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Original Article

Absence of poliovirus in apparently healthy school children in Bauchi state, Nigeria

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Abstract

Introduction: Poliovirus infections have been established to be in circulation in the remaining three polio-endemic nations. These pathogens have been associated with several chronic diseases, particularly acute flaccid paralysis of children.

This study sought to ascertain whether polioviruses are silently shed by apparently healthy schoolchildren in Bauchi, Katagum, and Misau local government areas of Bauchi state, Nigeria.

Methodology: This was a cross-sectional prospective study that involved 200 stool samples collected from apparently healthy schoolchildren. All samples were processed and inoculated onto rhabdomyosarcoma (RD) and L20B cell-lines. Inoculated cell lines were monitored for cytopathic effects (CPEs) for 10 days with one subculture after first 5 days.

Results: None of the samples came down with CPEs on L20B, and thus all samples were negative for poliovirus; however, three were positive for non-polio enteroviruses (NPEVs) on RD and not on the L20B cell line: one coxsackie B virus from a seven-year-old male, and two others were untypeable isolates, one each from a male and a female child. The coxsackie B virus was identified by microneutralization test using polyclonal sera as described by the World Health Organization.

Conclusions: Findings from this investigation indicate the absence of polioviruses in the children studied. This is an indication of good polio immunization coverage in these communities. However, more intensive and periodic surveillance is required to confirm the presence or exclude the absence of polioviruses in these communities and other parts of Nigeria.

Key words: Coxsackie B virus; microneutralization; poliovirus; non-polio enteroviruses; Nigeria.

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Introduction

Poliovirus (PV), the causative agent of poliomyelitis, is an enterovirus and member of the *Picornaviridae* family [1]. The virus exists in three well-defined serotypes: type 1, type 2, and type 3, which infects cells via a specific receptor known as poliovirus receptor CD155. Although only PV type 1 appears to be currently circulating, PV type 2 was last reported in 1999, and type 3 was last reported in 2012. Humans are the only reservoir of poliovirus [2]. The virus is transmitted person-to-person through fecal-oral and oral-oral routes, or less frequently through water and milk [3]. It multiplies in the intestine and spreads to the central nervous system, causing paralysis in 1 of every 200 infections [4], with a case fatality rate of 5% to 10% [5]. Because the poliovirus receptor is only expressed in the cells of humans and a few subhuman primate species, there are no known extrahuman reservoirs [6].

Following polio eradication, paralytic poliomyelitis will only occur as a result of continued use of oral polio vaccine [7]. This is because circulating vaccine-derived polioviruses (cVDPVs) commonly revert to the wild poliovirus phenotype, which increases transmissibility and high risk for paralytic disease. These cVDPVs have recently caused poliovirus infections and outbreaks of paralytic poliomyelitis in several countries [7]. Contacts between persons in communities with low polio vaccination coverage pose the potential for transmission of cVDPVs and outbreaks of paralytic poliomyelitis [7].

Polio typically occurs in outbreaks during the tropical rainy season or the temperate summer and autumn. The risk of infection is directly correlated with poor hygiene, poor sanitation, and overcrowding, typically among inadequately vaccinated populations. Young children under five years of age are at greatest risk of infection [7,8]. Apparently healthy school-aged

children are major reservoirs for all enteroviruses in most communities. Also, it has been shown that children on oral polio vaccine (OPV) may excrete these virus in their feces for several weeks [9].

Definitive diagnosis of polio is done by viral isolation. Poliovirus may be recovered from the stool or pharynx. Isolation of the virus from cerebrospinal fluid(CSF) is diagnostic but rarely accomplished. Oligonucleotide mapping or genomic sequencing is required to establish wild-like or vaccine-derived strains [10].

From 2003–2006, an outbreak of polio in northern Nigeria led to the national and international spread of the disease, eventually re-infecting 20 previously polio-free countries. Those outbreaks in Nigeria occurred because over 20% of children remain unimmunized in key polio-endemic areas in the northern part of the country [11]. Data compiled from comparable studies indicated that wild polioviruses were excreted by the majority of previously unvaccinated infants and young children for three to four weeks [12]. More than 75% of all cases of poliomyelitis occurred in the six highest-risk states in the north. These included Bauchi, Jigawa, Kaduna, Kano, Katsina, and Zamfara states [13]. In 2014, only three countries (Afghanistan, Nigeria, and Pakistan) remained polio endemic. In Nigeria, a total of six wild PV type 1 were confirmed, the most recent being in Sumaila local government area, Kano state [14]. Fortunately, Nigeria is now on the brink of being declared polio-free. Earlier in 2015, the World Health Organization (WHO) reported that Nigeria had last reported a wild poliovirus case in July 2014 and a cVDPV case in November 2014. If this status is sustained, Nigeria may be certified polio-free in the nearest future [14].

