Seroprevalence of chikungunya virus infection in India, 2017: a cross-sectional population-based serosurvey

Muthusamy Santhosh Kumar, Pattabi Kamaraj, Siraj Ahmed Khan, Ramesh Reddy Allam*, Pradip V Barde*, Bhagirathi Dwibedi*, Suman Kanungo*, Uday Mohan*, Suman Sundar Mohanty*, Subarna Roy*, Vivek Sagar*, Deepali Savargaonkar*, Babasaheb V Tandale*, Roshan Kamal Topno*, Chethrapilly P Girish Kumar, Ramasamy Sabarinathan, Velusamy Saravana Kumar, Sailaja Bitragunta†, Gagandeep Singh Grover†, Pinnaka V M Lakshmi†, Chandra Mauli Mishra†, Provash Sadhukhan†, Prakash Kumar Sahoo†, Shivendra K Singh†, Chander Prakash Yadav†, Elangovan Ramya Dinesh, Thiyagarajan Karunakaran, Chinnasamy Govindhasamy, Thomas Daniel Rajasekar, Annadurai Jeyakumar, Arunachalam Suresh, Duraisamy Augustine, Paparaju Ashok Kumar, Rajesh Kumar, Shanta Dutta, Gurudayal S Toteja, Nivedita Gupta, Hannah E Clapham, Sanjay M Mehendale, Manoj V Murhekar*

Summary

Background Since its re-emergence in 2005, chikungunya virus (CHIKV) transmission has been documented in most Indian states. Information is scarce regarding the seroprevalence of CHIKV in India. We aimed to estimate the age-specific seroprevalence, force of infection (FOI), and proportion of the population susceptible to CHIKV infection.

Methods We did a nationally representative, cross-sectional serosurvey, in which we randomly selected individuals in three age groups (5–8, 9–17, and 18–45 years), covering 240 clusters from 60 selected districts of 15 Indian states spread across all five geographical regions of India (north, northeast, east, south, and west). Age was the only inclusion criterion. We tested serum samples for IgG antibodies against CHIKV. We estimated the weighted age-group-specific seroprevalence of CHIKV infection for each region using the design weight (ie, the inverse of the overall probability of selection of state, district, village or ward, census enumeration block, and individual), adjusting for non-response. We constructed catalytic models to estimate the FOI and the proportion of the population susceptible to CHIKV in each region.

Findings From June 19, 2017, to April 12, 2018, we enumerated 117 675 individuals, of whom 77 640 were in the age group of 5–45 years. Of 17 930 randomly selected individuals, 12 300 individuals participated and their samples were used for estimation of CHIKV seroprevalence. The overall prevalence of IgG antibodies against CHIKV in the study population was 18·1% (95% CI 14·2–22·6). The overall seroprevalence was 9·2% (5·4–15·1) among individuals aged 5–8 years, 14·0% (8·8–21·4) among individuals aged 9–17 years, and 21·6% (15·9–28·5) among individuals aged 18–45 years. The seroprevalence was lowest in the northeast region (0·3% [95% CI 0·1–0·8]) and highest in the southern region (43·1% [34·3–52·3]). There was a significant difference in seroprevalence between rural (11·5% [8·8–15·0]) and urban (40·2% [31·7–49·3]) areas (p<0·0001). The seroprevalence did not differ by sex (male 18·8% [95% CI 15·2–23·0] *vs* female 17·6% [13·2–23·1]; p=0·50). Heterogeneous FOI models suggested that the FOI was higher during 2003–07 in the southern and western region and 2013–17 in the northern region. FOI was lowest in the eastern and northeastern regions. 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%).

Interpretation CHIKV transmission was higher in the southern, western, and northern regions of India than in the eastern and northeastern regions. However, a 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. Our survey findings will be useful in identifying appropriate target age groups and sites for setting up surveillance and for future CHIKV vaccine trials.

Funding Indian Council of Medical Research.

Introduction

Globally, 1·3 billion people living in 94 countries are estimated to be at risk of chikungunya virus (CHIKV) infection.[1] In India, the first wave of CHIKV outbreaks, from the Asian lineage of the virus, was reported from 1963 to 1973.[2] There were no published reports of CHIKV during the period from 1974 to 2004. CHIKV reappeared in 2005, with explosive outbreaks in the southern Indian states of Andhra Pradesh, Karnataka, Tamil Nadu, and Kerala, which affected nearly 1·4 million people, before spreading to western and northern states.[3] It was estimated that during the 2006 epidemic, 25 588 disability-adjusted life-years were lost, with an overall national burden of 45·26 disability-adjusted life-years per million.[4]

Lancet Microbe 2021; e41–47

*Contributed equally

†Contributed equally

Indian Council of Medical Research, National Institute of Epidemiology, Chennai, India (M S Kumar MPH, P Kamaraj MPhil, C P G Kumar PhD, R Sabarinathan BE, V S Kumar PhD, E R Dinesh PhD, T Karunakaran MSc, C Govindhasamy MSc, T D Rajasekar MSc, A Jeyakumar MPhil, A Suresh MA, D Augustine MA, P A Kumar BA, M V Murhekar MD); **Indian Council of Medical Research, Regional Medical Research Centre, Northeast Region, Dibrugarh, India** (S A Khan PhD); **Science Health Allied Research Education India, Hyderabad, India** (R R Allam MAE, S Bitragunta MAE); **Indian Council of Medical Research, National Institute of Research in Tribal Health, Jabalpur, India** (P V Barde PhD); **Indian Council of Medical Research, Regional Medical Research Centre, Bhubaneswar, India** (B Dwibedi MD, P K Sahoo PhD); **Indian Council of Medical Research, National Institute of Cholera and Enteric Diseases, Kolkata, India** (S Kanungo PhD, P Sadhukhan PhD, S Dutta PhD); **King George's Medical University, Lucknow, India** (U Mohan MD, C M Mishra MD, S K Singh PhD); **Indian Council of Medical Research, National Institute for Implementation Research on Non-Communicable Diseases, Jodhpur, India** (S S Mohanty PhD, G S Toteja PhD); **Indian Council of Medical Research, National Institute of Traditional Medicine, Belagavi, India** (S Roy PhD); **Postgraduate Institute of Medical Education**

