Neutralizing Antibodies Against Poliovirus Serotypes Among Children in Southwest Nigeria

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Summary

In May 1988, the World Health Assembly resolved to eradicate poliomyelitis globally by the year 2000. Despite the reported success in national immunization days, acute flaccid paralysis surveillance and accelerated efforts to meet the deadline including 'mopping-up' were executed in 1999 and subsequent years. Nigeria remains one of the major reservoirs for wild poliovirus transmission. Neutralizing antibody titre to the three poliovirus serotypes was determined among children from different communities in southwest of Nigeria, and analysed by age, gender and location. About 0.5–2 ml of blood sample was collected by venepuncture from each child. Aliquot of serum from each blood sample was inactivated prior to neutralization test by the beta method for poliovirus antibodies. A total of 347 (59.6 per cent) out of 500 and 82 children enrolled for the study had at least antibody titre of 1:8 against each of the three poliovirus serotypes. Immunity level to the three poliovirus serotypes increased with age and peaked in children aged 4–6 years. Seven (53.8 per cent) out of 13 unvaccinated children tested in the study had detectable neutralizing antibody to the three serotypes. Immunity pattern of P2 > P1 and P3 was observed but no correlation between gender and antibody to the poliovirus serotypes. The populations had 59.6 per cent herd immunity for the three poliovirus serotypes. In a country with high incidence of poliomyelitis this situation leaves a high number of non-immunized children at the risk of infection with one or more poliovirus serotypes.

Introduction

In May 1988, the World Health Assembly (WHA) resolved to eradicate poliomyelitis globally by the year 2000. In the African Region (AFRO) of the World Health Organization (WHO), eradication strategies were accelerated following supporting resolutions by the Regional Committee of the WHO for Africa in 1995 and the Organization for African Unity (now African Unity) in 1996.[1] Despite the reported success in National Immunization Days (NIDs), establishment of acute flaccid paralysis (AFP) surveillance and accelerated efforts to meet the year 2000 target including 'mopping-up' executed in 1999 and subsequent years, Nigeria, the most populous country in Africa, remains one of the major reservoirs for wild poliovirus transmission.

Although there has been improvement in the quality of surveillance for acute flaccid paralysis (AFP) and synchronized house-to-house NIDs conducted since late 2000 in Nigeria, the country remains one of the most significant poliovirus reservoirs with 998 reported cases of acute flaccid paralysis out of which 101 were due to poliovirus infection as of early August, 2002.

This study was conducted in order to evaluate the level of immunity against poliovirus serotypes 1, 2 and 3 by determining the neutralizing antibody in children within the age at greatest risk of poliomyelitis and the herd immunity as a true biological marker of protection against the virus rather than using solely the coverage rate during the national immunization campaigns as the situation in Nigeria.

Materials and Methods

Sampling method

Random sampling method was employed for subject selection in this study. Blood samples were collected from children aged one week to 10 years after due parental consent to enrol for the study.

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Healthy vaccinated and unvaccinated children were selected at random from designated health centres on immunization days as well as schools and households in different local government areas of the states included in the study. Subjects were selected from urban, semi-urban and rural communities and across different zones of the states.[2]

Sites and sample size

A total of 582 blood samples were collected from children from 3 states in southwestern, Nigeria (Oyo = 221, Ogun = 226, Osun = 135). The samples were collected from Abeokuta South/Owode, Ijebu-Ode/Shagamu and Ado-Odo-Ota/Yewa North in Ogun state; Ibadan Southwest, Egbeda, Ibadan North and Atiba Local Government Areas in Oyo state and Osogbo in Osun state (Fig. 1).

Collection, transport, preparation and storage of samples

About 0.5–2 ml of blood sample was collected by venepuncture from each child into a labelled sterile container free of anticoagulants or preservatives. Samples were transported to the laboratory immediately in a cold box with frozen ice packs to achieve condition of about 4–8°C. Serum samples were separated by low-speed centrifugation at 500 g for 5 min, or direct removal of the serum using a sterile disposable pipette after retraction of the clot. The serum was transferred into two labelled sterile cryovials per sample and stored at –20°C until ready for analysis.

The samples were inactivated at 56°C in water bath prior to use for neutralization assay. Virus suspensions of the laboratory strain of the three poliovirus serotypes (Sabin strains) were prepared in L20B cell line. Challenge dose of 100 $TCID_{50}$ 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 virology investigation of polio.[2]

Results

Five hundred and sixty-four (96.9 per cent) of the children enrolled in the study had complete polio vaccination (OPV_{0-3}); 5 (0.9 per cent) had incomplete OPV series vaccination while the other 13 (2.2 per cent) claimed unawareness or loss of vaccination record. Overall, a total of 347 (59.6 per cent) of the 582 children enrolled for the study had minimum neutralizing antibody titre of 1:8 to the three poliovirus serotypes.

