RESEARCH PAPER

Cross-sectional Serologic Assessment of Immunity to Poliovirus in Differential Risk Areas of India: India Seroprevalence Survey - 2014

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Objective: To assess the seroprevalence against all three poliovirus serotypes in traditional high risk areas in Bihar, lowest routine immunization coverage areas in Madhya Pradesh and migrant population living in Mumbai urban slums.

Design: Cross-sectional Survey.

Setting: Subjects selected by house to house visit (community based) and transported to government health facilities for further study procedures.

Participants: 1137 randomly selected healthy infants 6-11 months of age residing in the selected high-risk areas.

Methods: Serum samples from the study site were shipped to Enterovirus Research Centre (ERC), Mumbai to determine the neutralizing antibodies against all three poliovirus serotypes. Children with a reciprocal antibody titer ≥1:8 were considered seropositive to the specific poliovirus.

Results: Overall, seroprevalence in all the three study areas was 98%, 98% and 91% against poliovirus type-1, type-2 and type-3, respectively. Bihar had a seroprevalence of 99%, 99% and 92% against type-1, type-2 and type-3 respectively. Corresponding figures for Madhya Pradesh and Mumbai were 98%, 99% and 88% and 98%, 97% and 94%, respectively.

Conclusions: The study found high seroprevalence against all three poliovirus types not only in the traditional high-risk areas for polio in India, but even in the areas known to have low routine immunization coverage and among the migratory clusters living in Mumbai urban slums. Type-2 seroprevalence was found to be high. These findings are reassuring against the threat of emergence of circulating vaccine derived polioviruses (cVDPVs) in the country subsequent to switch from trivalent oral polio vaccine to bivalent oral polio vaccine in the routine immunization schedule from April 2016.

Keywords: *India, Poliovirus, Seroprevalence, Vaccine derived poliovirus.*

WHO South-East Asia Region was certified polio-free in March 2014, three years after the last polio case due to wild poliovirus in the Region was reported from India. [1]. However, India still faces the risk of wild poliovirus (WPV) importation and spread and the risk of paralysis from vaccine derived polio virus (VDPV) in areas with low population immunity [2,3]. To mitigate these risks, India continues its efforts to raise population immunity against polio through mass vaccinations and efforts to improve routine immunization coverage. To assess the population immunity against poliovirus, India conducted regular seroprevalence studies in the highest risk areas during 2007-2012 [4]. The serosurvey conducted in 2012 in high-risk areas of Uttar Pradesh (UP) and Bihar showed improving type-2 immunity even while sustaining high levels of seroprevalence to type 1 and 3 [5].

All previous serosurveys in India have been conducted in the traditional reservoirs of poliovirus transmission in UP and Bihar. Apart from these traditional risk areas, areas with low routine immunization coverage and moving populations pose additional risk of WPV importation and emergence of cVDPVs. This seroprevalence study against poliovirus was conducted among infants residing in Bihar, Madhya Pradesh (MP) and Mumbai. This serosurvey in India during August 2014 was the first after South East Asia Region of WHO including India was certified polio free in March 2014. The study assessed the seroprevalence to all three poliovirus serotypes in three categories of risk areas and compared the seroprevalence between 2014 and 2012.

METHODS

Study Design

The study was a cross-sectional seroprevalence of neutralizing antibodies against all three poliovirus types in infants 6-11 months of age staying in high risk areas for poliovirus transmission. Children with antibody levels ≥ 1:8 dilution for each poliovirus types were considered seropositive.

Target population: Risk area categories for the study

The study was conducted in three different categories of risk; 1- traditional high risk areas for polio (Bihar), 2- state with low routine immunization coverage (Madhya Pradesh) and 3- urban slum areas with large migratory population (Mumbai). Five highest risk districts from Kosi riverine areas of Central Bihar were selected. These districts posed extreme challenges to the polio eradication program in India and were among the last vestiges of polio transmission in India with maximum WPV circulation during 2005-2011 in Bihar [6,7]. Another five districts with lowest routine immunization coverage in MP as per the Annual Health Survey (AHS), 2011-2012 were included [8]. Eight municipal wards from Mumbai city were selected due to intense migration of population in the city. Almost 55% of Mumbai inhabitants live in slums with migration mainly from UP and Bihar and neighboring countries like Bangladesh, Nepal, Pakistan and Sri Lanka [9,10].