and Research, Chandigarh, India (V Sagar PhD, P V M Lakshmi MD, R Kumar MD); **Indian Council of Medical Research, National Institute of Malaria Research, New Delhi, India** (D Savargaonkar MBBS, C P Yadav PhD); **Indian Council of Medical Research, National Institute of Virology, Pune, India** (B V Tandale MD); **Indian Council of Medical Research, Rajendra Memorial Research Institute of Medical Sciences, Patna, India** (R K Topno MBBS); **Department of Health and Family Welfare, Government of Punjab, Punjab, India** (G S Grover MD); **Epidemiology and Communicable Diseases Division, Indian Council of Medical Research, New Delhi, India** (N Gupta PhD, S M Mehendale MD); **Oxford University Clinical Research Unit, Vietnam and Nuffield Department of Medicine, University of Oxford, Oxford, UK** (H E Clapham PhD); **Saw Swee Hock School of Public Health, National University of Singapore, Singapore** (H E Clapham)

Correspondence to:
Dr Manoj V Murhekar, Indian Council of Medical Research, National Institute of Epidemiology, Ayapakkam, Chennai 600077, India
mmurhekar@nieicmr.org.in

Research in context

Evidence before this study

We searched PubMed for estimates of seroprevalence of chikungunya virus (CHIKV) infection in India from database inception until Oct 31, 2019, using the search terms "chikungunya" AND "seroprevalence" AND "India" with no language restrictions. We identified 21 publications, of which four reported seroprevalence of CHIKV infection. Studies had reported seroprevalence of CHIKV ranging from 2·9% (Andaman Islands) to 68·0% (Kerala). In India, the first wave of CHIKV outbreaks was reported during 1963–73. Routine surveillance data from India's National Vector-Borne Disease Control Programme and the virus research and diagnostic laboratories network suggest continued transmission of CHIKV after its re-emergence in 2005. The few seroprevalence studies reported from India thus far were either done in a limited geographical area or after an outbreak. In this context, we tested the samples collected as part of a national serosurvey for IgG antibodies against CHIKV among individuals aged 5–45 years to estimate the age-specific seroprevalence of CHIKV infection in India.

Added value of this study

Our study indicated a wide variation in CHIKV seroprevalence across geographical regions in India, with higher transmission in southern, western, and northern regions and very low amounts of transmission in northeast and eastern regions. Age-related increase in seroprevalence in southern and western regions is consistent with the endemic pattern of CHIKV transmission, whereas uniform seroprevalence across all age groups in the northern region is suggestive of an epidemic pattern of transmission. CHIKV seroprevalence was higher in urban areas of India.

Implications of all the available evidence

Our findings will aid understanding of population susceptibility for CHIKV, help in the design of surveillance strategies, predict future outbreaks, and plan control measures. Estimated CHIKV seroprevalence will be helpful for vaccine developers in understanding the population-level immunity and deciding appropriate target age groups and sites for future CHIKV vaccine trials in India.

These outbreaks were caused by the Indian Ocean Lineage of the East-Central South African CHIKV genotype, which continued to circulate in India as of 2018.[5]

Although the National Vector-borne Disease Control Program reported a declining trend in CHIKV incidence during 2006–11,[6] laboratory confirmed cases continued to occur in India. Nationally, 3342 cases were reported in 2015 and 26 364 in 2016 with 37% reported in Delhi.[7] The Indian Council of Medical Research and the Department of Health Research established a network of virus research and diagnostic laboratories spread across all the regions of the country to strengthen laboratory capacity and provide timely diagnosis of disease outbreaks. During 2016–18, the 61 virus research and diagnostic laboratories tested serum samples from 49 380 patients with suspected CHIKV infection for IgM antibodies against CHIKV; 20·5% of the serum samples were seropositive. The laboratory network also diagnosed 28 CHIKV outbreaks mostly from the southern states of Andhra Pradesh and Tamil Nadu and the western state of Rajasthan.[8]

The data from the National Vector-borne Disease Control Program and virus research and diagnostic laboratory network suggest ongoing CHIKV transmission after its 2005 re-emergence and highlight the impending risk of outbreaks in different parts of India.[7,8] In a 2015 study to assess the global distribution of CHIKV, most Indian states were categorised as having good evidence for the presence of CHIKV transmission, except smaller states in the northeast, northwest, and Chhattisgarh in central India. Environmental suitability models for CHIKV transmission predicted a moderate risk for India.[1]

Serosurveys done during outbreaks highlighted the substantial burden of asymptomatic CHIKV infections.[9] Periodic seroprevalence surveys can supplement surveillance activity by providing insights on CHIKV circulation, population immunity, and the risk of future outbreaks.[10] Very few CHIKV seroprevalence surveys have been reported from India. These surveys were either done over a limited geographical area,[11–13] or immediately after CHIKV epidemics.[14] Nationwide data for seroprevalence of CHIKV in India is absent. In this study, we aimed to estimate age-specific seroprevalence, force of infection (FOI), and the proportion of the population susceptible to CHIKV infection by testing serum samples of individuals aged 5–45 years for CHIKV, collected as part of a nationally representative dengue serosurvey in India.[15]