Specifically, 460 (79.0 per cent), 521 (89.5 per cent) and 431 (74.1 per cent) children had minimum neutralizing antibody titre of 8 to poliovirus serotypes 1, 2 and 3 respectively while 347 (59.6 per cent) were seropositive to the three poliovirus serotypes.

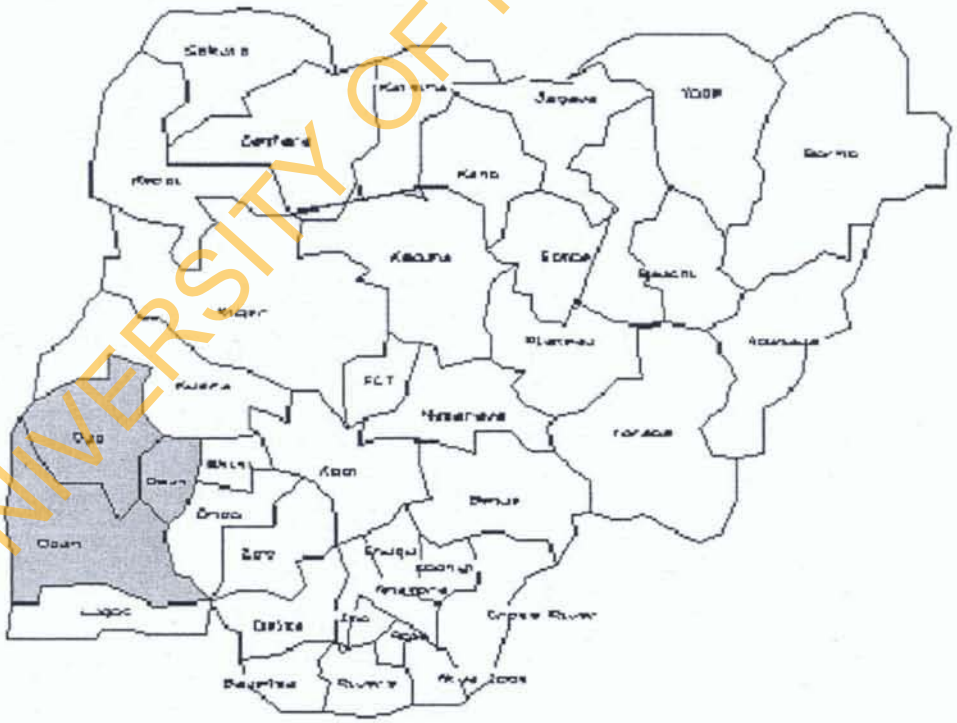

FIG. 1. Map of Nigeria showing the states included in the study.

Seventeen (3.0 per cent) of the children were seronegative to the three poliovirus serotypes. Seropositivity to poliovirus types 1, 2 and 3 increased with age and peaked in children aged 4–6 years (Table 1).

Eight (57.1 per cent) of the 14 children aged 0 ≤ 1 month tested in the study had neutralizing antibodies to the three poliovirus serotypes while 2 (14.3 per cent) were seronegative to the three poliovirus serotypes. The pattern of neutralizing antibody titre to poliovirus serotypes among children aged 0–1 month is shown in Table 2.

Out of 2 sets of twins (all females) enrolled in this study, one set was seronegative to P1 or P3. In addition, out of the 13 unvaccinated children tested, 12 (92.3 per cent), 10 (96.9 per cent) and 8 (61.5 per cent) had antibody titre ≥ 8 to poliovirus types 1, 2 and 3 respectively and 7 (53.8 per cent) were seropositive to the three poliovirus types.

Discussion

The pattern of seroconversion to each of the three poliovirus serotypes observed in this study was P2 > P1 > P3. In some similar previous surveys, the antibody level was reported to be lowest for type 3 followed by type 1 as found in the results of this work.

Ghosh, *et al.*[3], John and Jayabal[4] and Oduntan, *et al.*[5] reported low rates of seroconversion to poliovirus types 1 and 3 following administration of trivalent OPV in developing countries during the 1970s. In a review of data accumulated from developing countries over 25 years, Patriarca, *et al.*[6] also showed a wide variation in the seroconversion rate of children after vaccination with the three doses of OPV, with the percentage of children seroconverting being 73 per cent (range 36 per cent to 99 per cent) for type 1, 90 per cent (range 71 per cent to 100 per cent) for type 2, and 70 per cent (range 40 per cent to 90 per cent) for type 3.

