# Brief Original Article

# Poliovirus and other enteroviruses in children infected with intestinal parasites in Nigeria

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#### Abstract

Introduction: Poliovirus, an enterovirus, still persists in Nigeria despite the global efforts tailored towards its eradication. This study aimed to assess the impacts of poliovirus and other enteroviruses on the susceptibility of individuals to intestinal parasite infections.

Methodology: A cross-sectional study on the prevalence of intestinal parasites was conducted on two-sample stool specimens of 717 Nigerian children (between 1 and 19 years of age) whose poliovirus/other enteroviruses infection status had been determined.

Results: The overall prevalence of Sabin poliovirus and other related enteroviruses infections were 6.6% and 13.8%, respectively. The prevalence of *Ascaris lumbricoides* was significantly higher than that of other intestinal parasites (p < 0.05), with children in the 0–4 year age group being the most predisposed age group to intestinal parasitic infection (OR = 11.7, CI = 9.2–15.0). While the prevalence of all species of parasites except *S. mansoni* showed no significant variations in children with Sabin poliovirus (p > 0.05), the prevalence of hookworms and *Taenia* spp. was significantly higher in children with other enteroviral infections (p < 0.05).

Conclusions: The high risk of children of acquiring enteroviral infection through some intestinal parasites is an indication of possible association of the parasites in a more poliovirus-endemic population. A combined intervention approach for the two infections is advocated.

Key words: enteroviruses; poliovirus; intestinal parasites; susceptibility; children.

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#### Introduction

Intestinal parasitic infections are one of the most commonly distributed infections worldwide. The World Health Organization (WHO) estimated that around 3.5 billion people have experienced infections, with 450 million suffering from one or more illnesses associated with infections [1]. Morbidities resulting from infection by the causal parasites often include iron deficiency anemia, growth retardation, and physical and mental health impairment, the effects of which are more pronounced in children [2].

The factors of epidemiological importance in intestinal parasite transmission include sociodemographic variables associated with poverty such as reduced access to adequate sanitation, potable water, and healthcare, as well as the prevailing climatic and environmental conditions [3]. These, to a large extent, could also contribute to the spread of poliovirus, the causative agent of poliomyelitis.

Poliomyelitis, a disease characterized by acute flaccid paralysis of limbs, begins when the virus is ingested and multiplies in the intestinal and oropharyngeal mucosa [4]. Polio still remains a great public health problem in developing countries where sanitary conditions are poor [5], a factor that has been reported to influence the transmission of intestinal parasites. Nigeria alongside two other countries (Pakistan and Afghanistan) is the only country in the world that still suffers from the wild-type polio virus due to uninterrupted transmission [6]. In 2011, Nigeria was the global epicenter of poliovirus outbreaks, astonishing those who commended its success during 2010, when case numbers fell by 95% [7].

There have been several reports on intestinal parasites and viral co-infections with more emphasis on human immunodeficiency virus (HIV) and, to a lesser extent, hepatitis viruses (HBV and HCV) [8-10]. Although there have been no published reports on the co-infection patterns in poliovirus and intestinal parasites, the overlap in their geographical distributions is a strong indication of their possible coexistence. The involvement of the lower extremities in poliovirus infection in children may also increase the risk of exposure to soil-transmitted infections, since the vulnerable groups will likely have to crawl in the highly polluted environment in low-resource communities. This study aimed to assess the cooccurrence of poliovirus/other enteroviruses and various intestinal parasites with a view to determining the possible link between them.

# Methodology

#### Data source

This descriptive, cross-sectional study made use of total of 1,434 preserved stool samples (collected from 717 children) submitted between the months of September 2012 and January 2013 for poliovirus examination at the University College Hospital, Virology Department (Polio Laboratory), Ibadan, Nigeria. These samples were submitted by the poliomyelitis disease surveillance and notification officers (DSNOs) in Kaduna, Sokoto, Zamfara, Niger, Kwara, Benue, Federal Capital Territory (FCT), Abia, Delta, Kebbi, Kogi, and Nasarawa states to the WHO National Polio Laboratory, University College Hospital, Ibadan, Nigeria, where they were stored at -20°C until processed and subjected to examination. Two independent parasitologists examined all stool samples for the presence of intestinal parasites and were blinded for the virological status of specimens.

