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Archives of Applied Science Research, 2012, 4 (3):1432-1435
(http://scholarsresearchlibrary.com/archive.html)

ISSN 0975-508X
CODEN (USA) AASRC9

Polioviruses in Apparently Healthy Secondary School Students in FCT Abuja, Nigeria

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ABSTRACT

In an effort to complement the acute flaccid paralysis surveillance for wild poliovirus in FCT, Abuja, Nigeria stool samples from 200 apparently healthy secondary school students aged between 9-17 years were tested using cell culture technique. A Sabin strain poliovirus type 3 was isolated from a 13 year old female student while 4(0.02%) were positive for non polio enteroviruses consisting of coxsackie B, echovirus 2 and 2 indeterminate. The isolation of Sabin strain of poliovirus is advantageous to the polio eradication program as it is capable of inducing natural immunity in susceptible hosts. Presently there is no vaccine for non polio enteroviruses but as efforts are intensified on poliovirus eradication, there may be need to assess the impact of this group of viruses on the epidemiology of paralysis in children.

Key words: survey, Poliovirus, apparently healthy, students

INTRODUCTION

Poliovirus, an enterovirus in the family Picornaviridae, consists of antigenic types 1, 2, and 3 and all the three serotypes cause paralysis. In a bid to eradicate the wild poliovirus, vaccination with Oral Polio Vaccine (OPV) is adequate to stimulate adequate immunity in subjects. Poliomyelitis is still endemic in Nigeria. Reaching all the Nigerian children (0-5 years) remains a critical challenge that has pushed eradication efforts to explore increasingly research-driven and innovative communication strategies [1].

An efficient acute flaccid paralysis (AFP) surveillance system supported by a WHO accredited laboratory network for the virological diagnosis of wild poliovirus infections is essential not only for the detection of cases of poliomyelitis but also for documentation of the success of poliovirus eradication efforts [2]. It has been observed that supplementary surveillance involving healthy children above 5 years and screening of wastewater can be used effectively to demonstrate wild poliovirus transmission in the environment [3]. It can also serve as a good tool to assess the effectiveness of poliomyelitis immunization campaigns, especially during the last stages of poliovirus eradication [4]. However, the requirement of supplementary surveillance for the purpose of certification of poliovirus eradication is not yet clearly defined [5].

It is envisaged that survey of apparently healthy secondary school children, in selected communities for silent transmission of wild poliovirus may provide supplementary information on the persistent transmission of wild poliovirus in the country. This study aims at testing older children in Federal capital Territory (FCT) Abuja, for polioviruses.

MATERIALS AND METHODS

Study Area

The study was carried out in the Federal capital Territory (FCT) Abuja, Nigeria. Geographically, Abuja is centrally located in the country and any epidemic if it occurs can spread easily to many neighbouring States. Out of the secondary schools in Abuja, five were randomly selected for the study and they include Government secondary schools (GSS), Ijah, Kabusa,Takushara, Nyanya,and Karu III.

Study Population

Ethical approval from both Ministries of Health and Education in FCT, Abuja was obtained. Apparently healthy students whose ages ranged from 9 to 17 years were selected randomly from 5 Secondary schools. Initially 400 students were enlisted for the study, but after advocacy with the respective school authorities, each student received parental consent form and a universal container each. Only 50% of these students returned with endorsed consent forms and stool samples within the stipulated period. Freshly produced stool in universal containers was collected from each student between January and March 2011. The stool samples were transported in isothermal boxes packed with frozen ice packs to WHO National Polio/ITD Laboratory, University of Maiduguri Teaching Hospital, Maiduguri, Nigeria, where they were stored at -20^0C till tested.

Vaccination History of the Studied Population.

Relevant vaccination history of each student was not obtained partly because the students could not produce tentative vaccination records (either in written or oral form) and the sample collector could not have a direct access to the parent of each student for these information.

Virus Isolation and Identification / Microneutralization tests for Non Polio enteroviruses.