In a prospective study conducted in Japan over 5 years among children after two doses of trivalent OPV (at 3 and 6 months of age) or administration of a booster dose of OPV at 5 years of age, Nishio, *et al.*[7] reported that neutralizing antibody titre for types 1 and 2 declined gradually. However, a more rapid and statistically significant decline in the antibody level was observed with type 3. Krugman, *et al.*[8] and Trivello, *et al.*[9] in separate prospective studies in the United States and Italy respectively also presented result in support of seroconversion pattern of P2 > P1 > P3.

Gelfand, *et al.*[10], McKay, *et al.*[11], Magrath, *et al.*[12] and Nishio, *et al.*[7] in different studies from different places consistently showed that children with low but detectable serum neutralizing antibody could be re-infected with wild or vaccine virus. These investigations also suggest that 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. Domok, *et al.*[13] and Munube and Mutanda [14] in different studies reported the presence of inhibitors in the gut which may prevent the multiplication of vaccine virus and this may be partly responsible for the variation in the seropositivity found with the sets of twins in the study.

Ashley, *et al.*,[15] carried out a population based study involving more than 2500 children and adolescents aged up to 19 years and showed that among children 1–4 years that had never received polio vaccine, 53 per cent, 77 per cent and 55 per cent were seropositive for P1, P2 and P3 respectively, probably reflecting circulation of both wild and secondary vaccine virus strains in the country. Circulation of poliovirus due to poor sanitation and existence of unhygienic environment could be contributing factors.[16]

Immunity level as determined by the antibody titre was higher in children within the age range of 0–6 years who were seropositive to the three poliovirus serotypes, with some of them in this study presenting with high antibody titre of 512 to the three serotypes. This could be attributed to routine and complementary, most of the time repeated vaccine administration during NID program for children within the age group.

TABLE 1

Age distribution of children with complete seropositivity to P1, P2 and P3 in southwestern Nigeria

Age (years)	Total	
	No.	No. (per cent) + VE
0–2	177	85 (48.0)
2⁺–4	127	81 (63.8)
4⁺–6	110	83 (75.5)
6⁺–8	70	47 (67.1)
8⁺–10	98	51 (52.0)
Total	582	347 (59.6)

TABLE 2

Pattern of neutralizing antibody titre to poliovirus serotypes among children aged 0–1 month in southwestern Nigeria

Titre / serotype	8	16	32	64	128	256	512
P1	5	1	4	–	–	–	–
P2	4	3	1	1	2	1	–
P3	1	2	–	5	1	–	–

Seropositivity variation in the infants could be attributed to passively transferred immunity (maternal antibody) from mother to foetus via the placenta. Gelfand, *et al.*,[17] Ananthakrishnan, *et al.*[18] and WHO[19] had reported higher concentration of types 1 and 2 IgG neutralizing antibody in the newborn as approximately equal to that of the mother and type 3 titres lower than that of the mother suggesting differential transplacental transfer.

This study showed that children from the three south western states of Nigeria included in this study had 59.6 per cent herd immunity for the three poliovirus serotypes. In a country with high incidence of poliomyelitis, this situation leaves a high number of non-immunized children at the risk of infection with one or more poliovirus serotypes. The number of non-immunized children found in this study as against NPI reports of 108 per cent immunization coverage in the second round of the 1999 NIDs,[1,20] favours continued spread and outbreak of poliovirus infection.

Conclusions

This study showed the presence of high number of children without detectable neutralizing antibodies to one or more poliovirus serotypes in southwestern Nigeria. Such children who are not immunized may be potential link in the maintenance and circulation of the virus in the population. It therefore shows the need to do the following:

- Re-evaluate the vaccination procedure including monitoring vaccine handling procedures before and during administration.
- Further study the peculiar factors that may affect seroconversion by vaccinees such as malnutrition, helminth infection, immunosuppression due to infections like HIV, epidemiological patterns of the disease in the country, rate of vaccine associated paralytic poliomyelitis (VAPP) and intestinal protection by secretory IgA antibody.
- Improve on the environmental sanitation and hygiene to control poliovirus circulation in the country.
- Continuously evaluate the herd immunity against the three serotypes of the virus among children at risk of infection in the country.

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