# Isolation and identification of poliovirus and other enteroviruses

The fecal samples were processed according to protocols for virus isolation standard and characterization as described by the WHO [11-13]. Briefly, a healthy monolayer of L20B (mouse L cells expressing the human CD155 poliovirus receptor [PVR]) and human rhabdomyosarcoma (RD) cell lines maintained in Eagle's minimum essential medium (MEM) supplemented with 2% fetal calf serum (FCS) were seeded at 105 cells per tube 48 hours prior use. The inoculated monolayers were observed daily for characteristic enterovirus cytopathic effects (CPE), and tubes with CPE  $\geq$  75% were harvested and stored at -20°C. These were then passaged in a fresh monolayer of the second cell lines in order to increase the titer. Re-passaging was repeated on L20B cell line if infected cells were seen negative following a fiveday incubation. A negative result was confirmed after another five-day incubation period. The tubes positive for poliovirus-specific cytopathic effects were freezethawed three times, spun at 4°C, and the supernatants were aliquoted and kept frozen at -20°C as poliovirus isolates. The virus was titrated and its titer value was calculated.

## Intratypic differentiation of the poliovirus

Characterization of isolates was achieved by reverse transcriptase polymerase chain reaction (RT-PCR) protocols using enterovirus-specific and poliovirus group-, serotype-, and Sabin strain-specific primer sets and enzyme-linked immunosorbent assays using highly specific cross-absorbed hyperimmune rabbit sera [11,14,15].

# Parasitological examination

Stool samples collected from children between 1 and 19 years of age  $(3.47 \pm 2.61 \text{ years})$  whose status poliovirus/other enterovirus had been determined by standard methods [11-13] were examined using direct wet smear and formol-ether concentration technique to identify parasitic ova and cysts as described by Martinez [16] and Cheesbrough [17]. Briefly, 2 g of the preserved stool was emulsified in 7 mL of 10% formalin in a centrifuge tube, mixed, and strained using a wire sieve. The filtrate was then poured into a test tube to which 3 mL of ether was added and mixed for 15 seconds. The formol-ether suspension was centrifuged at 1,500 g for one minute. The fatty plug was loosened using an applicator stick and the centrifuge tube quickly inverted to discard the supernatant, allowing only a few drops of the sediment to remain, which was well mixed, and a drop of it was placed on a clean glass slide. A drop of Lugols iodine was added to clarify cysts present and examined under a cover slip at x40 objective of the light microscope.

The study protocol was reviewed and approved by the joint ethical review committee of the University College Hospital/University of Ibadan, Nigeria, and was conducted in compliance with approved ethical guidelines of the committee. Permission to carry out the study was also obtained from the Director and Staff of Department of Virology, University College Hospital, Ibadan, Nigeria.

# Data analysis

The data were keyed into Microsoft Excel and exported to the Statistical Package for Social Sciences (SPSS) version 21.0 for further analysis. The statistical significance of differences in prevalence of infections by age and sex was determined via Chi-square analysis. Multivariate logistic regression analysis was used to predict the extent of association between disease occurrence and the age and sex of the children. P < 0.05 was considered statistically significant.

#### Results

The overall prevalence of Sabin-type poliovirus and other related enteroviruses were 6.6% and 13.8%, respectively. Only one stool sample was positive for wild-type poliovirus. The prevalence of poliovirus was neither age nor sex dependent (p > 0.05) (Table 1). The prevalence of *Ascaris lumbricoides* infection was significantly higher than that of any other intestinal parasites (p < 0.05). Children in the 0–4 year age group were most predisposed to intestinal parasitic infections (OR = 11.7, CI = 9.2–15.0) (Table 2). Although there were no associations between parasitic infection status and age/sex of the children (p > 0.05), the risk of infection was higher in the male subjects (OR = 1.2, CI = 1.0–1.5) than in the female subjects (OR = 0.8, CI = 0.7–1.0) (Table 2). The prevalence of co-infections of *A. lumbricoides* with other intestinal parasites varied, with *Entamoeba histolytica* and *Taenia* spp. showing the highest (1.7%) and the lowest (0.1%) prevalence, respectively. While the prevalence of all species of parasites (except *S. mansoni*) showed no significant variations in children with Sabin poliovirus (p > 0.05), the prevalence of hookworms and *Taenia* spp. was significantly higher in children with other enteroviral infections (p < 0.05) (Table 3).