Methods

Study design and participants

We did a nationally representative, cross-sectional, population-based serosurvey, which estimated the seroprevalence of dengue virus infection. We tested the serum samples collected as part of the dengue study for CHIKV. The age-stratified serosurvey covered three age groups (ie, 5–8, 9–17, and 18–45 years) across five geographical regions (north, northeast, east, south, and west) of India. In each region, three states were randomly selected, and from each state, four districts were selected by probability proportional to population size. We considered wards in urban areas and villages in rural areas as clusters. Four clusters (two from urban and two from rural areas) were selected randomly from each district. One census enumeration block was selected randomly from each cluster. In India, during decennial

census operations, an enumerator is allotted one census enumeration block, which has about 120–150 households. The survey team visited the selected census enumeration block. First they visited all households in the area, numbered the houses, and listed all the family members. The enumeration data were uploaded to the server. We randomly selected 25 individuals from three age groups (ie, 5–8, 7–17, and 18–45 years) from the enumerated population using an Android application developed specifically for the survey. The teams visited all randomly selected individuals and invited them to participate in the study. Age was the only inclusion criterion. To summarise, the survey was done in 240 clusters (118 rural, 122 urban; all clusters from two districts of the National Capital Territory of Delhi were urban) from 60 selected districts of 15 Indian states spread across all five regions of India. The Institutional Ethics Committees of the Indian Council of Medical Research, National Institute of Epidemiology, and all the participating institutes approved the study protocol. We obtained written informed consent from adults, and parental permission and assent from children. The survey design has been described in our publication on dengue seroprevalence.[15]

Procedures

All serum samples were tested for the presence of IgG antibodies against CHIKV using $CHIK_{ij}$ Detect IgG ELISA (InBios International, Seattle, WA, USA). The assay was developed by generating recombinant viruses, which contained the structural protein genes of pathogenic CHIKV and the non-structural protein genes of an insect-specific alphavirus, Eilat virus.[16] The assay was found to have a sensitivity and specificity of more than 90%. As per the manufacturer's instructions, the immune status ratio (ISR) value for each serum sample was calculated by dividing the mean test sample optical density (OD_{450}) by the cutoff control value. Serum samples with ISR values of 1·0 or more were considered positive, values less than 1·0 were considered negative, and those between 0·9–1·1 were retested with the same assay. As part of quality control, each serological test was done with positive and negative controls provided by the manufacturer. As an additional quality measure, we randomly retested 538 serum samples and calculated the agreement between the two IgG results.

Statistical analysis

We estimated weighted age-group-specific seroprevalence of CHIKV infection along with 95% CIs for each region with design weight and adjusting for non-response (appendix p 2). We calculated 95% CIs with Taylor-linearised variance estimation for SEs. We estimated the overall seroprevalence based on seroprevalence in different regions and considering the proportion of the population aged 5–8, 9–17, and 18–45 years in each region as weights. We used inverse distance weighting, a spatial interpolation method using ArcGIS, version 10 software to map the seroprevalence observed in the study. We calculated Spearman's rank correlation between the variables ISR and age.

We used Muench's catalytic model to estimate the FOI for CHIKV transmission.[17–19] We stratified the CHIKV ELISA results into eight age groups, which were 5–9, 10–14, 15–19, 20–24, 25–29, 30–34, 35–39, and 40–45 years, denoted by *n*.

The contribution to the likelihood of λ from an individual who was seronegative in age group *n* was:

$$L_n^{Neg} = \exp\left(-5\sum_{i=1}^{n-1}\lambda_i - 2\cdot 5\lambda_n\right)$$

The contribution to the likelihood of λ from an individual who was seropositive in age group *n* was:

$$L_n^{Pos} = 1 - L_n^{Neg}$$

The value 2·5 in the likelihood function in any age group, for example:

$$L_{5-9}^{pos} = 1 - \exp\left[-(5\lambda_1 + 2\cdot 5\lambda_2)\right]$$

contributes 5·0 years from birth and 2·5 years (half) from the present age group to FOI (where λ_i [i=1, 2, 3, and so on up until 8] is the mean annual FOI in the 5-year time periods going backward from 2013–17, 2008–12, 2003–07, up until 1977–82). We built two different models: constant FOI and heterogeneous FOI. The Akaike Information Criteria values between the two models were used to assess the best model fit for each region. FOI estimates reflect CHIVK transmission by time period as well as age, although the contribution of time period is greater.