Sample size and study area selection

Keeping in view the objectives of the study, 360 subjects were proposed to be enrolled in each risk area category, giving a total sample of 1080 subjects in the whole study. This gave a power of 80%, α error at 5% and 95% confidence level and compensating for subjects with dry tap or hemolysis during blood collection and processing. Microplans of the supplementary immunization activity (SIA) teams carrying out polio vaccination campaigns were used to randomly select study areas and houses to be visited to screen and select study subjects. Of all the SIA team areas in the study districts/wards, a total of 60 SIA team areas were randomly chosen in Bihar, MP and Mumbai. A random house from all the houses in the selected SIA team area was allotted to the study staff to start the screening for the subjects. Moving consecutively as per the SIA microplan, the study staff selected six children from each SIA team area to enroll 360 infants in each study location.

Inclusion criteria, screening and selection of subjects

All 6-11 month old, healthy infants residing in the study area and whose parents provided consent were eligible for the study. About 3-5 days prior to actual enrollment, the field study staff visited the households in their respective polio vaccination team areas to screen age-eligible children, starting with a randomly allotted first house. On actual study days, a surveillance medical officer (SMO) of WHO India-NPSP visited households of age-eligible children to select study-eligible subjects and transported them to the designated study sites with their family members.

Study procedures

The study was implemented after approval from the Institutional Ethics Committee (IEC) of Enterovirus Research Center (ERC), Mumbai and the Ethics Review Committee (ERC) at WHO, Geneva. Study sites were set up at government health facilities (PHC/health posts etc.). At the study site, a study physician obtained written signed consent from participants' parents and administered a short questionnaire. A pediatric resident then collected blood by venepuncture in a vaccutainer tube with clot separator. Blood samples were allowed to clot at room temperature and centrifuged. Serum was separated in cryovials and stored below -20°C. At the end of study, all cryovials with serum were shipped in dry ice to the Enterovirus Research Centre (ERC), Mumbai where samples were processed to determine neutralizing antibodies against all three poliovirus serotypes using Sabin polioviruses in a modified micro-neutralization assay following a standard protocol. Serial two-fold dilutions of test serum sample are reacted with 100 CCID50 of each of the three poliovirus types to determine the highest dilution that neutralizes the virus infectivity 50% of the time. HEp-2(C) cells are used to detect virus infectivity. The test requires an incubation period of 5 days. All samples for polio antibodies were tested in triplicate beginning at a 1:8 dilution. Specimens were randomized by district and site across the test runs.

Analysis of Results

Based upon an estimated 28-30 days half-life of maternal antibodies for polio, it is assumed that maternal antibodies will have decayed to undetectable levels by 6 months. Therefore, all children with a reciprocal antibody titer ≥1:8 were considered seropositive to the specific poliovirus.

RESULTS

Enrolment

A total of 76,822 houses were visited during initial screening and 2,890 age-eligible infants were identified. Of this, 1,137 infants were enrolled in the study. Test results for poliovirus neutralizing antibodies were available from 1,110 subjects (***Fig.* 1**). These subjects were almost equally distributed in the age group of 6-7 months (*N*=374), 8-9 months (*N*=366) and 10-11 months (*N*=370).

OPV doses received by children

Overall, the study children in Bihar, MP and Mumbai received a median of 3 routine doses (tOPV) and 4 doses of OPV (2 bOPV+ 2 tOPV) through polio campaigns. Infants in Bihar received maximum median OPV doses (4

