction of a dengue vaccine in India. To address the data gap, we conducted a national level serosurvey during 2017-18, in 60 randomly selected districts of 15 Indian states covering 5 geographic regions (north, east, west, south, and north-east) of India.[5] From each district, four clusters (villages/wards) were selected randomly, the population of the entire cluster was enumerated to prepare the sampling frame, and 25 individuals each from

[5] Murhekar MV et al. Burden of dengue infection in India, 2017: a cross-sectional population based serosurvey. Lancet Glob Health. 2019 Aug;7(8):e1065-e1073. doi: 10.1016/S2214-109X(19)30250-5.

the age group of 5–8 years, 9–17 years, and 18–45 years were randomly selected from the sampling frame. The randomly selected individuals were visited in their households consenting individuals were included in the study. The sera samples were tested for the presence of IgG antibodies against dengue using indirect ELISA, and a sub-sample was tested by plaque reduction neutralization test (PRNT90) against four DENV serotypes and the PRNT data were used to adjust the ELISA kit cutoff.

The overall seroprevalence of dengue infection was 48.7% (95% CI: 48·7% (95% CI 43·5–54·0), increasing from 28·3% among children aged 5–8 years to 41·0% among children aged 9–17 years and 56·2% among individuals aged between 18–45 years. The seroprevalence was high in the southern (76·9%), western (62·3%), and northern (60·3%) regions (Fig 2).

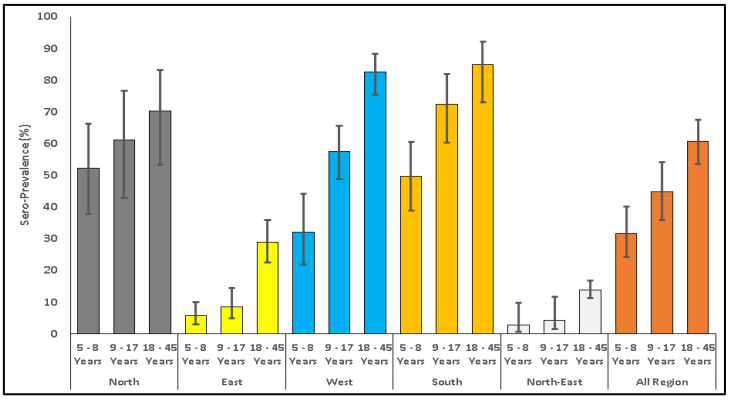

Fig 2: Age specific seroprevalence of dengue infection by region, India, 2017-18

A catalytic model constructed (Fig 3) based on the age specific seroprevalence indicated that in 2017, there were about 9 million (based on age dependent force of infection)– 13 million (based on constant force of infection) primary dengue infections in 2017 among individuals aged 5–45 years from 30 Indian states. This study also generated data about the profile of dengue serotype-specific neutralizing antibodies. In eastern and northeastern regions, where dengue seroprevalence was low, most infections were

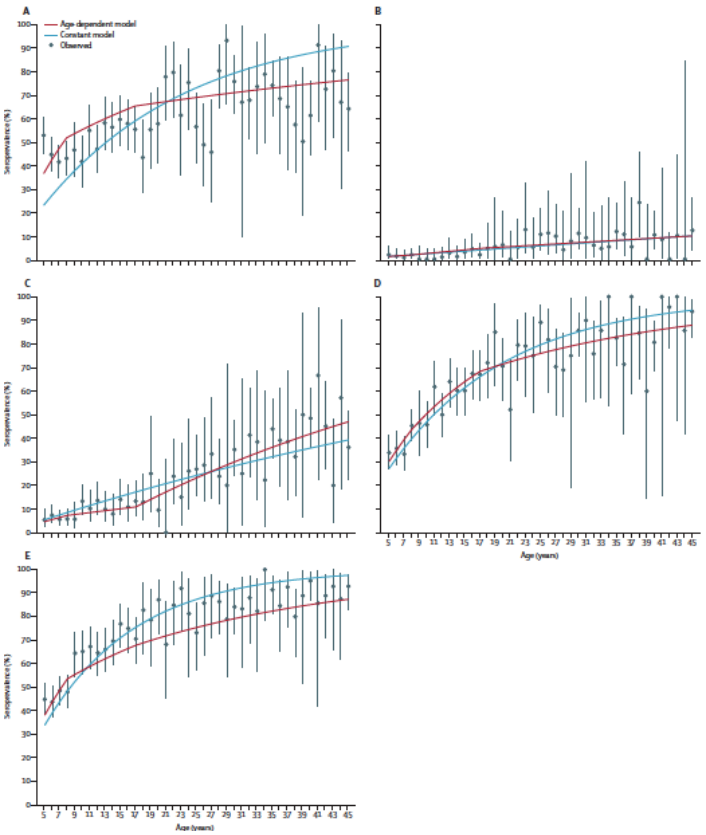

Fig 3: Observed and model-predicted seroprevalence of dengue by age Data presented with 95% CIs. (A) Northern region. (B) Northeastern region. (C) Eastern region. (D) Western region. (E) Southern region.

monotypic in nature; while in northern, western, and southern regions most dengue infections were multitypic in nature.

