Prevalence of non-polio enteroviruses infections among children in Northern Nigeria

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Summary

Background: Human non-polio enteroviruses (NPEVs) have been associated with certain life-threatening disorders in children. However, there is paucity of NPEV infection data in most developing countries. This study determined the 3-year prevalence of non-polio enteroviruses (NPEVs) among children in some Northern States of Nigeria.

Materials and Methods: Duplicate stool samples were collected from 27778 children ≤15 years. These samples were processed and analyzed for characteristic NPEVs cytopathic effects (CPE) on L20B and RD cell lines. Tests were considered positive if the duplicate samples produced distinct CPE on both cell lines.

Results: Of the 27778 samples processed, 3991 (14.4%) NPEVs were isolated. Participants of the male gender (14.5%) within the age range of 0-5 years (14.7%) from Yobe state (15.3%) whose samples were received in the month of June (22.2%) and in the year 2015 (18.8%) had the highest prevalence of NPEVs. June had significant risk factors of NPEVs ($p<0.001$, OR=1.95 [95%CI: 1.60-2.34]). However, there was no significant association between age, sex and location of sample collection with the prevalence of NPEV ($p>0.05$)

Conclusions: This study revealed a relatively high prevalence of NPEVs among the study population. This calls for the need for government implementation of consistently improved water, food and environmental hygiene.

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Introduction

Non-polio enteroviruses (NPEVs), which belongs to the *Picornaviridae* family with four out of fifteen species and more than a hundred immunologically unique serotypes that most frequently infect humans and cause a broad of spectrum of mild or asymptomatic disorders (1, 2). However, NPEVs have been implicated to cause acute flaccid paralysis (AFP) which is similar to that caused by polio. In addition, certain strains of enteroviruses have been implicated in myocarditis, hand-foot-and-mouth disease, type-I diabetes mellitus and encephalitis (3).

The transmission of NPEVs are facilitated through the fecal-oral routes and its isolation can be done using stool and respiratory samples. High risk individuals are mainly children that reside in crowded accommodations and who are faced with challenges of their low immunity, increased exposure and poor hygiene in such situations (2, 4).

Scientific evidence has revealed that these high-risk asymptomatic individuals play a vital role in the circulation of these pathogens within their various communities (4). The isolation and identification of NPEV isolates from these high-risk group is done using the cell culture and microneutralization assay which has a high sensitivity and specificity for detecting functional strain-specific neutralizing antibodies to NPEVs.

Since October 2016, Nigeria has not recorded any case of wild poliovirus (WPV) following the massive campaigns on oral poliovirus vaccine (OPV) which intensified during August 2016 till December 2017 (5). However, evidence exist which suggests that WPV as well as NPEV can impose resistance against immunity (6), replicate asymptomatically (7) and can be transmitted from a well-

immunized individual to susceptible persons, thus establishing a continuous trend of person-to-person transmission (8, 9). Hence, the need to determine the prevalence of NPEVs among children in some Northern States in Nigeria.

This study is aimed at determining the prevalence of NPEVs among children aged 15 years and younger within a 3 years period in 10 selected Northern states of Nigeria. Information generated from this study will guide on the urgent need for better water supply, food and environmental hygiene.

Materials and Methods

Study area

Two hundred and thirty-four local government areas across ten Northeastern states of Nigeria were selected for the study. The states include Adamawa, Bauchi, Borno, Gombe, Jigawa, Katsina, Kano, Plateau, Taraba, and Yobe. The study was conducted from January, 2015 to December, 2017. The locations were selected based on overcrowding and poor sanitation.

Ethical issues

Ethical approval was received from the Ethical Committee of the Ethical Research Committee of University of Maiduguri Teaching Hospital, Maiduguri, Nigeria. Informed consent and accent were sought from the parents and children, respectively before the study was commenced. All ethical considerations based on confidentiality in anonymizing participant identity as well as data securing and ensuring no harm either on the part of the participant or the investigators were adhered to.

Study population

Twenty-seven thousand, seven hundred and seventy-eight (27778) children aged 15 years and below were enrolled in this study. Fecal samples were collected from symptomatic children between January, 2015 to December, 2017. A symptomatic child refers to a child whose medical history as well as physical examination present with sign and symptoms that may be related to enteroviral infections including diarrhea, pyrexia, cough, cold, and conjunctivitis. Most of the study participants received the complete vaccination for routine vaccination as confirmed by their parents/guardians during their informed consent for inclusion into the study. The overall number of participants vaccinated with OPV was not known since the vaccination cards were not presented to reveal the doses given during the national immunization day. Stool samples were collected from every participant with demographic data including age, gender, geographical location and immunization history. Since the virus shedding may be intermittent, the isolation rate was increased by collecting two samples (24-48 hours) apart.