To ensure that wild poliovirus transmission has been completely interrupted, surveillance for polioviruses among apparently healthy children in the population to supplement acute flaccid paralysis (AFP) surveillance is essential. This helps to identify gaps where poliovirus transmission could occur undetected and allows for the timely detection of an outbreak in a previously polio-free area [14]. In an effort to complement the global eradication of polioviruses, we conducted this study using cell culture-based isolation and identification of polioviruses and other non-polio enteroviruses (NPEVs) in order to identify apparently healthy schoolchildren who may harbor and shed these viruses in Bauchi, Katagum, and Misau local government areas of Bauchi state, Nigeria.

Methodology

Study area

This cross-sectional prospective study aimed to identify the number of children shedding poliovirus and other non-polio enteroviruses in three local government areas of Bauchi state. These local government areas include Bauchi from the south, Katagum from the north, and Misau from the central zone. Bauchi state has been known and established to be one of the major states with frequent polio cases in Nigeria. The weather experienced in the south and north areas of Nigeria varies considerably; it is humidly hot during the early part of the rainy season in the south, and the hot and dry dusty weather lingers up in the north (900 km apart).

Study population

Two hundred fresh stool samples were collected from apparently healthy primary school children in selected schools from Bauchi, Katagumm, and Misau local government areas of Bauchi state. Forty-six samples were collected from Katagum, fifty-four from Misau, and one hundred from Bauchi. Only apparently healthy male and female children 3–12 years of age were randomly recruited for the study because this age range represents individuals who are at high risk of contracting polio. Crippled children were not selected for the study. Children udner 3 or over 12 years of age were excluded, and samples other than stool were not required for the study because of polio's mode of transmission (fecal-oral). Two children from Katagun, five from Misau, and nine from Bauchi were excluded from the study because of their ages. The samples were transported in cold chain (maintained at 4°C) within 48 hours of collection to the WHO National Polio Laboratory, University of Maiduguri Teaching Hospital, for virological analysis.

Sample size calculation

The sample size was determined using data from a prevalence study conducted in Nigeria with a prevalence of 8.8%, as demonstrated by Baba *et al.* [15]. Therefore, the minimum sample size for a sample proportion with 5% margin of error and 95% confidence level was 126.

Questionnaire

Structured questionnaires were used to obtain some sociodemograhic data of children from their parents or guardians. These included age of child, history of polio vaccination, and previous history of child's complaint of muscles and/or bone pains.

Informed consent

The purpose of this work was explained to parents of the children before they voluntarily consented to allow their children to participate in the research. The consent forms were appropriated completed by children's class teachers, after which each parent/guardian signed their corresponding forms on behalf of their children. All data were analyzed confidentially throughout the study.

Ethical approval

This study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by ethical and human research committee of Bauchi state Ministry of Health before the research was undertaken. The approval number was MOH/GEN/S/1409/I.

All sample collection, transportation, virological processing and analysis, and findings reported were based on the WHO polio laboratory manual [16].

Sample collection and storage

Two separate pea-size stool samples were individually collected 24 hours apart from primary school children 3–12 years of age. Each sample was placed in clean screw-capped universal container. It was labeled with the name, study number (laboratory number), and age of the pupil, using a water-resistant pen. The specimen was placed in a cold box below 4ºC between frozen ice packs. The name, age, and sex of the pupils were also recorded in a work book. The samples were transported to the WHO National Polio Laboratory, University of Maiduguri Teaching Hospital, in cold chain within 48 hours of collection for processing and laboratory analyses.

Laboratory analyses

Passaging cell lines, stool extraction/processing, inoculation and isolation, microneutrilization tests, and identification of virus growth on cell culture were conducted according to the procedures in the WHO polio laboratory manual [16].

Identification of virus growth in cell culture

According to the WHO laboratory manual for polio and NPEV isolation, cytopathic effects (CPEs) on recombinant murine L20B cell line (provided by the USA Centers for Disease Control and Prevention), characterized by rounding off of cells from their monolayer, is typical for polioviruses and considered negative if there are no CPEs for 10 days with one subculture after the first 5 days. Samples' CPEs in rhabdomyosarcoma (RD) cells (provided by the USA Centers for Disease Control and Prevention) were presumptive for NPEVs. Thereafter, samples containing the unknown virus were identified by microneutralization using polyclonal sera containing specific antibodies to 21 of 68 known enteroviruses. This serology kit was provided by the National Institute for Public Health and the Environment (RIVM), Netherlands [16].

Statistical analysis

Data were analyzed by descriptive statistics using SPSS, version 18 (SPSS, Chicago, USA) to determine the frequency and percentage of children with polio and other enterovirus infections. Results are presented in tabular form.