See **Online** for appendix

	Northern region	Northeastern region	Eastern region	Southern region	Western region	All regions
Age group, years						
5–8	794; 16·9% (6·9–35·9)	722; 1·3% (0·3–5·2)	815; 3·1% (1·8–5·5)	960; 10·7% (7·3–15·6)	768; 7·0% (4·7–10·3)	4059; 9·2% (5·4–15·1)
9–17	826; 14·0% (3·9–38·9)	805; 0·5% (0·1–1·8)	874; 4·6% (2·7–7·9)	936; 36·4% (28·9–44·7)	824; 16·4% (10·0–25·7)	4265; 14·0% (8·8–21·4)
18–45	782; 19·9% (7·9–41·8)	833; 0·03% (0·005–0·19)	797; 4·5% (2·6–7·7)	820; 50·2% (37·3–63·1)	744; 30% (21·2–40·6)	3976; 21·6% (15·9–28·5)
All, 5–45	2402; 17·9% (9·4–31·5)	2360; 0·3% (0·1–0·8)	2486; 4·4% (3·0–6·3)	2716; 43·1% (34·3–52·3)	2336; 23·3% (17·5–30·3)	12 300; 18·1% (14·2–22·6)
Sex						
Male	1145; 18·0% (10·6–29·0)	1028; 0·6% (0·2–2·1)	1192; 5·9% (3·8–8·9)	1289; 42·1% (32·1–52·9)	1159; 23·8% (16·7–32·6)	5813; 18·8% (15·2–23·0)
Female	1257; 18·0% (8·5–34·2)	1332; 0·1% (0·02–0·21)	1294; 3·3% (1·7–6·2)	1427; 43·9% (34·1–54·1)	1177; 23·0% (17·4–29·8)	6487; 17·6% (13·2–23·1)
Area of residence						
Rural	1117; 3·8% (1·7–8·0)	1196; 0·3% (0·08–0·92)	1280; 4·2% (2·8–6·3)	1415; 38·6% (27·6–50·9)	1229; 20·0% (13·5–28·6)	6237; 11·5% (8·8–15·0)
Urban	1285; 48·1% (33·5–62·9)	1164; 0·6% (0·2–1·6)	1206; 5·3% (2·1–12·8)	1301; 53·2% (44·1–62·0)	1107; 37·2% (26·2–49·8)	6063; 40·2% (31·7–49·3)

Data are number tested; prevalence (95% CI). n=12 300.

Table 1: **Weighted seroprevalence of IgG antibodies against chikungunya virus**

	5–8 years	9–17 years	18–45 years	Overall
Northern region				
Punjab	13/286 (5%)	17/288 (6%)	17/260 (7%)	47/834 (6%)
National Capital Territory of Delhi	81/253 (32%)	85/257 (33%)	100/252 (40%)	266/762 (35%)
Uttar Pradesh	31/255 (12%)	42/281 (15%)	50/270 (19%)	123/806 (15%)
Northeastern region				
Assam	2/242 (1%)	5/270 (2%)	1/260 (<1%)	8/772 (1%)
Meghalaya	3/231 (1%)	4/255 (2%)	1/282 (<1%)	8/768 (1%)
Tripura	2/249 (1%)	4/280 (1%)	1/291 (<1%)	7/820 (1%)
Eastern region				
Bihar	5/243 (2%)	9/286 (3%)	6/256 (2%)	20/785 (3%)
West Bengal	14/248 (6%)	21/257 (8%)	24/255 (9%)	59/760 (8%)
Odisha	2/324 (1%)	3/331 (1%)	11/286 (4%)	16/941 (2%)
Southern region				
Andhra Pradesh	69/327 (21%)	149/345 (43%)	173/313 (55%)	391/985 (40%)
Karnataka	29/300 (10%)	87/275 (32%)	114/261 (44%)	230/836 (28%)
Tamil Nadu	27/333 (8%)	140/316 (44%)	148/246 (60%)	315/895 (35%)
Western region				
Rajasthan	13/243 (5%)	29/266 (11%)	56/253 (22%)	98/762 (13%)
Madhya Pradesh	26/264 (10%)	59/277 (21%)	81/261 (31%)	166/802 (21%)
Maharashtra	25/261 (10%)	128/281 (46%)	136/230 (59%)	289/772 (37%)

Data are n/N (%).

Table 2: **Unweighted seroprevalence of IgG antibodies against chikungunya virus in different states of India**

	Northern region	Northeastern region	Eastern region	Southern region	Western region
Constant force of infection	0·012 (0·011–0·013)	0·0006 (0·0003–0·0008)	0·002 (0·002–0·003)	0·028 (0·026–0·029)	0·017 (0·016–0·018)
AIC	2983·49	362·33	1357·50	5131·93	3742·54
Heterogeneous force of infection					
2013–17	0·028 (0·018–0·036)	0·001 (0·0002–0·002)	0·004 (0·0007–0·006)	0·007 (0·0004–0·022)	0·007 (0·0005–0·018)
2008–12	0·009 (0·0008–0·024)	0·0008467 (0·00006–0·002)	0·0029 (0·0003–0·007)	0·05452 (0·024–0·072)	0·02309 (0·004–0·039)
2003–07	0·004 (0·0002–0·012)	0·0005 (0·00003–0·002)	0·001 (0·00008–0·004)	0·057 (0·034–0·083)	0·043 (0·023–0·063)
1998–2002	0·004 (0·0002–0·012)	0·0003 (0·00002–0·001)	0·0009 (0·00005–0·004)	0·008 (0·0005–0·029)	0·009 (0·0006–0·027)
1993–97	0·003 (0·0002–0·011)	0·0004 (0·00002–0·002)	0·001 (0·00007–0·005)	0·009 (0·0007–0·032)	0·007 (0·0005–0·022)
1988–92	0·003 (0·0002–0·009)	0·0005 (0·00003–0·002)	0·002 (0·0001–0·007)	0·012 (0·0008–0·038)	0·005 (0·0003–0·019)
1983–87	0·003 (0·0002–0·013)	0·0007 (0·00004–0·003)	0·003 (0·0002–0·009)	0·016 (0·001–0·047)	0·005 (0·0003–0·019)
1977–82	0·004 (0·0002–0·014)	0·0009 (0·00006–0·004)	0·003 (0·0002–0·011)	0·017 (0·001–0·054)	0·008 (0·0005–0·028)
AIC	2387·86	420·18	928·34	3236·35	2456·28

Data are force of infection (95% CI) unless specified. AIC=Akaike Information Criteria.