Table 1. Age- and sex-related	prevalence of poliovirus	s and other enterovirus infection	patterns among Nigerian children

Variable		No. examined		OR (95% CI)	P value		
		-	Polio negative examined (%)	Sabin polio positive (%)	Other enteroviruses positive (%)	-	
Age (years)	0–4	527	425 (80.6)	36 (6.8)	66 (12.5)	0.8 (0.5–1.2)	0.749
	5–9	156	118 (75.6)	10 (6.4)	28 (17.9)	1.3(0.9-2.0)	
	10-14	32	26 (81.3)	1 (3.1)	5 (15.6)	0.9 (0.4–2.2)	
	$\geq 15$	2	2 (100.0)	0 (0.0)	0 (0.0)	0.0	
Sex	Male	387	307 (79.3)	27 (7.0)	53 (13.7)	1.0 (0.7–1.5)	0.961
	Female	330	264 (80.0)	20 (6.1)	46 (13.9)	1.0 (0.7–1.4)	
	Overall	717	571 (73.5)	47 (6.6)	99 (13.8)		

Note<sup>\*</sup> Odd ratio OR: compared risk of Sabin poliovirus/other enterovirus predisposition by age and sex

Variable		No. examined	No. infected (Prevalence)						OR (95% CI)	P value		
			Al	Hw	Tt	Ss	Ts	Sm	Eh	Ec		
Age (years)	0–4	555	529 (95.3)	2 (0.4)	2 (0.4)	3 (0.5)	1 (0.2)	2 (0.4)	12(2.2)	1 (0.2)	11.7 (9.2–15.0)	0.147
0 /	5-9	130	129 (99.2)	1 (0.8)	0 (0.0)	0 (0.0)	0 (0.0)	2 (1.5)	2 (1.5)	0 (0.0)	0.05 (0.04-0.06)	
	10-14	30	29 (99.2)	0 (0.0)	0 (0.0)	1 (3.3)	0 (0.0)	0 (0.0)	1 (3.3)	0 (0.0)	0.002 (0.001- 0.003)	
	≥15	2	2 (100.0)	0 (0.0)	0 (0.0)	1 (50.0)	0 (0.0)	1 (50.0)	1 (50.0)	0 (0.0)	0 (0-0.0001)	
Sex	Male	377	364 (96.6)	2 (0.5)	0 (0.0)	1 (0.3)	0 (0.0)	4 (1.1)	9 (2.4)	0 (0.0)	1.2 (1.0–1.5)	0.877
	Female	340	324 (95.3)	1 (0.3)	2 (0.6)	3 (0.9)	1 (0.3)	1 (0.3)	6 (1.8)	1 (0.3)	0.8 (0.7-1.0)	
	Overall	717	688 (96.0)	3 (0.4)	2 (0.3)	4 (0.6)	1 (0.1)	5 (0.6)	15 (2.1)	1 (0.1)		

Al: Ascaris lumbricoides; Hw: hookworm; Tt: Trichuris trichiura; Ss: Strongyloides stercoralis; Ts: Taenia species; Sm: Schistosoma mansoni; Eh: Entamoeba histolytica; Ec: Entamoeba coli. Odds ratio (OR) compared risk of intestinal parasite predisposition by age and sex.

Intestinal parasites —	Polio negative (n = 571)	Sabin polio (n = 47)	Other enteroviruses (n = 99)	P value
	Examined (%)	No. infected (%)	No. infected (%)	
A. lumbricoides	531 (93.0)	46 (97.9)	96 (97.0)	0.055
S. mansoni	1 (0.2)	1 (2.1)	2 (2.0)	0.007
E. histolytica	10 (1.8)	1 (2.1)	3 (3.0)	0.441
S. stercoralis	3 (0.5)	0 (0.0)	1 (1.0)	0.563
Hookworm	4 (0.7)	1 (2.1)	4 (4.0)	0.008
<i>Taenia</i> spp.	4 (0.7)	0 (0.0)	3 (3.0)	0.035
T. trichuria	1 (0.2)	0 (0.0)	0 (0.0)	0.613
E. coli	3 (0.5)	0 (0.0)	0 (0.0)	0.380