We also estimated that 10.7% (10.7%; 95% CI: 9.4-12.1) individuals with evidence of past dengue infection (sera positive IgG Indirect ELISA) had high antibody titers on dengue IgG capture ELISA, suggestive of acute or recent secondary infection. Such individuals were more likely from northern and southern regions of India.[6]

Public health implications: Besides understanding the disease burden, the findings of our survey will be useful in making informed decisions about the introduction of newer dengue vaccines in the country.

Population immunity against diphtheria among children aged 5-17 years

Diphtheria is re-emerging as a public health problem in several Indian states, with most cases occurring among children older than 5 years, indicating lower coverage of diphtheria booster doses as well as decline in immunity acquired due to partial vaccination. Information about population level immunity against diphtheria is scanty. In this study, we tested the residual serum samples from the national serosurvey for dengue infection to estimate the age group-specific seroprevalence of antibodies to diphtheria in children aged 5–17 years in India. We considered children with antibody concentrations of 0·1 IU/mL or greater as immune, Individuals with levels less than 0·01 IU/mL as non-immune (and hence susceptible to diphtheria), and those with levels in the range of 0·01 to less than 0·1 IU/mL as partially immune.

The findings of this study indicated that less than one-third (29·7%, 95% CI 26·3–33·4) of children aged 5–17 years were immune to diphtheria, 10·5% (8·6–12·8) were non-immune, and 59·8% (56·3–63·1) were partially immune. The proportion of children non-immune to diphtheria was higher in northeast region (16.8%, 95% CI: 11.2-24.4) and

[6] Santhosh Kumar M, Kamaraj P, Khan SA, Allam RR, Barde PV, Dwibedi B, Kanungo S, Mohan U, Sundar Mohanty S, Roy S, Sagar V, Savargaonkar D, Tandale BV, Topno RK, Kumar CPG, Sabarinathan R, Kumar VS, Karunakaran T, Jose A, Sadhukhan P, Toteja GS, Dutta S, Murhekar M. Seroprevalence of Dengue Infection Using IgG Capture ELISA in India, 2017-2018. Am J Trop Med Hyg. 2021 Aug 9:tpmd210386. doi: 10.4269/ajtmh.21-0386.

urban areas (13.1%, 95% CI: 10.2-16.6) (Fig 4). A higher proportion of girls than boys were non-immune to diphtheria in the northern and northeastern regions.[7]

Public health implications: This was the first study documenting the population level immunity against diphtheria at the national level. The study findings have important programmatic implications. With a substantial proportion of children non-immune or partially immune to diphtheria, transmission is likely to continue in India until the immunity gap is bridged through adequate coverage of primary vaccination and booster doses administered as a part of universal immunization and school health programs. Being a nationally representative survey, the study findings could serve as a baseline of population immunity for assessing the effect of introduction of tetanus-diphtheria vaccine in India.

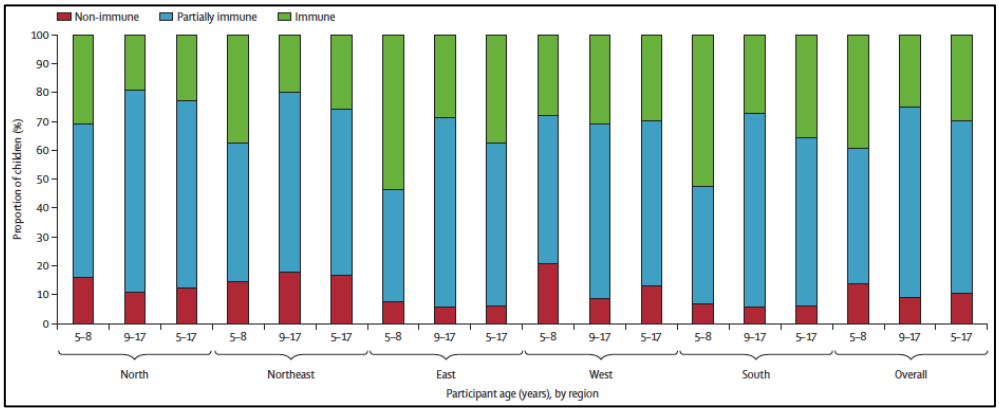

Fig 4: Diphtheria immunity among children aged 5-17 years, by age group and geographical region, 2017-18