Table 3: **Estimated force of infection**

We estimated the age-specific proportion of seropositive individuals based on the FOI estimates obtained from the catalytic model. All of those individuals who were seronegative were assumed to be susceptible. We projected the 2017 age-structured population data from the 2011 census. We estimated the susceptibility for the whole population with the sum of the total number of susceptible people from all ages divided by the total population. For children aged younger than 5 years, we used the FOI estimated during the period 2013–17. As we did not have data for FOI before 1976, we assumed two scenarios for the population aged 45–70 years to estimate the susceptible proportion: (1) using mean FOI estimated during 1977–82 and (2) using mean FOI estimated during 2013–17. We did the analysis with use of the survey data analysis module in Stata SE, version 13.0 and R, version 3.5.1 software.

Role of the funding source

The funder of the study had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. All authors had full access to all the data in the study and the corresponding author had final responsibility for the decision to submit for publication.

Results

From June 19, 2017, to April 12, 2018, we enumerated 117 675 individuals, of whom 77 640 were aged between 5 years and 45 years. Of the 17 930 randomly selected individuals, 2980 (16·6%) were not available during the survey. Of the 14 950 individuals who were available for participation, 1213 (8·1%) refused to participate in the survey, 1405 (9·4%) refused to provide a blood specimen, and 32 (0·2%) were excluded because their actual age was different from the age group for which they were randomly selected. Thus, data for 12 300 individuals were used for estimation of CHIKV seroprevalence.[15]

Of the 12 300 individuals enrolled, 4059 (33·0%) were aged 5–8 years, 4265 (34·7%) were aged 9–17 years, and 3976 (32·3%) were aged 18–45 years. 77·9% of participants were Hindu, 52·7% were women, 50·7% were residents of rural areas, and 8·2% had no formal education. Further details of the study profile and sociodemographic characteristics of the population surveyed are described elsewhere.[15]

Of the 12 300 serum samples tested, 2043 were positive for IgG antibodies against CHIKV, indicating past exposure to CHIKV, with the weighted overall seroprevalence of 18·1% (95% CI 14·2–22·6). The seroprevalence was highest in the southern (43·1% [95% CI 34·3–52·3]) region, followed by the western (23·3% [17·5–30·3]), and the northern (17·9% [9·4–31·5]) regions. The seroprevalence was low in the eastern (4·4% [3·0–6·3]) and northeastern (0·3% [0·1–0·8]) regions (table 1, appendix p 3). The overall seroprevalence was 9·2% (5·4–15·1) among individuals aged 5–8 years, 14·0% (8·8–21·4) among individuals aged 9–17 years, and 21·6% (15·9–28·5) among individuals aged 18–45 years. The overall seroprevalence was higher in urban (40·2% [95% CI 31·7–49·3]) than in rural (11·5% [8·8–15·0])

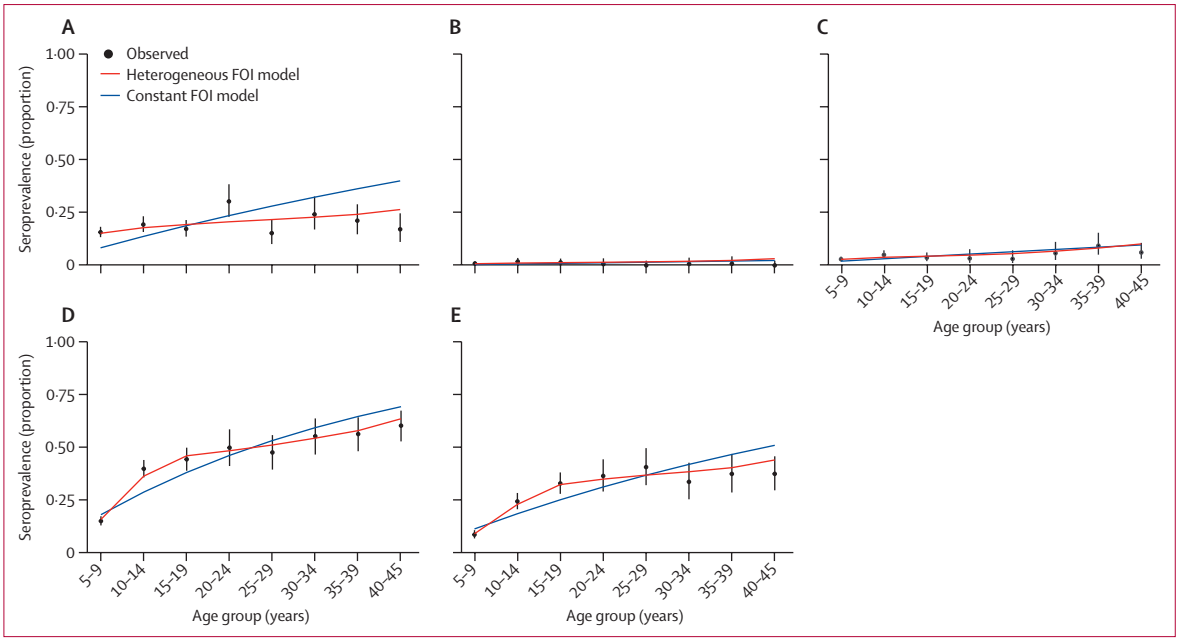

Figure: **Observed and model-predicted seroprevalence of chikungunya virus by age**
Seroprevalence by age group in the northern (A), northeastern (B), eastern (C), southern (D), and western (E) regions. Error bars indicate 95% CIs. FOI=force of infection.