Sample collection, processing and transportation

A fecal specimen size of about two adult thumbnails (*i.e.* 4 to 8g) was collected in a dry clean, leak proof universal container with a screw cap with labels, absorbent material, a specimen referral form and zip lock plastic bags provided by World Health Organization. These samples were collected and sent at 4 ^{0}C using jab low or vaccine carrier box containing ice packs to the laboratory by designated Surveillance Officers to the WHO National Polio Reference Laboratory, University of Maiduguri Teaching Hospital, Maiduguri, Borno State-Nigeria for storage at -20°C until the period for analysis.

Viral isolation

Stool specimens received at the WHO National Polio Reference Laboratory, University of Maiduguri Teaching Hospital, were analyzed according to WHO Polio laboratory manual (9). Four to eight grams of the sample was briefly treated in phosphate buffered saline with 10% chloroform and inoculated on Rhabdomyosarcoma (RD) cell line and L20B derived from mouse cell line (L-cells) genetically engineered to express the human poliovirus receptor CD155. These monolayer cell lines were seeded in culture tubes with 10% fetal calf serum (FCS) and switched to maintenance medium prior to inoculation. Cultures were inoculated at 37°C and observed on a daily basis for cytopathic effect (*i.e.* rounded, refractile cells detaching from the surface of the tube). Positive cultures were harvested and stored at -20°C, while negative cultures were observed for five days and re-passaged on a new monolayer. Tests were considered positive if the duplicate samples produced distinct CPE on both cell lines.

Data analysis

Epi-Info 6.04d was used to compute the prevalence rate of NPEV and to compare these rates on the basis of age and gender, year of sample receipt, month of sample receipt, and State of sample receipt.

Statistical analysis

Data obtained from the study was presented in tabular forms, pie chart and the percentage of positive samples in relation to site of sample collection. Multivariate Logistic regression was used to determine the risk factors (Odd ratios) of NPEVs. Data were analyzed using Statistical Package for Social Sciences (SPSS) version 26 (California Inc., USA). P values less and/or equal to 0.05 at 95% confidence interval were considered statistically significant.

Results

Demographic features

Fecal specimens were collected from a total of twenty-seven thousand, seven hundred and seventy-eight (27778) children aged ≤15 years to determine the prevalence of human non-polio enteroviruses (NPEVs) in 10 selected Northeastern states of Nigeria. A total of 15, 236/27, 778 (54.8%) of the participants were female and their mean age was 9.5±2.8 years, while a total of 12, 542/27, 778 (45.2%) of the participants were male (Table 2). Out of the total

Table 1. Prevalence of NPEV based on year of sample collection.

Year of sample collection	No. tested	No. of NPEV positive (%)	No. of NPEV negative	OR (95% CI)	p value
2015	48	9 (18.8)	39	1.41 (0.68-2.93)	0.351
2016	19655	2849 (14.5)	16806	1.04 (0.96-1.11)	0.317
2017	8075	1133 (14.0)	6942	Referent	-

Significance determined by Multivariate Logistic regression.

number of participants, the youngest participants aged five years and below [24, 197/27, 778 (87.1%)] were the largest of individuals recruited for the study, while the oldest participants aged between eleven to fifteen years [1, 033/27, 778 (3.7%)] were the least of individuals involved in the study (Table 2).

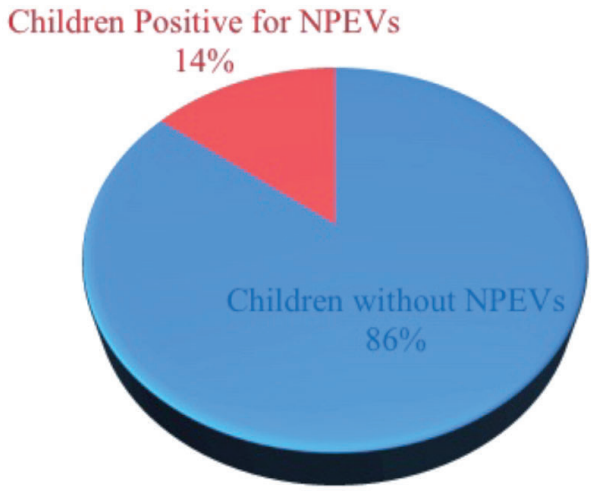

Figure 1. Prevalence of NPEVs among children in Northern Nigeria.

