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## IMMUNITY TO POLIOVIRUS SEROTYPES IN CHILDREN POPULATION OF SELECTED COMMUNITIES IN SOUTH-WEST, NIGERIA

Johnson Adekunle Adeniji<sup>1</sup>, Folakemi Abiodun Osundare<sup>2</sup>, Olubusuyi Moses Adewumi<sup>1\*</sup>, Anyebe Bernard Onoja<sup>1</sup>, Ademola Hezekiah Fagbami<sup>3</sup>

Department of Virology, College of Medicine, University College Hospital, University of Ibadan, Ibadan, Nigeria<sup>1</sup>

Department of Science Laboratory Technology, School of Applied Science, The Federal Polytechnic, Ede, Nigeria<sup>2</sup>

Department of Biological Sciences, Faculty of Science, Ondo State University of Science and Technology, Okitipupa, Nigeria<sup>3</sup>

\* E-mail: [adewumi1@hotmail.com](mailto:adewumi1@hotmail.com)

### Abstract

**Background:** Poliovirus outbreaks are still reported in Nigeria despite renewed efforts to improve vaccine coverage, thus suggesting the existence of susceptible hosts. Also, there is anecdotal evidence of variation in vaccine coverage by region and specifically between urban and rural communities. Consequently, this study assessed neutralizing antibodies to poliovirus serotypes among children in selected urban and rural communities in south western Nigeria. **Methodology:** Two hundred and forty-four {(M=119, F=125); Urban: 142 (M=63, F=79); Rural: 102 (M=56, F=46)} children of consenting parent/guardian aged one week to 15 years were enrolled for the study. About 2-3ml of blood was collected from each child by venepuncture into a labelled sterile container free of anticoagulants. Subsequently, questionnaire was administered to the parent/guardian of each child to retrieve relevant information. Recovered sera were analysed for detectable neutralizing antibodies to poliovirus serotypes by the standard method of constant virus, varying serum dilutions.

**Results:** Overall, 64.3% (n=157) of the children had detectable neutralizing antibodies to the three poliovirus serotypes. Also, 84.8% (n=207), 91.0% (n=222) and 75.0% (n=183) of the children had detectable antibodies to poliovirus serotypes 1, 2 and 3 respectively. Eighty seven (35.7%) of the children had no detectable neutralizing antibody to at least one of the three poliovirus serotypes, while 9 (3.7%) children had no detectable neutralizing antibody to the three poliovirus serotypes. Geometric mean titre (GMT) of neutralizing antibodies to the three poliovirus serotypes varied significantly (p=0.0005).

**Conclusion:** Disparity in immunity to poliovirus infection and existence of children with low or zero neutralizing antibody levels were confirmed.

**Key words:** Immunity, Neutralizing antibody, Nigeria, Poliovirus serotypes.

### Introduction

The World Health Assembly launched the Global Polio Eradication Initiative (GPEI) in 1988 (WHO, 1988). Subsequently, the completion of polio eradication; a programmatic emergency for global public health was declared in 2012 (WHO, 2012). Adopted strategies include routine immunization using Oral Polio Vaccine (OPV), surveillance of acute flaccid paralysis (AFP) cases, national immunization days (NIDs) and mopping-up immunization campaigns. In Nigeria, the National Programme on Immunization was established in 1979 with the mandate to control poliomyelitis among other deadly diseases through effective immunization (USAID, 1999). In 1999, a new drive to sustainably re-vitalize the immunization system commenced in synergy with the accelerated strategy on polio eradication. In Nigeria, OPV is administered at birth (OPV<sub>0</sub>), and subsequently at 6 (OPV<sub>1</sub>), 10 (OPV<sub>2</sub>) and 14 (OPV<sub>3</sub>) weeks of age. In addition, children aged less than five years are specifically targeted during supplementary immunization activities (SIAs) (Alyward *et al.*, 2000; CDC, 2009).

In 2002, three states in northern Nigeria boycotted vaccination due to some erroneous beliefs. Consequently, outbreaks of polio were recorded in 2003. In response to the menace, Immunization Plus Days (IPDs) was implemented in May 2006 to improve eradication efforts. The policy offered substantially beneficial health interventions to increase the uptake of OPVs.

In 2008, the World Health Assembly (WHA) called for increased commitment by the Nigerian government to increase vaccination coverage (CDC, 2008). However, despite renewed efforts aimed at improving the level of vaccine coverage, outbreaks are still reported in the country (WHO, 2013). Such development suggests existence of susceptible hosts with low or zero levels of immunity against poliovirus in the population. Furthermore, there is anecdotal evidence of variation in vaccine coverage by region and specifically between urban and rural communities. Consequently, this study was designed to assess neutralizing antibodies to poliovirus serotypes among children in selected urban and rural communities in south western Nigeria.