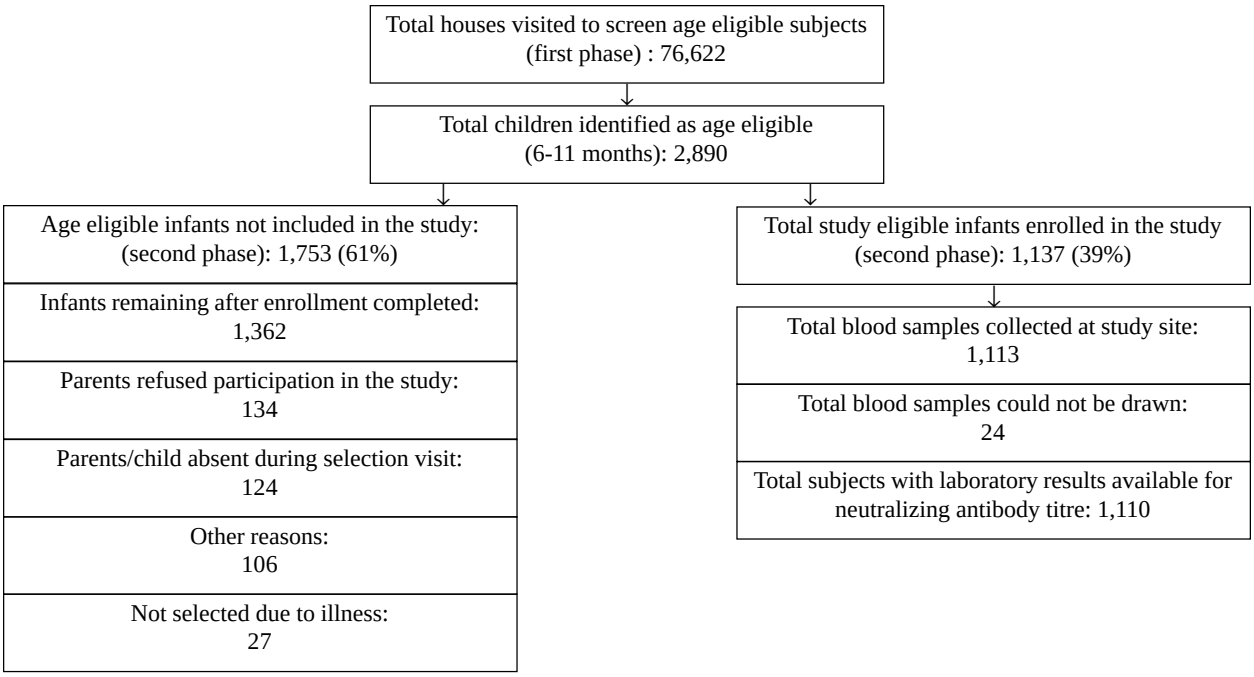

Fig. 1 *Subject enrolment overview.*

through routine and 5 through polio campaigns) compared to Mumbai (3 and 4) and MP (3 and 2). Overall, only 6.4% infants in the study received less than 4 OPV doses. No infant from Bihar received less than 4 doses of OPV (tOPV+bOPV through routine immunization and polio campaigns), whereas 5.6% infants from Mumbai and 13.8% infants from MP received less than 4 OPV doses. Three quarter infants from Bihar, about one-third from Mumbai and none from MP received 8 or more OPV doses (***Table* I**).

Risk-area category wise seroprevalence

Overall, seroprevalence across the study areas in Bihar, MP and Mumbai was 98.3%, 98.1% and 91.1% against poliovirus type-1 (P1), type-2 (P2) and type-3 (P3) respectively. Bihar had a seroprevalence of 98.9%, 98.9% and 91.6% against P1, P2 and P3, respectively. Corresponding figures for MP and Mumbai were 97.8%, 99.2% and 88.0% and 98.1%, 97.3%, and 93.6% respectively (***Fig.* 2**).

Age-wise seroprevalence

The youngest age group (6-7 months) had seropositivity rates of 97.3%, 97.9% and 86.9% against P1, P2 and P3 respectively, compared to 98.7%, 97.3% and 93.5% among infants 10-11 months of age. P3 seroprevalence was significantly high in 10-11 months infants compared to 6-7 months subjects (***Table* II**).