### Discussion

This study shows the persistence of oral poliovirus vaccine (OPV)-like viruses (Sabin-like polio virus strain) in Nigeria with a prevalence as high as 6.6% in the child population. Although OPV is capable of inducing immunity in unvaccinated children, thus promoting herd immunity [18], its low coverage along with poor personal hygiene, overcrowding, tropical conditions, and prior eradication of the corresponding serotype of wild poliovirus are said to be the main risk factors for circulating vaccine-derived poliovirus (cVDPV) emergence [19,20]. Several studies have shown the occurrence of vaccine-associated paralytic poliomyelitis (VAPP) among OPV recipients who excreted vaccine-derived poliovirus [21-23]. In Nigeria, there have also been several reports of acute flaccid paralysis cases associated with an outbreak of cVDPV, mostly in northern and central parts of Nigeria [22,24]. The prevalence of wild-type poliovirus in this study is negligible; it was isolated in only one sample, and was thus excluded from the overall analysis. The approximately 99% vaccination status of the children could have been responsible for the very low number of cases of wild-type poliovirus. The lack of association between enterovirus infection and sex in the present study is similar to that in an earlier report in Nigeria [25], signifying equal predisposition of the two sexes to enteroviral infection.

The prevalence of *A. lumbricoides* in this study was very high. There have been inconsistent reports on the degree of transmission dynamic of *A. lumbricoides* compared with other intestinal parasites. While many reports favored a higher prevalence of *A. lumbricoides* [26-28], some reported higher prevalence of parasites such as hookworms and *Trichuris trichiura* [29,30]. The high prevalence in *A. lumbricoides* could have resulted from its easy route of human contamination coupled with the parasite eggs' (which bear the infective stage) ability to survive in both favorable and adverse environmental conditions. The latter feature of *A. lumbricoides* could also be the reason for the success of *Entamoeba histolytica* being the secondmost prevalent parasite in this study.

The prevalence pattern of intestinal parasites in relation to age and sex of the children is similar to observations in enterovirus infection patterns, with younger children being at higher risk and a lack of association with sex. These observations further confirm the strong overlap in the transmission dynamic of the two infections. It is known that *A. lumbricoides* infections are rarely found alone in human communities [31], hence the coexistence of *A.* 

*lumbricoides* with other intestinal parasites like *E. histolytica*, which had the highest co-infection rate in this study.

Several studies have observed the effects of viral infections on susceptibility to parasitic infections or vice versa. Studies have been conducted on HIV/HBV and parasites such as *Taenia crassiceps* [32], Strongyloides stercoralis [33], Cryptosporidium spp. [34], and Schistosoma japonicum [10]. The major cause of susceptibility has been attributed to impairment of cell-mediated immunity often initiated by the viral agent [9,32]. Even though the occurrence of Sabin poliovirus seemed not to be associated with most of the intestinal parasite species in the present study (except S. mansoni), the parasite species association observed with other enteroviruses is of public health importance. Since poliovirus belongs to the enterovirus group, it is naturally expected that they might share similar course in their pathogenesis. It is therefore not surprising that other enteroviruses have been incriminated in acute flaccid paralysis, a common morbidity characterized with poliovirus [35]. In the presence of more poliovirus cases, more possible associations other than with S. mansoni could be predicted with other intestinal parasites. In addition, the species-specific association of enteroviruses with Taenia species and hookworms may also play a role. These species of intestinal parasites seem to inflict greater damage to the intestinal caecum, thus providing easy route for enterovirus establishment and proliferation. However, this needs further investigation.

# Conclusions

This study has reported the persistence of Sabinlike poliovirus and other enteroviruses in Nigeria, with higher risk found in the younger population. The higher prevalence of Sabin-like poliovirus and enteroviruses in children infected with S. mansoni and hookworms/Taenia spp., respectively, could pose some public health threats. However, the public health implications of concomitant occurrence of enteroviruses intestinal and parasites remain suggestive, owning to the low prevalence of enterovirus-associated parasites reported in this study. Further epidemiological studies in this regard are therefore recommended. Concerted efforts targeted at eradicating poliovirus- or other enterovirus-prone populations should integrate campaigns against intestinal parasites because of the foreseeable synergistic effects of these parasites. Therefore, a mass deworming program may complement a communitybased poliovirus vaccination program. Public enlightenment and education on the importance of prompt vaccination and hygienic living will further strengthen positive outcomes.

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