### Methodology

#### Study area

Osun state is located in the south western region of Nigeria. It is situated within latitude 6.55 and 8.10 North and longitude 3.55 and 5.05 East. It covers a total landmass of about 12,820 square kilometers. The state has a population of 2.158 million inhabitants (Male: 1.043 million; Female: 1.11million), and it is largely classified as a rural state with 19 out of 30 Local Government Areas (LGAs) being rural local government councils (Ajala *et al.*, 2005). Osogbo, the state capital city is a fairly busy commercial centre. Ethical approvals were obtained from the two Local Government Authorities prior to initiation of the study.

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### Study locations

Samples analysed in this study were collected from two independent populations in Osun state, Nigeria. One from Olorunda LGA and the other from Isokan LGA. Olorunda LGA is situated in the northern part of Osogbo. It is a typical urban setting with educational institutions, health facilities and social amenities suitable for economic development. Inhabitants are predominantly civil servants, entrepreneurs and retail traders. The population enjoys considerable enlightenment being at the epi-center of most government activities. Thus, they are relatively sensitized to health issues including vaccination and its implication on maternal and infant morbidity. Index of accessibility to health service from the computation and hospital management board records shows that inhabitants are fairly disposed to good health facilities (Ajala *et al.*, 2005)

Isokan LGA on the other hand is a typical rural setting located in the south western region of Osun state. It lacks most public amenities characteristic of urban settings. Inhabitants are predominantly farmers, timber dealers, artisans and petty traders. They are less sensitized about health issues including vaccination and its implication on maternal and infant morbidity. Also, most of them still engage in child delivery at home, mission homes or traditional birth attendant places. The index of accessibility to health service from computation and hospital management board shows that the population is poorly disposed to good health facilities (Ajala *et al.*, 2005)

### Study population

The study population included children attending primary schools and healthcare centres in communities within Olorunda (urban) and Isokan (rural) Local Government Areas (LGAs) of Osun state in south western Nigeria. The children were randomly selected during visits to schools and health care centres in the communities between July and December, 2010. Children aged between one week and 15 years whose parent/guardian consent to participate in the study were included. Children whose parent/guardian did not consent to participate in the study were excluded. A total of 244 (M=119, F=125) children were enrolled for the study. The study population include 142 (M=63; F=79) and 102 (M=56; F=46) children enrolled from the urban and rural communities respectively. Age profile shows that the population includes age  $\leq$  30 days (n=6), >1-9 months (n=19) and 1-15 years (n=219). Vaccination records (or recall by parents or guardian) of children enrolled for the study showed that 90.6% (n=221) of the children had four doses (OPV<sub>0.3</sub>) of OPV, while 2.9% (n=7), 2.5% (n=6) and 2.5% (n=6) missed 1, 2 or 3 doses of vaccine respectively. Four (1.6%) children had never received OPV. Only, children who received four doses (OPV<sub>0.3</sub>) were considered to have complete polio vaccination.

### Sample collection, processing and storage

After parental consent to participate in the study was obtained, about 2-3ml of blood specimen was collected from each child by venepuncture. The specimen was collected into a labelled sterile container free of anticoagulants or preservatives. Subsequently, questionnaire was administered to parent/guardian of each child to retrieve relevant information. Samples were transported to the laboratory immediately in a cold box with ice packs to maintain cold chain at about 4–8°C. Serum was recovered from each sample by low-speed centrifugation at 500 g for 5 minutes, followed by direct removal of the serum using a sterile disposable pipette. The serum was transferred into two labelled sterile cryovials per sample and stored at –20°C until ready for analysis.

### Neutralization assay

Samples were inactivated at 56°C for 30 minutes in a water bath before they were used for neutralization assay. Virus suspensions of laboratory strain of the three poliovirus serotypes (Sabin strains) were prepared in L20B cell line. Challenge dose of 100 TCID<sub>50</sub> of poliovirus serotypes 1, 2 and 3 was determined and used for the neutralization test by the standard method of constant virus, varying serum dilutions as described in the manual for the virological investigation of polio (WHO, 2004).

### Statistical analysis

Statistical analysis was performed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL.). Results were presented using descriptive statistics. Chi square statistical test was used to establish association between participants' variables. Indicator of statistical significance was set at  $p \leq 0.05$ .