Burden of Chikungunya virus infection

Following the re-emergence of Chikungunya virus (CHIKV) in 2005, transmission has been documented in most Indian states. Information is scarce regarding the seroprevalence of CHIKV in India. In this study, we estimated the age- specific seroprevalence, force of infection (FOI), and proportion of the population susceptible to CHIKV infection, using the residual sera from the national serosurvey for dengue virus infection.

[7] Murhekar MV et al. Immunity against diphtheria among children aged 5-17 years in India, 2017-18: a cross-sectional, population-based serosurvey. Lancet Infect Dis. 2021 Jun;21(6):868-875. doi: 10.1016/S1473-3099(20)30595-8

The overall prevalence of IgG antibodies against CHIKV was 18·1%. The seroprevalence was lowest in the northeast region (0·3%) and highest in the southern region (43·1%), with a significant difference in seroprevalence between rural (11·5%) and urban (40·2%) areas. The FOI was lowest in the eastern and northeastern regions (Fig 5). The estimated proportion of the population susceptible to CHIKV in 2017 was lowest in the southern region (56·3%) and highest in the northeastern region (98·0%).[8]

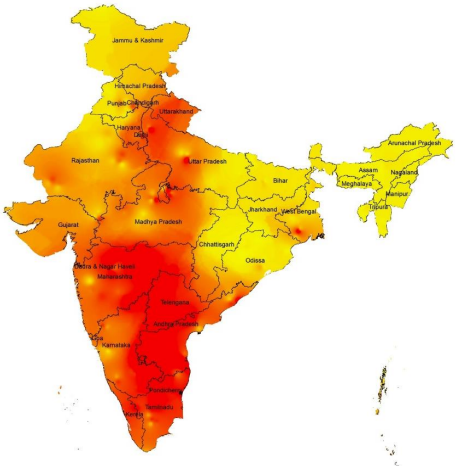

Fig 5: Seroprevalence of CHIKV infection across different geographic regions in India, 2017-18

Public health Implications: Higher proportion of the population susceptible to CHIKV in the eastern and northeastern regions suggests a susceptibility of these regions to outbreaks in the future. With several CHIKV vaccine candidates in different phases of development, this serosurvey findings would be useful in identifying appropriate target age groups and sites for setting up surveillance and for future CHIKV vaccine trials in India.

Impact of hepatitis B vaccination in India

Hepatitis-B (HB) vaccine was introduced in the Universal Immunization Program in India during 2002–2003 on a pilot basis, expanded to ten states in 2007–2008 (phase-1), and the entire country in 2011–2012 (phase-2). Except for a few small-scale studies, there are no data regarding the impact of the hepatitis B vaccine introduction at the national level in India. This study assessed the impact of HB vaccination by comparing the prevalence of different markers of HB infection among children born before and after

[8] Santhosh Kumar M, Kamaraj P, Khan SA, Allam R, Barde PV, Dwibedi B, Kanungo S, Mohan U, Mohanty SS, Roy S, Sagar V, Savargaonkar D, Tandale BV, Topno RK, Girish Kumar CP, Sabarinathan R, Saravana Kumar V, Bitragunta S, Grover GS, Lakshmi PVM, Mishra CM, Sadhukhan P, Sahoo PK, Singh SK, Yadav CP, Ramya Dinesh E, Karunakaran T, Govindhasamy C, Daniel Rajasekar T, Jeyakumar A, Suresh A, Augustine D, Ashok Kumar P, Kumar R, Dutta S, Toteja GS, Gupta N, Clapham HE, Mehendale SM, Murhekar MV. Seroprevalence of chikungunya virus infection in India, 2017: a cross-sectional population-based serosurvey. Lancet Microbe. 2021, 2: e41-e47

introduction of HB vaccine in India, using the sera from the nationally representative serosurvey.