Results

A virus isolation technique was used for all the treated and extracted stool samples. A total of 200 samples were collected from 170 vaccinated and 30 unvaccinated apparently healthy primary school children from selected primary schools in Bauchi, Katagum, and Misau local government areas of Bauchi state. Of these, 110 (55%) were males and 90 (45%) were females (Table 1). Of the subjects, 170 (85%) had

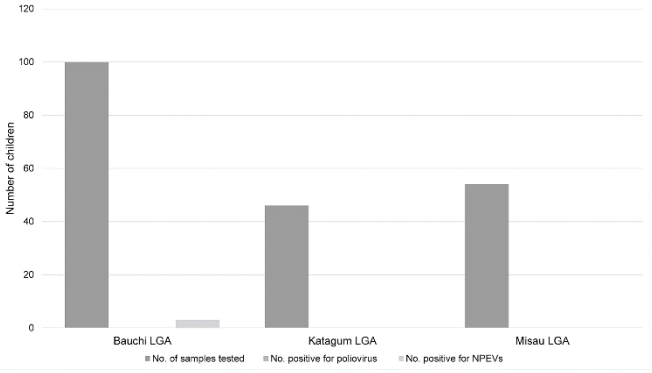
Table 1. Distribution of polio and non-polio enteroviruses among apparently healthy children across age and gender.

Biodata	No. of subjects tested (%)	No. positive for poliovirus (%)	No. positive for NPEVs (%)
Gender			
Male	110 (55)	0 (0.0)	2 (1.0)
Female	90 (45)	0 (0.0)	1 (0.5)
Total	**200 (100)**	**0 (0.0)**	**3 (1.5)**
Age (years)			
0–4	8 (4)	0 (0.0)	0 (0.0)
5–9	67 (33.5)	0 (0.0)	2 (1.0)
10–14	125 (62.5)	0 (0.0)	1 (0.5)
Total	**200 (100)**	**0 (0.0)**	**3 (1.5)**

NPEVs: non-polio enteroviruses.

been immunized with the three doses of OPV. None of the samples was positive for poliovirus, but three of the samples were found to be positive for non-polio enteroviruses (Figure 1). One sample from a male child yielded coxsackie B virus; in addition, one female and one male had untypeable non-polio enteroviruses (Figure 1). All the three positive samples were from children not immunized against OPV.

Figure 1. Polio and non-polio enteroviruses (NPEVs) isolated from the three local government areas (LGA) in Bauchi state.



Discussion

Nigeria is one of three remaining poliovirus-endemic nations. Thus, it is crucial to embark on supplementary surveys of locations previously known for poliovirus activities, especially among children, who are often susceptible to polio.

The low number of NPEVs isolated in our study was, however, lower than the 34% and 20% NPEVs isolated from acute poliomyelitis cases, both reported in India, and the 5.3% reported in Nigeria [15,17,18]. The variations observed may be attributed to factors such as differences in sample size; geographical location of the study; specificity and sensitivity of laboratory methods; stool specimen collection, handling, and transportation; stage/degree of poliovirus endemicity in the study area(s); and the time when those studies were conducted. However, all disparities that could be attributable to technical issues are not applicable to our study because all procedures, starting from specimen collection to the post-analytical phase, were handled by highly competent personnel. The implication of this low NPEV rate is that circulating wild polioviruses might be in drastically low circulation (if any) in our study area. This is evident because there have been no polio cases in these communities at the time of the present study to date [14].

Polio vaccine coverage among children within this study indicated that coverage levels with three doses of oral polio vaccine was 85%. This high vaccination coverage perhaps might have left no room for susceptible children who may help to sustain the transmission of poliovirus in the community. It is important to note that lowered immunization coverage may also have serious consequences in countries that use OPV; this was recently demonstrated by outbreaks of poliomyelitis due to cVDPVs in Nigeria [19]. However, the most important reason for the recent recognition of cVDPV-associated outbreaks is that most cVDPV outbreaks occurred when immunization programs were very incomplete, with less than 50% of children receiving three doses of OPV. In such situations, there are enough susceptible children to sustain widespread infection, leading to outbreaks of paralytic polio [19]. Due to the low frequency of isolates from this study, we cannot categorically establish gender and age predilections of NPEVs isolated from our subjects.

As far as contemporary epidemiology of NPEVs is concerned, many authorities consider them unimportant as human pathogens because most apparently healthy children and recipients of OPV could excrete NPEVs in their feces [20]. What is of great public health significance is that no poliovirus was isolated from children in our study area.

Conclusions

At this point, we may assert that there has been remarkable progress in the move to eradicate polio in Bauchi, Misau, and Katagum local government areas of Bauchi state, Nigeria. This may be attributed to the maximum cooperation of parents/guardians in fully vaccinating their children with the three required doses of OPV. The remaining few unvaccinated children might have gotten herd immunity from their classmates when they played and mingled together. Since enteroviruses are fecal-oral transmitted pathogens, personal hygiene and sanitary household and school conditions should be encouraged. Hence, schoolchildren, especially in rural communities, need to be educated on frequent handwashing practice and discouraged from indiscriminate defecation. Nonetheless, more intensive and periodic surveillance is required to confirm the presence or exclude the absence of polioviruses in these communities and other parts Nigeria.

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