The methods adopted for virus isolation and microneutrlization for identification of isolates were described in WHO Polio Laboratory Manual 2004 and the supplemental manual of 2006 for the New Algorithm Technique currently used for poliovirus isolation. In brief, stool suspensions were prepared using chloroform and phosphate buffered saline, centrifuged at 3000 rpm for 30 minutes. 200ul of the supernatant was inoculated onto healthy monolayer of L20B and Rhabdomyosarcoma (RD) cell lines (Source: Centre for Disease Control and Prevention, Atlanta. USA) in maintainance medium (Eagle's MEM Supplemented with 2% Fetal Calf Serum (FCS). The cells were seeded 48 hours prior to inoculation with growth medium (Eagle's MEM supplemented with 10% FCS). The inoculated monolayers were incubated at 36^0C and observed daily for the characteristic enterovirus cytopathic effects (CPE) of rounded refractile cells and detaching from the surface of the tube. The tubes with CPE up to 75% and above were harvested and kept at -20^0C to be passaged to a fresh monolayer of the second cell line. While those negative after 5 days of incubation at 36^0C were re-passaged on the same cell line. Tubes showing no CPE after five days were considered as negative.With the New Algorithm Technique [6], Positive isolates usually end in RD cell line because virus titre seems higher in RD than L20B. Positive samples on RD cells were passaged onto L20B, the selective cell line for poliovirus. Identication of isolates on RD cells was carried out by microneutralization technique using antisera raised in horse against coxsackie and echoviruses prepared by the National Institute of Public Health and Enviroment (RIVM), Netherlands.

Poliovirus Intratypic Differentiation by Real Time RT-PCR.

The intratypic differentiation (ITD) of poliovirus isolates were as described by Kilpatrick et.al. (2009), and [9] to either Sabin or Wild or cVDPV on isolates from L20B arm and sometimes RD arm. The principles of the technique involved the conversion of viral RNA (VRNA) to complementary DNA (cDNA) using reverse transcriptase. The cDNA was amplified in a PCR reaction using Taq polymerase. The PCR products were detected and identified by hybridization with specific Taqman prmers / probes. Both the cDNA synthesis and the PCR reaction used multiple sets of oligonucleotide primers that were tagged with probes with different specificities. This combination of primers and probes resulted in serotype identification and intratypic differentiation of poliovirus isolates.

RESULTS

The aim of this study was to determine the type of poliovirus excreted by apparently healthy secondary school students into the environment. These students were found to shed Sabin poliovirus type 3, and non polio enteroviruses. In addition, among four positive samples on RD cell line (but negative on L20B) were 2 echovirus type 2 and 2 indeterminate by microneutralization technique. The shedding of the Sabin poliovirus promotes herd immunity and therefore favours polio eradication program in Nigeria.

Table I Shows the result of ITD assay for the poliovirus

TABLE I: ITD ASSAY RESULTS INTERPRETATION

PE	PP	PV1	PV2	PV3	SAB1	SAB2	SAB3	RESULTS
+	+	-	-	+	-	-	+	PV3 Sabin

Table II shows the sex distribution of students who shed poliovirus and non polio enteroviruses. The Sabin poliovirus type 3 was shed by a female. However, both sexes had non-polio enteroviruses.

TABLE II: SEX DISTRIBUTION OF STUDENTS THAT SHED POLIO AND NON POLIO ENTEROVIRUS

Sex	No tested	Sabin Polioviruses (%)	Non Polio Viruses
Male	99	0(0.0%	2 (0.01%)
Female	101	1(0.005%)	2 (0.01%)
Total	**200**	**1(0.005%)**	**4(0.02%)**

Table III shows the age distribution of students with polio and non-polio enteroviruses shed.

TABLE III: AGE DISTRIBUTION OF STUDENTS WITH POLIO AND NON POLIO ENTEROVIRUSES

Age group	No tested	Sabin poliovirus (%)	Non polio enterovirus
9 – 11	94	0(0.0%)	1(0.005%)
12 – 14	78	1(0.005%)	3(0.01%)
15 – 17	28	0(0.0%)	0(0.00%)
Total	**200**	**1(0.005%)**	**4(0.02%)**

The fact that a female student shed the poliovirus does not point to sex bias as male-to-female ratio for acute infection is 1:1 [10]. However, the female sex is more predisposed to post polio syndrome which is not an infectious process, and persons experiencing the syndrome do not shed poliovirus [11].