areas (p<0·0001) and a similar pattern was observed in all regions. The seroprevalence was not different by sex (male 18·8% [95% CI 15·2–23·0] *vs* female 17·6% [13·2–23·1]; p=0·50). In the southern region, the seroprevalence increased from 10·7% (95% CI 7·3–15·6) among children aged 5–8 years to 36·4% (28·9–44·7) among children aged 9–17 years, and 50·2% (37·3–63·1) among those aged 18–45 years. A similar pattern of age-specific increase in prevalence was observed in the western region. By contrast, in the northern region, the seroprevalence was not different across age groups and ranged between 14·0% and 19·9%.

There was a wide variation in seroprevalence across states within the southern, western, and northern regions. In the northern region, overall seroprevalence was lowest in the state of Punjab (47 [6%] of 834) and highest in the National capital Territory of Delhi (266 [35%] of 762). In the western region, the seroprevalence ranged between 13% (98 of 762) in Rajasthan and 37% (289 of 772) in Maharashtra, whereas in the southern region, the seroprevalence ranged between 28% (230 of 836) in Karnataka and 40% (391 of 985) in Andhra Pradesh (table 2). As part of quality control, 538 randomly selected samples were retested; the agreement between IgG results in the initial and subsequent retesting was 98·9% (95% CI 97·6–99·5).

The Akaike Information Criteria of constant and heterogeneous FOI models are shown in table 3. The heterogenous FOI models were the best fit models for all regions except the northeastern region, as the Akaike Information Criteria of these models were lower than constant FOI models. In the southern and western regions, the mean annual FOI was highest during 2003–07. In the southern region, the FOIs were 0·057 for 2003–07 and 0·054 for 2008–12, indicating that, on average, 5·5% individuals who were seronegative from this region seroconverted every year for 2003–07 and 5·2% for 2008–12. In the northern region, the peak transmission was observed during 2013–17 with an FOI of 0·028. Very low to no transmission was observed in the northeast and eastern regions during all periods (table 3, figure). Estimates of FOI for different states of India are given in the appendix (p 4).

The estimated proportion of the population who were susceptible in 2017 with the first assumption (mean FOI estimated during 1977–82) was lowest in the southern region (56·3%) and highest in the northeastern region (98·0%). Similarly, with assumption two (mean FOI estimated during 2013–17), the proportion who were susceptible was lowest in the southern region (57·0%) and highest in the northeastern region (98·0%; appendix p 5). The proportion who were susceptible was high in the younger age group and declined with increasing age (appendix p 6).

Of the 12 300 samples tested, 1739 (14·1%) were positive for dengue and CHIKV. The proportion of individuals with co-infection of dengue and CHIKV ranged between 0·0% (0 of 2360) in the northeast region and 30·8% (836 of 2716) in the southern region. Co-infection increased with age from 5·8% (235 of 4059) in the 5–8 years age group to 21·6% (859 of 3976) in the 18–45 years age group. A significant difference was

observed in the proportion of individuals with co-infection between rural (643 [10·3%] of 6237) and urban areas (1096 [18·1%] of 6063; p<0·0001; appendix p 7)

There was no correlation between ISR values and age among individuals who were seropositive (r=–0·16) and individuals who were seronegative (r=–0·06). The distribution of ISR values by age group showed a clear separation of seronegative and seropositive using manufacturer recommended ISR cutoff values (appendix pp 8–9).

Discussion

Our population-based serosurvey of 15 Indian states indicated that 18% of the study population had past exposure to CHIKV. The seroprevalence was less than 5% in the northeastern and the eastern regions but was higher in the southern, northern, and western regions. The geographical variations in seroprevalence observed for CHIKV were similar to that of dengue.[15] The ongoing transmission of CHIKV in India after its re-emergence in 2005 could be due to the fact that between 56·3% and 98·0% of the Indian population in different regions is still susceptible to infection. By contrast, the Indian Ocean islands did not report any CHIKV cases after initial outbreaks, due to the development of herd immunity.[20]

The age-specific seroprevalence of CHIKV showed different patterns by region, with an increase in seroprevalence with age in southern and western regions and uniform seroprevalence across age groups in the northern region. Increase in seroprevalence with age observed in southern and western regions indicates an endemic pattern of CHIKV transmission, whereas uniform seroprevalence across age groups observed in the northern region suggests an epidemic pattern of transmission.[13] Consistent with the dengue seroprevalence observed in India, the CHIKV seroprevalence was also higher in urban areas than rural areas. However, in the southern and western regions, 20–39% of the population showed evidence of exposure to CHIKV infection in rural areas as well.