TABLE I PERCENT SUBJECTS BY OPV (TOPV+BOPV) DOSES RECEIVED

Doses received	*Bihar*	*MP*	*Mumbai*	*Total*	*Total cumulative %*
0	0.0	0.3	0.3	0.2	0.2
1	0.0	1.4	0.0	0.5	0.6
2	0.0	3.3	2.1	1.8	2.4
3	0.0	8.8	3.2	4.0	6.4
4	1.6	11.8	5.4	6.2	12.6
5	3.0	34.8	10.7	16.0	28.7
6	5.9	39.7	19.8	21.7	50.4
7	13.5	0.0	23.0	12.3	62.6
8	27.2	0.0	19.5	15.7	78.3
9	26.2	0.0	15.8	14.1	92.3
10	22.6	0.0	0.3	7.7	100.0
Total subjects (N)	371	365	374	1110	100

Comparison of seroprevalence against poliovirus during 2012 and 2014

In 2012, a similar seroprevalence study was conducted in the high risk areas of UP and Bihar. A significant improvement in 2014 was observed in overall seroprevalence against all three poliovirus serotypes compared to the 2012 serosurvey (***Table* III**). Bihar was

TABLE II PERCENT SEROPREVALENCE AGAINST POLIOVIRUSES BY AGE GROUPS

Age Poliovirus type	*6-7 mo*	*8-9 mo*	*10-11 mo*	*P value**
Type 1	97.3	98.9	98.7	0.20
Type 2	97.9	99.2	97.3	0.62
Type 3	86.9	92.9	93.5	0.00
Total subjects (N)	374	366	370	

**P value for seroprevalence between 6-7 & 10-11 months.*

included in 2012 and 2014 serosurvey. The seroprevalence in Bihar increased from 96.7% in 2012 to 98.9% in 2014 against type-1 (P=0.03). Similar increase was also observed for type-2 (98.9%, 89.4%, P<0.01) and type-3 (91.6%, 86.2%, P<0.001).

Type 2 seroprevalence by number of tOPV doses

Seroprevalence against type-2 poliovirus steadily increased with number of tOPV doses, reaching as high as 97.0% with 4 tOPV doses. Infants from Madhya Pradesh showed the highest seroprevalence of 98.0% with 3 or lesser tOPV doses; reaching 100% with 5 or more tOPV doses. Four doses of tOPV gave a seroprevalence of 98.3%, 96.6% and 95.4% in Mumbai, Bihar and MP respectively (***Table* IV**).

DISCUSSION

Unlike previous seroprevalence studies in India, the current seroprevalence study included additional areas with low RI coverage and areas with sizable mobile/ migrant populations, apart from traditional reservoir areas of poliovirus transmission. Due to intensive SIAs in Bihar, 76% infants in the present study received ≥8 doses of OPV. Infants in Bihar received a median of 4 routine tOPV doses compared to 3 in MP and Mumbai. In the post-polio-free certification period the levels of humoral immunity against type-1 poliovirus not only continues to be high in the traditional high risk areas of Bihar (99%) but also high in the lowest routine coverage areas of MP (98%) and in slum areas with migratory populations in Mumbai (98%). This high seroprevalence should be protective against any poliovirus spread from any potential type-1 importations from polio endemic countries [19]. Type-2 seroprevalence is high (>97%) in all the three risk areas; the highest seroprevalence against type-2 recorded since 2007 [20]. This improvement in the type-2 immunity is also a testimony of India's efforts to improve routine immunization in the recent years [21]. Type-3 immunity takes the maximum effort to develop as evident in the present study also [22]. Infants in the age group of 10-11 months had significantly higher seroprevalence for type-3 compared to 6-7 month olds. However, no such differences were observed between the age groups for type -1 and type-2 seroprevalence. This could be attributed to better opportunity of 10-11 months infants to be covered with bOPV through the SIAs compared to 6-7 month infants.