DISCUSSION

In 2012, only three countries (Afghanistan, Nigeria and Pakistan) remain polio-endemic, down from more than 125 in 1988. Persistent pockets of polio transmission in northern Nigeria and the border between Afghanistan and Pakistan are the current focus of the polio eradication initiative. As long as a single child remains infected, children in all countries are at risk of contracting polio. For instance, in 2009-2010, 23 previously polio-free countries were re-infected due to imports of the virus. A report has shown that 95% of wild polioviruses are being transmitted in 8 high risk States (Kano, Jigawa, Borno, Zamfara, Sokoto, Kebbi, Yobe and Kasina) in Nigeria between 20011 and 2012 [12]. The limitation of this study lies on the fact that the students tested failed to give their vaccination details either in written or oral form and meeting the parent of each student for the same information was a difficult task to accomplish.

In this study, the young adults tested only excreted Sabin Poliovirus and non-Polio-enteroviruses. This result is in consistent with the fact that FCT is not an endemic State in Nigeria for wild poliovirus transmission. However, the lack of wild poliovirus in this study may not necessarily reflect the true situation because many parents refused their wards to participate in the study by refusing to endorse the consent forms. It could be assumed that if more students were tested, probably wild-type poliovirus would have been detected.

The excretion of one Sabin type Poliovirus gives hope of success to polio eradication program in Nigeria. According to Sutter et al (2004), Sabin strain limits the exposure of the unvaccinated to infection with wild-type especially the younger siblings of those shedding the virus. Also, the excretion of Sabin type Poliovirus from these students agreed favorably with a report that environmental surveillance in populations immunized with Oral Polio vaccine (OPV) should reveal Sabin-like strains. Although the study population showed no evidence of vaccination history, it could be assume that before the age of 9 years most children in Nigeria are vaccinated or acquire immunity from the environment. Unlike this study, a report [14] has revealed wild poliovirus from apparently healthy children in Borno State (a high risk State for persistent wild polio virus transmission). However, the children used for that study were from primary schools whose ages ranged from 5-16 years while Secondary school children with ages 9-17 years were the target population in this study. Also, the geographical location, cultural beliefs, religion and literacy rate of the residents of the study sites in both studies may play significant role in respect to polio vaccination. Whilst compliancy to polio vaccination is high in FCT, it is low in Borno State probably because the residents (from 6- 39 years) of the former are comparatively better educated [15]. This observation conformed favorably with a recent report of the 23rd ERC Meeting (2012) which showed that 68% of wild polio viruses in Nigeria were obtained from Borno State. However, FCT has achieved 85% polio vaccination coverage [16]. The possibility of importing or re-establishment of wild polioviruses in previously non-endemic States should keep all hands in FCT on deck to ensure a total coverage of the city as advised by the Hon. Minister of State for Health [17]. As long as travelling from

endemic States to and fro FCT continues daily, polio could be re-established in the city unless appropriate vaccination strategies are sustained.

However, this result should not be a ground for complacency, as outbreaks of paralytic poliomyelitis have occurred in poorly or non-immunized subgroups within otherwise well-vaccinated populations [18]. In the outbreak in Finland where more than 90% of the population were immunized, there was evidence indicating that the Finnish poliovirus strains had undergone antigenic evolution because the virus was found to be poorly neutralized by antibodies to the classical poliovirus type 3 vaccine strain [19]. Therefore, all effort should be made to achieve as near 100% coverage as possible and, at the same time, seroepidemiological surveys should be carried out periodically to monitor the changing pattern of the immunity of the population to the three types of poliovirus [20]. Moreso relying on disease notification or outbreak and immunization coverage can be misleading. Since Abuja is situated in the midst of states that are still endemic for poliomyelitis, wild poliovirus strains can still be introduced by visitors.

CONCLUSION

The shedding of Sabin / Vaccine strain of Poliovirus type 3 is advantageous for global polio eradication initiative in Nigeria as contact with susceptible un- immunized individuals can be infected positively as indirect immunization which increases the populace herd immunity. In addition, high literacy rate, good urban and regional planning with proper aeration improves the hygiene standard of populace and reduces disease spread.

Acknowledgements
We thank the WHO for their technical support.

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