Virologic detection of NPEVs by cell culture

Two stool samples were collected from each participant. Of the 27, 778 samples collected, three thousand nine hundred and ninety-one (14.4%) were positive for non-polio enteroviruses (Figure 1), while twenty-three thousand, seven hundred and eighty-seven (85.6%) were negative for non-polio enteroviruses, from their samples inoculated on Rhabdomyosarcoma (RD) and L20B cell lines. Male participates had a slightly higher NPEV prevalence [2, 213/15, 236 (14.5%)] compared to their female counterparts [1778/12542 (14.2%)] (Table 2). There was a higher isolation rate among the youngest participants [3, 546/24, 197 (14.7%)] aged five years and below compared to their older counterparts [304/2, 548 (11.9%)] aged between six to ten years (Table 2).

Samples received in 2015 and 2017 had the highest [9/48 (18.8%)] and lowest [1, 133/8, 075 (14.0%)] prevalence respectively (Table 1). Samples obtained in September and March had the highest [371/1, 874 (19.8%)] and lowest [413/4, 333 (9.5%)] prevalence rates respectively (Table 3), while samples received from Jigawa and Bauchi had the highest [368/2, 462 (14.9%)] and lowest [259/2, 004 (12.9%)] prevalence rates respectively (Table 4).

Month of June was significant risk factor of NPEVs ($p<0.001$, OR=1.95 [95%CI: 1.60-2.34]) (Table 3). However, there was no significant association between age, gender and NPEV prevalence ($p>0.05$) (Table 2), and but there is significant association between location of sample collection in Bauchi and Gombe and NPEV prevalence ($p<0.05$) (Table 4).

Table 2. Prevalence of non-polio enterovirus (NPEV) by age and sex.

	No. Tested	No. of NPEV positive (%)	No. of NPEV negative	OR (95% CI)	p value
Age group (years)					
0 – 5	24197	3546 (14.7)	20651	1.10 (0.91-1.32)	0.371
6 – 10	2548	304 (11.9)	2244	0.86 (0.69-1.06)	0.158
11 – 15	1033	141 (13.6)	892	Referent	-
Sex					
Male	15236	2213 (14.5)	13023	1.02 (0.96-1.1)	0.410
Female	12542	1778 (14.2)	10764	Referent	-

Significance determined by Multivariate Logistic regression.

Table 3. Prevalence of non-polio enterovirus (NPEV) based on month.

Month of sample receipt	No. tested	No. of NPEV positive (%)	No. of NPEV negative	OR (95% CI)	p value
January	2166	265 (12.2)	1901	0.95 (0.77-1.17)	0.639
February	2633	254 (9.6)	2379	0.73 (0.59-0.89)	0.003*
March	4333	413 (9.5)	3920	0.72 (0.59-0.87)	0.0008*
April	3717	467 (12.6)	3250	0.96 (0.79-1.16)	0.671
May	3712	629 (16.9)	3083	1.39 (1.16-1.67)	0.0005*
June	1771	394 (22.2)	1377	1.95 (1.60-2.34)	0.0001*
July	1642	244 (14.9)	1398	1.19 (0.96-1.47)	0.108
August	1871	272 (14.5)	1599	1.16 (0.94-1.42)	0.161
September	1874	371 (19.8)	1503	1.68 (1.38-2.06)	0.0001*
October	1616	245 (15.2)	1371	1.2 (0.99-1.51)	0.07
November	1392	273 (19.6)	1119	1.67 (1.34-2.05)	0.0001*
December	1051	164 (15.6)	1119	Referent	-

*Significance determined by Multivariate Logistic regression.

Discussion

Although NPEVs are common in children, their clinical importance in Nigeria has not been properly documented. A total of 3, 991 out of 27, 778 samples tested was observed to grow on the cells at an NPEV rate of 14.4%. Similar results were obtained in Egypt and Ghana were NPEV rate ranged from 17.6% to 24.2% with no isolation of polio from 1000 and 273 apparently healthy participants (10, 11). The high prevalence rate as observed in this study could be due to the sensitivity of RD cell line used (11), their exposure, hygiene, and immunity status of the participants.

Gender distribution of NPEV in males [2, 213/15, 236 (14.5%)] compared to their female counterparts [1, 778/12, 542 (14.2%)] are in line with studies in India (12) and Ghana (11). These studies suggest that biological explanations are responsible for the high prevalence rate among males which include longer period of virous excretion and elevated titer of the virus in feces of males.