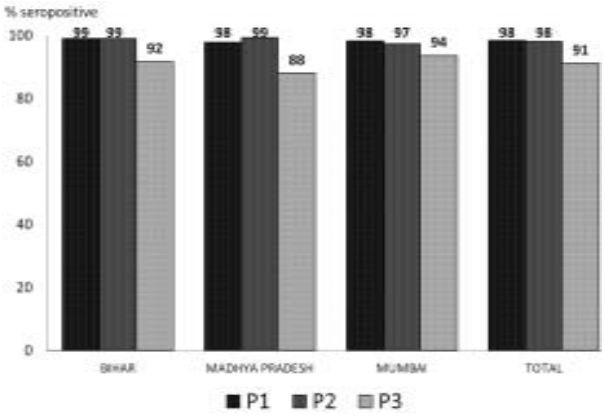

Fig. 2 *Seropositivity against three polioviruses types.*

TABLE IV PERCENT TYPE-2 SEROPREVALENCE AGAINST POLIOVIRUSES BY TOPV DOSE

tOPV doses *Study area*	*0-3 doses*	*4 doses*	*>=5 doses*
Bihar	93.8	96.6	98.4
Madhya Pradesh	98.0	95.4	100.0
Mumbai	91.8	98.3	98.8
Total	94.2	97.0	99.0

TABLE III PERCENT SEROPREVALENCE AGAINST POLIOVIRUSES DURING 2012 AND 2014 SEROSURVEYS

	Overall seroprevalence			*Seroprevalence in Bihar*		
Study areas (Year) *Poliovirus types*	*UP, Bihar (2012)*	*Bihar, MP & Mumbai (2014)*	*P value*	*Bihar (2012)*	*Bihar (2014)*	*P value*
Type 1	96.8	98.3	0.02	96.7	98.9	0.03
Type 2	88.8	98.1	0.00	89.4	98.9	0.00
Type 3	87.0	91.1	0.00	86.2	91.6	0.01
Number of subjects (N)	1250	1110		620	371	

WHAT IS ALREADY KNOWN?

- Prior to polio-free certification, the seroprevalence against poliovirus types 1 & 3 was high in the traditional high risk areas of transmission in India.

WHAT THIS STUDY ADDS?

- In 2014 (post-polio-free period), the seroprevalence is high not only against poliovirus types 1 & 3 but also against type-2 in the traditional high risk areas in India, as well as in the areas with low routine coverage and migratory clusters living in urban slums.

Bihar was common study area for the serosurveys during 2012 and 2014. The seroprevalence in Bihar significantly improved during 2014 compared with 2012 for all three poliovirus serotypes (type-1: 98.9% Vs 96.7%, P=0.03; type-2: 98.9% *vs* 89.4%, P<0.001; type-3: 91.6% *vs* 86.2%, P=0.01) [5]. Similar improvement is observed when all the areas of 2012 serosurvey (UP and Bihar) are compared with the entire risk category areas of 2014 serosurvey (Bihar, MP and Mumbai). This improved seropositivity is seen despite reduced number of OPV doses in 2014. Further analysis of results in this study shows that for children in Bihar who received 5 doses, OPV efficacy has significantly improved in 2014 compared to 2012 (P<0.01). The immunogenicity of OPV seems to have improved in countries of the Indian subcontinent during the past 5–10 years, from fairly low baseline values. Improvements in access to clean water, improved sanitation or rising socioeconomic status could have contributed to this [23].

We would have liked to understand the population immunity against polioviruses in areas with known social, political and geographical challenges where polio surveillance quality is also known to be weak. But due inherent operational complexity and security concerns, this was an important limitation of our study.

High seroprevalence rates against all three poliovirus types in all risk areas as demonstrated in the present study would mitigate India's most important risks of poliovirus spread upon any importation and emergence of cVDPVs. In areas with low RI coverage, the additional tOPV doses given through NIDs have provided adequate back-up to keep type-2 immunity high in these areas. Low type-2 seroprevalence had led to outbreaks of cVDPV2 in India in 2009-10 [19]. Along with strengthened routine immunization, NIDs should be continued in India until global polio free certification to mitigate the risk of VDPV emergence/circulation. Similar seroprevalence studies are recommended in India after the tOPV-bOPV switch to understand the population immunity in dynamically changing vaccine and vaccination scenario during the polio endgame.

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Contributors: MA: conception and design of the project, finalising the study protocol, SOPs, trainings of study staff, leading the field implemetation of the project including monitoring and supervision, data management and data analysis and manuscript writing; SB: conception and design of the project, finalising the study protocol, data management and data analysis and manuscript writing; AK: finalising the study protocol, SOPs, trainings of study staff, facilitating field implemetation, monitoring and supervision and manuscript writing.

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