## Results

### Prevalence of neutralizing antibodies to poliovirus serotypes among the entire study population

Overall, 64.3% (n=157) of the children had minimum neutralizing antibody titre of 8 to the three poliovirus serotypes. Also, 84.8% (n=207), 91.0% (n=222) and 75.0% (n=183) children had minimum neutralizing antibody titre of 8 to poliovirus serotypes 1, 2 and 3 respectively (Table 1). Eighty seven (35.7%) of the children had no detectable neutralizing antibody to at least one of the three poliovirus serotypes, while 9 (3.7%) children had no detectable neutralizing antibody to the three poliovirus serotypes. Ten (4.1%) children had no detectable neutralizing antibody to poliovirus serotypes 1 and 2; 13 (5.3%) children lacked detectable neutralizing antibody to poliovirus serotypes 2 and 3, while neutralizing antibody to poliovirus serotypes 1 and 3 were not detected in 18 (7.4%) children. All the four children without record of vaccination had at least neutralizing antibody titre of 8 to the three poliovirus serotypes. Thirty-one (57.4%) and 190 (100%) of children aged  $\leq$  5 years and >5 years respectively had four doses of poliovirus vaccine. Geometric mean titre (GMT) of neutralizing antibodies to the three poliovirus serotypes varied significantly ( $p=0.0005$ ). Also, significant variation was observed in the GMT of neutralizing antibodies to the three polioviruses among children in the urban ( $p=0.0005$ ) and rural ( $p=0.0005$ ) communities. No significant difference ( $p>0.05$ ) was observed in the GMT of neutralizing antibodies to the poliovirus serotypes among children aged  $\leq$ 30 days. However, significant differences were observed among ages >1-9 months ( $p=0.003$ ) and 1-15 years ( $p=0.0005$ ).

**Table 1:** Neutralizing antibodies to poliovirus serotypes 1, 2 and 3 among children in an urban and rural community in South-west Nigeria

Titre	P1		P2		P3	
	Urban (%)	Rural (%)	Urban (%)	Rural (%)	Urban (%)	Rural (%)
<8	23 (16.2)	14 (13.7)	20 (14.1)	2 (2.0)	44 (31.0)	17 (16.7)
8	6 (4.2)	8 (7.8)	10 (7.0)	2 (2.0)	7 (4.9)	13 (12.7)
16	18 (12.7)	19 (18.6)	18 (12.7)	10 (9.8)	21 (14.8)	21 (20.6)
32	22 (15.5)	19 (18.6)	20 (14.1)	22 (21.6)	31 (21.8)	19 (18.6)
64	26 (18.3)	16 (15.7)	37 (26.1)	23 (22.5)	25 (17.6)	12 (11.8)
128	18 (12.7)	20 (19.6)	13 (9.2)	18 (17.6)	10 (7.0)	14 (13.7)
256	18 (12.7)	3 (2.9)	11 (7.7)	20 (19.6)	4 (2.8)	3 (2.9)
512	9 (6.3)	3 (2.9)	8 (5.6)	4 (3.9)	0 (0)	3 (2.9)
1024	2 (1.4)	0 (0)	5 (3.5)	1 (1.0)	0 (0)	0 (0)
<b>Total</b>	<b>142</b>	<b>102</b>	<b>142</b>	<b>102</b>	<b>142</b>	<b>102</b>

#### Prevalence of neutralizing antibodies to poliovirus among children in the urban community

Out of the 142 children enrolled in the urban community, 59.9% (n=85) had minimum neutralizing antibody titre of 8 to the three poliovirus serotypes. Furthermore, 83.8% (n=119), 85.9% (n=122), 69.0% (n=98) of the children had minimum neutralizing antibody titre of 8 to poliovirus serotypes 1, 2 and 3 respectively (Table 1). Fifty seven (40.1%) of the children had no detectable neutralizing antibody to at least one of the three poliovirus serotypes, while 8 (3.3%) children had no detectable neutralizing antibody to the three poliovirus serotypes. Nine (6.3%) children lacked detectable neutralizing antibodies to poliovirus serotypes 1 and 2. Also, 12 (8.5%) children had no detectable neutralizing antibodies to poliovirus serotypes 2 and 3, while neutralizing antibodies to poliovirus serotypes 1 and 3 were not detected in 16 (11.3%) children.