Virus previous studies have observed that age is one of the vital determinants of enteroviral infection cases, with dissimilar age groups having different degrees of susceptibilities to infection (13). In this study, the prevalence rate among the various age groups reveals that the youngest participants within the age range 0 to 5 years have the highest isolation rate which conforms with the study in Ghana (11) and India (12) with higher isolation rates in their respective participant age ranges of 2-5 years and 0-2 years respectively. Young children have developing immunity against each of the numerous circulating Human Enteroviruses (HEV) types and are thus highly susceptible to NPEV infections (14). This is in agreement with the study reported by Nijhuis *et al* (15), that children younger than 5 years are more susceptible because of poor hygiene habits and lack of prior immunity.

The variation in NPEVs distribution is observed in different geographical locations. Some serotypes could be endemic, with small gradual change in the range of NPEV serotypes present from one year to another. The Prevalence of Non-Polio enterovirus based on Year of Sample Receipt revealed that, the year 2015, had the highest prevalence rate of 18.8% for non-polio enteroviruses which depreciated in successive years. This could suggest a gradual improvement in reducing the transmission of enterovirus infection by good hygiene, a possible communal decongestion (11) as these factors are important preventive measures (16).

In temperate climates, there is reported increased circulation in summer to early fall (*i.e.* June to September) (11). This study also revealed that the month of June, had the highest prevalence rate of (22.2%), followed by the month of September (19.8%). Echovirus has been reported to be responsible for summer respiratory infections in children, and this pathogen has a higher prevalence during the summer and autumn months (17). Similarly, Laxmivandana *et al* (13), reported in their findings on characterization of NPEVs Infections associated with acute flaccid paralysis in South-Western India, that, NPEV positive AFP cases were detected throughout the year, significantly more number of cases were found during April–June (summer months) (146/564; 25.9%) than in July–September (monsoon months) (122/638; 19.1%, p<0.005), October–December (post-monsoon months) (99/510; 19.4%, p<0.02) and January–March (winter months) (55/474; 12%, p<0.0000001). Further, the winter months showed significantly lower NPEV positivity than monsoon/post-monsoon months (p<0.0008).

Although Northeastern Nigeria has historically been at a high risk of the transmission of polio (8) reports from Bassey and colleagues (18) revealed the least NPEV of 20.6% in Yobe state with 215 AFP cases compared to Adamawa state with 338 AFP and the highest NPEV of 22.2%. This outcome contrasts with reports from this study which revealed the highest prevalence rate of NPEV in Yobe State (15.3%). These results indicated that there is high prevalence rate of non-polio enteroviruses in Yobe State (15.3%), which showed a wide spectrum of viruses' virulence compared to other states. These might be attributed to poor standard of hygiene, unsafe drinking water, defecation in the open field, because the viruses are shed in the feces, and the environmental condition of the concerned State and communities (11, 19).

The finding in this study could be limited by the use of two cell lines to detect NPEVs. A number of studies have recommended the use of MRC5 to produce the best outcome [20]. However, our laboratory chose to use Rhabdomyosarcoma (RD) and L20B cell lines due to the recommendation by the WHO as the ideal cell culture protocol for isolation of NPEVs and Polioviruses.

Conclusions

This study was able to establish baseline cases of NPEV with an overall prevalence of 14.4% among apparently healthy and unhealthy children aged 15 years and below in 10 selected Northeastern states of Nigeria. This high prevalence of NPEV among healthy and unhealthy participants indicates a continuous fecal-oral transmission. This calls for the need for government implementation of consistently improved water, food and environmental hygiene.

Table 4. Prevalence of non-polio enterovirus (NPEV) based on location of sample collection.

State of sample collection	No. tested	No. of NPEV positive (%)	No. of NPEV negative	OR (95% CI)	p value
Adamawa	3697	541 (14.6)	3156	0.95 (0.83-1.08)	0.428
Bauchi	2004	259 (12.9)	1745	0.82 (0.69-0.97)	0.017*
Borno	4834	688 (14.2)	4146	0.92 (0.81-1.04)	0.183
Gombe	1380	182 (14.2)	1198	0.84 (0.70-1.02)	0.007*
Jigawa	2462	368 (14.9)	2094	0.97 (0.84-1.13)	0.696
Kano	5103	752 (14.7)	4351	0.95 (0.84-1.08)	0.472
Katsina	2846	408 (14.3)	2438	0.92 (0.80-1.07)	0.287
Plateau	1358	180 (13.3)	1178	0.84 (0.70-1.01)	0.07
Taraba	1106	155 (14.0)	951	0.90 (0.74-1.09)	0.296
Yobe	2988	458 (15.3)	2530	Referent	-

*Significance determined by Multivariate Logistic regression.

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