Among children aged 5-8 years, 1.1% were chronic carriers (positive for HBsAg and anti-HBc), 5.3% immune due to past infection (positive for anti-HBc and negative for HBsAg), and 23.2% vaccinated (positive for anti-HBs but negative for anti-HBc and HBsAg). The corresponding proportions among children aged 9-17 years were 1.1%, 8.0%, and 12.0%, respectively. In the seven phase-1 states, the proportion of children chronically infected was not different among children aged 11–17 years (born before the introduction of the HB vaccine) and children aged 5–10 years (born after the introduction of the hepatitis B vaccine) (χ^2 = 0.255, p = 0.614). On the other hand, the proportion of children immune due to past infection was lower among children born after the introduction of vaccine (4.9%) as compared to those born before the introduction of vaccine (7.6%; χ^2=12.56, p < 0.001). A higher proportion of children born after the introduction of the HB vaccine had evidence of vaccination (37.7% vs. 14.7%; χ^2= 249.0, p < 0.001) (Table 1).[9]

Table: Status of hepatitis B virus infection among children from phase-1 states before and after the introduction of hepatitis B vaccination.

Hepatitis B status	Age group		P
	5-10 years (n=2448)	11-17 years (n=1594)	0.614
% Chronically infected (95% CI)	0.7% (0.4-1.1)	0.6% (0.3-1.1)	0.614
% Immune due to past infection (95% CI)	4.9% (4.1-5.9)	7.6% (6.4-9.1)	<0.001
% Immune due to vaccination (95% CI)	37.7% (35.8-39.6)	14.7% (13.1-16.6)	<0.001

Public health significance: The findings of this study could be considered an interim assessment of the impact of the hepatitis B vaccine introduction, which indicates that India is on track for achieving the South East Asia Regional goal of 1% HBsAg prevalence among 5- year-old children.

[9] Murhekar MV et al. Hepatitis-B virus infection in India: Findings from a nationally representative serosurvey, 2017-18. Int J Infect Dis. 2020 Nov;100:455-460. doi: 10.1016/j.ijid.2020.08.084.

Burden of congenital rubella syndrome (CRS) in India

India is committed to eliminate measles and rubella/Congenital Rubella Syndrome (CRS) by 2023. Towards this goal, India conducted nationwide supplementary immunization activity (SIA) with measles-rubella containing vaccine (MRCV) targeting children aged between 9 months to <15 years and established a hospital-based sentinel surveillance for CRS. Reliable data about incidence of CRS is necessary to monitor progress towards the elimination goal. As an adjunct activity to CRS surveillance, serosurveys were conducted among pregnant women attending the antenatal clinics of the 12 sentinel hospitals where CRS surveillance is ongoing. This study estimated the burden and incidence of CRS using catalytic models constructed based on the age-specific seroprevalence data of IgG antibodies against rubella.

The seroprevalence of IgG antibodies against rubella among pregnant women attending antenatal clinics of the sentinel surveillance sites ranged between 70% - 89% (Fig 6). Based on the constant and age-dependent force of infection models, the annual incidence of CRS in India was 225.58 per 100,000 live births (95% CI: 217.49–232.41) and 65.47 per 100,000 live births (95% CI: 41.60–104.16) respectively (Table 5). This translated to an estimated 14,520 (95% CI: 9,225–23,100) and 50,028 (95% CI: 48,234–51,543) infants with CRS every year based on age-dependent and constant force of infection models respectively.[10]

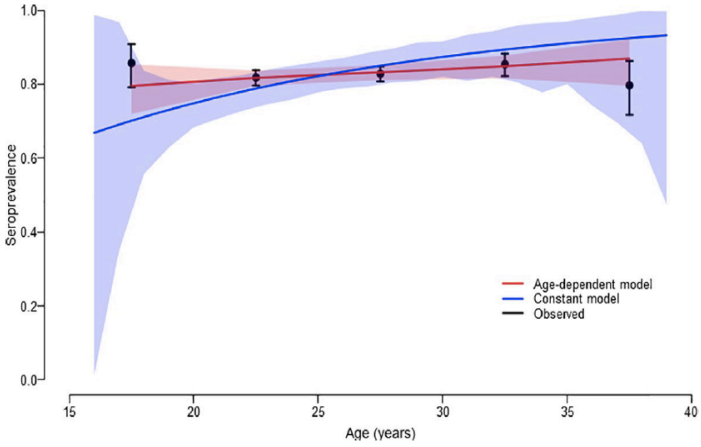

Fig 6: Seroprevalence of CHIKV infection across different geographic regions in India, 2017-18

[10] Shanmugasundaram D, Awasthi S, Dwibedi B, Geetha S, Jain M, Malik S, Patel B, Singh H, Tripathi S, Viswanathan R, Agarwal A, Bonu R, Jain S, Jena SK, Priyasree J, Pushpalatha K, Ali S, Biswas D, Jain A, Narang R, Madhuri S, George S, Kaduskar O, Kiruthika G, Sabarinathan R, Sapakal G, Gupta N, Murhekar MV. Burden of congenital rubella syndrome (CRS) in India based on data from cross-sectional serosurveys, 2017 and 2019-20. PLoS Negl Trop Dis. 2021 Jul 23;15(7):e0009608. doi: 10.1371/journal.pntd.0009608.