Previous serosurveys from different geographical settings in India have reported CHIKV seroprevalence ranging from 2·9% to 68·0%.[11–14] Serosurveys reported from Andaman Islands (2·9%) and Kolkata (4·4%) detected a low level of seroprevalence. These serosurveys were from before the 2005 outbreak.[11,12] The seroprevalence for the southern region observed in our study was similar to the seroprevalence of 44% reported in Chennai, a metropolitan city in south India, in 2011[13] and lower than the seroprevalence reported from Kerala (68%) immediately after the 2007 outbreak.[14] Although seroprevalence data are not available, large CHIKV outbreaks were reported from northern states such as Delhi and Punjab.[21,22]

In India, the first wave of CHIKV transmission was reported in 1963–73.[2] Our study population aged 5–45 years in 2017, was born after the year 1972. The FOI of CHIKV in the southern and western regions indicated that transmission peaked during 2003–07, corresponding to the introduction of the East-Central South African genotype of the virus in India. Although large outbreaks of CHIKV were not reported after 1973, our analysis suggested low amounts of transmission with an FOI ranging between 0·008 and 0·017 in the southern region during the period between 1973 and 2004. In the northern region, CHIKV transmission peaked during 2013–17. This finding is consistent with the surveillance data from the National Vector-borne Disease Control Program, with reported CHIKV outbreaks or upsurges in the number of CHIKV cases from northern states including Delhi, Punjab, and Uttar Pradesh. Very low seroprevalence and FOI in the northeastern region suggest a relatively recent (2014–17) introduction of the infection in the region.[23,24] An entomological survey done in seven northeastern states during 2004–05 observed an abundance of *Aedes aegypti* and *Aedes albopictus* in all states. Such a conducive environment coupled with high CHIKV susceptibility estimated by our study position both northeast and eastern regions of India at higher risk of CHIKV outbreaks in future.[25] Bangladesh, which shares a border with eastern and northeastern Indian states such as West Bengal, Assam, Tripura, and Meghalaya, reported its first large CHIKV outbreak in Dhaka in 2017.[26]

Our serosurvey has some limitations. First, the sample size for the serosurvey was calculated assuming a dengue seroprevalence of 60% in various regions and age groups.[15] This sample size was adequate to capture CHIKV seroprevalence in a given age group within a region of 20% with an absolute precision of 5%, design effect of 2, and a confidence level of 95%. Our sample size, however, might not have been adequate to capture very low seroprevalence in the northeast and eastern regions of the country. Second, we did not include children aged younger than 5 years and adults older than 45 years, meaning that our results are not necessarily representative of all age groups. Third, due to resource constraints, we could not test the samples for the CHIKV Plaque Reduction Neutralization Test. Although CHIKV is known to show cross-reactivity with Eastern equine encephalitis virus and Mayaro virus, the viruses or antibodies to them have not been reported from India.

The study findings will be useful to improve the public health preparedness in tackling future outbreaks in regions with a high susceptibility for CHIKV. In our study, co-infection of dengue and CHIKV was found to be higher in the southern region followed by the western and northern regions. In addition to co-circulation of CHIKV and dengue in India,[22] detection of indigenous transmission of Zika virus infection in 2016[27] highlights the importance of novel vector control approaches.[28] Several CHIKV vaccine candidates are under development with a few already in phase 1/2 trials (CTRI/2017/02/007755, NCT02562482). Information about disease burden and the level of population

immunity is useful for vaccine developers in identifying appropriate target age groups and sites for CHIKV vaccine trials in India.[29]

In conclusion, our study indicates heterogeneous transmission of CHIKV in India with a higher prevalence in three of the five regions under investigation. The pattern observed for CHIKV was similar to dengue transmission reported in our earlier study.[16] Our data indicated the possibility of continued transmission of CHIKV in all regions of India, especially in the northeast and eastern regions.

Contributors

MVM was the principal investigator of the survey. MVM, PK, MSK, NG, and SMM conceived and designed the study. MVM, PK, MSK, SAK, RRA, PVB, BD, SK, UM, SSM, SR, VS, DS, BVT, RKT, SB, GSG, PVML, CMM, PS, PKS, SKS, CPY, RK, SD, GST, CG, TDR, AJ, AS, DA, and PAK coordinated the field operations. CPGK oversaw all laboratory procedures with the support of ERD and TK. PK, MSK, RS, and MVM managed and analysed the data. VSK and HEC developed the catalytic models and estimated susceptibility proportion. PK, RS, VSK, MSK, and MVM accessed and verified the data. MSK and MVM drafted the first version of the manuscript and all authors contributed, reviewed, and approved this Article.

Declaration of interests

We declare no competing interests.

Acknowledgments

We thank T Magesh, Annamma Jose, G-H Arun, P Jayasree, K Gayathri, K Satishkumar, K Ramu, D Murugan, I Kalaimani, P Lourdu Stella Mary, V Ramachandran, G Elavarasu, S Satheesh Kumar, M Mohanraj, P Vaishnavi, S Ragupathi, S Satish, and D Nallathambi for technical support, and Sudipto Bora, Purvita Chowdhury, Varun Shahi, Satinder Bharti, Lakhwinder Singh, Rajat Chauhan, Suchandra Chowdhury, Arshad Shamim, Pravin Suryakantrao Deshmukh, and Shashi Shekhar Suman for their support in field operations. We thank the Director General of the Indian Council of Medical Research for his support and encouragement for the study. We thank all the field staff involved in the implementation of the survey. We thank the state and district health officials who supported and facilitated field operations. We thank all the study participants for taking part in the study.

References

1 Nsoesie EO, Kraemer MU, Golding N, et al. Global distribution and environmental suitability for chikungunya virus, 1952 to 2015. *Euro Surveill* 2016; **21:** 21.

2 Weaver SC, Lecuit M. Chikungunya virus and the global spread of a mosquito-borne disease. *N Engl J Med* 2015; **372:** 1231–39.

3 WHO. Outbreak and spread of chikungunya. *Wkly Epidemiol Rec* 2007; **82:** 409–15.

4 Krishnamoorthy K, Harichandrakumar KT, Krishna Kumari A, Das LK. Burden of chikungunya in India: estimates of disability adjusted life years (DALY) lost in 2006 epidemic. *J Vector Borne Dis* 2009; **46:** 26–35.