#### Prevalence of neutralizing antibodies to poliovirus among children in the rural community

Out of the 102 children enrolled from the rural community, seventy two (70.6%) had minimum neutralizing antibody titre of 8 to the three poliovirus serotypes. Also, 86.3% (n=88), 98.0% (n=100) and 83.3% (n=85) children had minimum neutralizing antibody titre of 8 to poliovirus serotypes 1, 2 and 3 respectively (Table 1). Thirty (29.4%) children had no detectable neutralizing antibody to at least one of the three poliovirus serotypes, while one (0.98%) child had no detectable neutralizing antibody to the three serotypes. One (0.98%) child had no detectable neutralizing antibodies to poliovirus serotypes 1 and 2; one (0.98%) child also lacked detectable poliovirus neutralizing antibodies to serotypes 2 and 3, while two (2.0%) children had no detectable neutralizing antibodies to poliovirus serotypes 1 and 3.

## Discussion

Results of this study confirm previous reports (Adewumi et al., 2006; Donbraye *et al.*, 2011; Baba et al., 2012; Giwa et al., 2012) documenting disparity in immunity to poliovirus infection between and within communities in Nigeria. It also corroborates reports of possible low national vaccine coverage and variation within states, and the risk of possible reintroduction of poliovirus into polio-free regions within and beyond Nigeria (CDC, 2003). The results further support previous reports (Wassilak et al., 2011; Burns et al., 2013) suggesting gaps in surveillance and uncertain improvement in immunization campaign quality. The overall neutralizing antibody prevalence among children in the studied communities is similar to the results obtained by Ghosh et al., (1980), Oduntan *et al.*, (1978) and Adewumi et al., (2006), supporting a sero-conversion pattern of P2>P1>P3. Similarly, John and Jayabal (1972) and Oduntan et al., (1978) reported low rates of sero-conversion to poliovirus serotypes 1 and 3 subsequent to administration of oral polio vaccine in developing countries. Also, in a study among children in the south-western Nigeria Adewumi and his colleagues reported highest prevalence of neutralizing antibody to poliovirus serotype 2 and lowest to serotype 3 (Adewumi et al., 2006). In contrast, Baba et al., (2012) and Giwa et al., (2012) in separate studies in northern Nigeria reported sero-conversion pattern of P1>P3>P2. The change in sero-conversion pattern may be as a result of emphasis on mOPV1, mOPV3, and bivalent OPV (types 1 and 3) in SIAs since January 2010 (Burns et al., 2013)

Children without detectable neutralizing antibodies to the three poliovirus serotypes or to at least one poliovirus serotype, as well as those with persistently low immunity to any of the poliovirus serotypes could serve as pockets of children at risk of re-infection and continued transmission of the virus in the community. Previous studies have consistently showed that children with low but detectable serum neutralizing antibody could be re-infected with wild or vaccine virus (Magrath et al., 1981; Nishio et al., 1984). Children in this category may not be in danger of developing clinical poliomyelitis but may be re-infected with poliovirus and possibly provide a source of infection for others who have not been vaccinated (Thompson et al., 2013). It is worth mentioning that identification and vaccination of chronically missed children during SIAs have been identified as some of the key challenges impeding realization of immunization coverage needed to eliminate poliovirus transmission in Nigeria.

This study showed that children from the selected communities had 64.3% immunity to the three poliovirus serotypes. It also showed disparity in population immunity between the rural and urban communities. Higher population immunity was recorded among children in the rural community and this may be due to secondary vaccination. Significantly, despite the claimed high vaccination coverage in the country, the

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low sero-conversion rate and the low population immunity observed in this study suggest existence of children susceptible to poliovirus infection in the population. In a country with high incidence of poliomyelitis, such situation leaves a high number of non-immunized children at risk of infection with one or more poliovirus serotypes. Also, the number of non-immunized children found in the study may favour continued spread and outbreak of poliovirus infection. Specifically, with the on-going wild poliovirus (WPV) transmission in northern Nigeria, reintroduction into southern Nigeria and surrounding polio-free countries is a possibility.

## Conclusions

This study shows the presence of high number of children without detectable neutralizing antibodies to one or more poliovirus serotypes in the selected communities. Significantly, children who are not immunized may be potentially at risk of infection. Additionally, they may covertly participate in the maintenance and circulation of the virus in the population. Equally significant, existence of children with low or zero neutralizing antibody levels in a country without a satisfactory AFP surveillance mechanism suggests high probability of silent transmission and maintenance of poliovirus circulation.

**Competing interest:** The authors declare that we have neither financial nor non-financial competing interests that may influence the interpretation of the results of the study.

## Author's contribution:

JAA participated in the design and coordination of the study, and review of the manuscript. FAO participated in data collection, laboratory procedures and review of manuscript. OMA participated in the design of the study and laboratory procedures. He also drafted the manuscript. ABO participated in the laboratory procedures and review of the manuscript. AHF participated in the design and coordination of the study, and review of the manuscript. All authors read and approved the final manuscript.

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