Public health significance: The estimates of CRS incidence will serve as a baseline to monitor the impact of MRCV SIAs, as well progress towards the elimination goal of rubella/CRS.

Infections among contacts of patients with Nipah virus, India, 2018

Nipah virus (NiV) infection is an emerging zoonotic disease which can cause severe disease in both animals and humans. Although NiV is known to cause subclinical infections, the extent of these infections among close contacts showed wide variations between outbreaks in Bangladesh (no subclinical infection) to about 5-15% subclinical infections during outbreaks in Malaysia and Singapore.

This study documents the extent of subclinical infections among close (hospital and household/community) contacts during the 2018 NiV outbreak in Kozhikode and Malappuram districts, Kerala.[11] The overall seroprevalence of subclinical infections among 279 contacts was 1.08% (95% CI 0.37–3.11). Two of the three contacts were family members of a laboratory-confirmed patient, and the third was an HCW in the emergency medicine department. All 3 had a history of exposure to body fluids of >1 NiV patient. None of the NiV seropositive contacts had history of febrile illness.

Public health significance: Subclinical infections among close contacts of Nipah patients were less infrequent and occurred only among individuals who had a definite history of exposure to body fluids of laboratory confirmed patient. These findings have important implications for contact tracing among the close contacts of laboratory confirmed NiV cases in future outbreaks.

[11] Kumar CPG, Sugunan AP, Yadav P, Kurup KK, Aarathee R, Manickam P, Bhatnagar T, Radhakrishnan C, Thomas B, Kumar A, Jayasree J, Philomina B, Kumar KGS, Thulaseedharan NK, Gupta N, Rajendran R, Saritha RL, Mourya DT, Gangakhedkar RR, Murhekar MV. Infections among Contacts of Patients with Nipah Virus, India. Emerg Infect Dis. 2019 May;25(5):1007-1010. doi: 10.3201/eid2505.181352.

Scrub Typhus as an etiology of Acute Febrile Illness among children in Gorakhpur, Uttar Pradesh, India.

Gorakhpur and the adjoining districts of Indian state of Uttar Pradesh have been witnessing seasonal outbreaks of acute encephalitis syndrome (AES), causing high morbidity and mortality especially among children. Investigations conducted during the 2014 and 2015 outbreaks revealed presence of immunoglobulin M (IgM) antibodies against O. tsutsugamushi, a causative agent of scrub typhus, in more than 60% of AES cases, suggesting a role for scrub typhus among the etiological agents of AES. Studies also indicated that AES cases were hospitalized late.

In order to better understand the epidemiology of scrub typhus/AES in the area, we conducted a study to estimate the proportion of fever cases attending peripheral health facilities because of scrub typhus. We established a facility-based surveillance among children attending peripheral health facilities in Gorakhpur during August – October. Analysis of 224 consecutive patients with acute febrile illness (AFI) enrolled in the surveillance, indicated that scrub typhus was one of the commonest etiology of acute febrile illness in monsoon and post-monsoon season, accounting for about one-fifth (18%, 95% CI: 13.3–23.3) of all patients (Fig 7).[12] Children with scrub typhus were more likely to have hepatomegaly (adjusted odds ratio, AOR = 3.2, 95% CI: 1.3–7.8), or lymphadenopathy (AOR: 2.9, 95% CI: 1.3–7.3) and did not have cough (AOR: 2.5, 95% CI: 1.1–6.9).

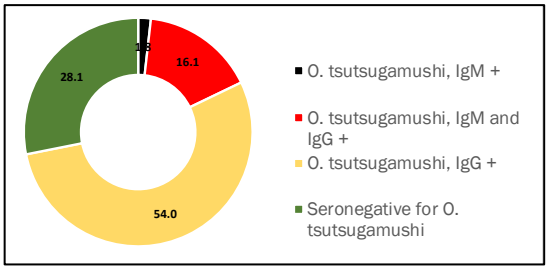

Fig 7: IgM and IgG seropositivity against O. tsutsugamushi

Public health implications: The evidence generated by this study, as well as other investigations (including studies among AES patients and entomological investigations) conducted in the region, was the basis of ICMR recommendations for early treatment of suspected scrub typhus cases with doxycycline or azithromycin in order to prevent their progression to AES.

[12] Vivian Thangaraj 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. doi: 10.4269/ajtmh.17-0135.