5 Newase P, More A, Patil J, et al Chikungunya phylogeography reveals persistent global transmissions of the Indian Ocean Lineage from India in association with mutational fitness. *Infect Genet Evol* 2020; **82:** 104289.

6 Muniaraj M. Fading chikungunya fever from India: beginning of the end of another episode? *Indian J Med Res* 2014; **139:** 468–70.

7 National Vector Borne Disease Control Programme, Directorate General of Health Services, Ministry of Health and Family Welfare, Government of India. CHIKV situation in India. 2020. https://www.nvbdcp.gov.in/index4.php?lang=1&level=0&linkid=486&lid=3765 (accessed April 5, 2019).

8 Murhekar M, Kanagasabai K, Shete V, et al. Epidemiology of chikungunya based on laboratory surveillance data-India, 2016–2018. *Trans R Soc Trop Med Hyg* 2019; **113:** 259–62.

9 Gay N, Rousset D, Huc P, et al. Seroprevalence of Asian lineage chikungunya virus infection on Saint Martin Island, 7 months after the 2013 emergence. *Am J Trop Med Hyg* 2016; **94:** 393–96.

10 Ang LW, Kam YW, Lin C, et al. Seroprevalence of antibodies against chikungunya virus in Singapore resident adult population. *PLoS Negl Trop Dis* 2017; **11:** e0006163.

11 Neogi DK, Bhattacharya N, Mukherjee KK, et al. Serosurvey of chikungunya antibody in Calcutta metropolis. *J Commun Dis* 1995; **27:** 19–22.

12 Padbidri VS, Wairagkar NS, Joshi GD, et al. A serological survey of arboviral diseases among the human population of the Andaman and Nicobar Islands, India. *Southeast Asian J Trop Med Public Health* 2002; **33:** 794–800.

13 Rodriguez-Barraquer I, Solomon SS, Kuganantham P, et al. The hidden burden of dengue and chikungunya in Chennai, India. *PLoS Negl Trop Dis* 2015; **9:** e0003906.

14 Kumar NP, Suresh A, Vanamail P, et al. Chikungunya virus outbreak in Kerala, India, 2007: a seroprevalence study. *Mem Inst Oswaldo Cruz* 2011; **106:** 912–16.

15 Murhekar MV, Kamaraj P, Kumar MS, et al. Burden of dengue infection in India, 2017: a cross-sectional population based serosurvey. *Lancet Glob Health* 2019; **7:** e1065–73.

16 Erasmus JH, Needham J, Raychaudhuri S, et al. Utilization of an Eilat virus-based chimera for serological detection of chikungunya infection. *PLoS Negl Trop Dis* 2015; **9:** e0004119.

17 Muench H. Catalytic models in epidemiology. Cambridge, MA: Harvard University Press, 1959.

18 Quan TM, Phuong HT, Vy NHT, et al. Evidence of previous but not current transmission of chikungunya virus in southern and central Vietnam: results from a systematic review and a seroprevalence study in four locations. *PLoS Negl Trop Dis* 2018; **12:** e0006246.

19 Salje H, Cauchemez S, Alera MT, et al. Reconstruction of 60 years of chikungunya epidemiology in the Philippines demonstrates episodic and focal transmission. *J Infect Dis* 2016; **213:** 604–10.

20 Staples JE, Breiman RF, Powers AM. Chikungunya fever: an epidemiological review of a re-emerging infectious disease. *Clin Infect Dis* 2009; **49:** 942–48.

21 Kaur N, Jain J, Kumar A, et al. Chikungunya outbreak in Delhi, India, 2016: report on coinfection status and comorbid conditions in patients. *New Microbes New Infect* 2017; **20:** 39–42.

22 Kaur M, Singh K, Sidhu SK, et al. Coinfection of chikungunya and dengue viruses: a serological study from north western region of Punjab, India. *J Lab Physicians* 2018; **10:** 443–47.

23 Dutta P, Khan SA, Khan AM, Borah J, Chowdhury P, Mahanta J. First evidence of chikungunya virus infection in Assam, Northeast India. *Trans R Soc Trop Med Hyg* 2011; **105:** 355–57.

24 Dutta P, Khan SA, Phukan AC, et al. Surveillance of chikungunya virus activity in some north-eastern states of India. *Asian Pac J Trop Med* 2019; **12:** 19–25.

25 Dutta P, Mahanta J. Potential vectors of dengue and the profile of dengue in the north-eastern region of India: an epidemiological perspective. WHO Regional Office for South-East Asia, 2006. https://apps.who.int/iris/handle/10665/170212 (accessed Dec 19, 2019).

26 Hossain MS, Hasan MM, Islam MS, et al. Chikungunya outbreak (2017) in Bangladesh: clinical profile, economic impact and quality of life during the acute phase of the disease. *PLoS Negl Trop Dis* 2018; **12:** e0006561.

27 Sapkal GN, Yadav PD, Vegad MM, Viswanathan R, Gupta N, Mourya DT. First laboratory confirmation on the existence of Zika virus disease in India. *J Infect* 2018; **76:** 314–17.

28 Paixão ES, Teixeira MG, Rodrigues LC. Zika, chikungunya and dengue: the causes and threats of new and re-emerging arboviral diseases. *BMJ Glob Health* 2018; **3** (suppl 1)**:** e000530.

29 Smalley C, Erasmus JH, Chesson CB, Beasley DWC. Status of research and development of vaccines for chikungunya. *Vaccine* 2016; **34:** 2976–81.