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Vaccine Associated Paralytic Poliomyelitis Cases From Children Presenting With Acute Flaccid Paralysis in Uganda

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A retrospective study to identify VAPP cases from the entire Uganda was conducted between January 2003 and December 2011. Eleven of the 106 AFP cases were VAPPs. The VAPP rate ranged from 0 to 3.39 cases per 1,000,000 birth cohorts and the peak was in 2009 when there was scaling up of OPV immunization activities following an importation of wild poliovirus in the country. All the subsequent polio suspect cases since then have been vaccine-associated polio cases. Our data support the strategy to withdraw OPV and introduce IPV progressively in order to mitigate against the paralysis arising from Sabin polioviruses. ***J. Med. Virol. 87:2163–2167, 2015***.

KEY WORDS: OPV; adverse event; Uganda

INTRODUCTION

Poliomyelitis is caused by *Poliovirus* 1, 2, and 3 which belong to the family *Picornaviridae* and the genus *Enterovirus*. Paralytic polio usually affects one lower limb and presents with hypotonia, reduced reflexes, wasting of muscles but with no loss of sensorium. Plans to eradicate poliomyelitis are intensively on-going and these are being achieved by the use of both oral polio vaccine (OPV) as well as the inactivated polio vaccine (IPV). The viral strains in OPV (Sabins) are unstable (Agol, 2006) for example Sabin poliovirus 1 harbors an attenuating mutation of A480G in the 5′UTR whereas that of Sabin *Poliovirus* 2 and 3 are at the G481A and C472U sites respectively [Kew et al., 2004; Kew, 2009]. The mutations at these specific sites are not infrequent. They are associated rarely with increased neurovirulence and only infrequently cause AFP. This underrates their importance.

Vaccine associated paralytic poliomyelitis (VAPP) and vaccine derived *Polioviruses* (VDPVs) cases have been described [Shulman et al., 2006; Kapusinszky B et al., 2010; Diop et al., 2014]. VDPVs are OPV mutants that are genetically divergent and may have reverted to increased neurovirulence. VAPPs are categories of poliomyelitis that are identified by clinical signs, history of recent exposure to OPV, and virological investigations. The VAPP cases arise rarely from the genetic instability within the 5′UTR of the Sabin *Poliovirus*. They mostly occur after the first dose of OPV and are more prevalent in children with primary immune-deficiencies (Kim et al., 2007; Shahmahmoodi et al., 2010). VAPP is a rare event and from these rare cases VAPP type 2 and 3 are most frequent (Pliaka et al., 2010). In Latin America the estimated overall risk of VAPP was one case per 1.19 million newborns (Landaverde et al., 2014) and in Russia it was one case per 2.2 million doses of OPV recipients (Ivanova et al., 2007). The risk of VAPP following the first OPV dose in India and USA was reported at one case per 2.8 million children and one case per 1.4 million children respectively (WHO Global Vaccine safety, 2014).

The African Region has embarked on massive immunization campaigns with multiple rounds of OPV in supplementary immunization activities in pursuit of the polio eradication agenda. Incidentally this scenario coupled with variable rates of

Grant sponsor: VAPP cases were identified from the on-going WHO AFP surveillance system in the country.

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population immunity, makes for the adverse effects of OPV virus strains mutations to be of major concern. The objective for this study was to survey for the cases of VAPP in Uganda.

MATERIALS AND METHODS

An AFP case was defined as any case of acute-onset flaccid paralysis in a person less than 15 years of age for any reason other than severe trauma. VAPPs cases were classified into recipient and contact VAPPs. In this study a recipient VAPP was defined as a person who 4 to 30 days after receiving OPV dose developed AFP compatible with poliomyelitis, which persists for $\geq$60 days following onset and further associated with Sabin *Poliovirus* isolation from stool. A contact VAPP case was defined as a person with paralytic poliomyelitis that has a known contact with a vaccinee who received OPV within 7–70 days before paralysis onset and the contact occurred 4–30 days before the onset of paralysis.

The study was performed using stored specimens from an on-going WHO AFP surveillance system in the country. It was a retrospective study of AFP cases identified as part of the national AFP surveillance system from January 2003 to December 2011 in Uganda. Cases of AFP were identified from the field by health workers using the Uganda Surveillance AFP case definition adapted from WHO Regional office for Africa protocol. Two stool specimens were collected from the cases within 24–48 hr interval and transported to the Expanded Programme on Immunization Laboratory at Uganda Virus Research Institute (EPI LAB-UVRI) in a reverse cold chain for *Poliovirus* isolation. The EPI LAB-UVRI is a WHO-accredited reference laboratory for isolating *Polioviruses* from stool specimens. Cases that were identified with Sabin viruses were followed up and examined at 60 days after the onset of the AFP by a member of the National Polio Expert Committee to ascertain whether there was residual paralysis or not. The affected children were examined for the typical neurological manifestations of poliomyelitis.

Stool specimens were processed according to standard procedures (WHO, 2004, Polio laboratory manual). Real-time intra-typic differentiation of the isolated *Poliovirus* was performed. Briefly, the viral RNA in the isolate was reverse transcribed to complementary DNA (cDNA) which was then amplified in a PCR reaction using Taq polymerase. Multiple sets of *Poliovirus* type-specific oligonucleotide primers that are tagged with probes were used for intra-typic differentiation of the *Poliovirus* as described (Poliovirus Real-Time RT-PCR, 2010).

One hundred and forty microliters (140 μl) of the cell culture supernatant was used for RNA extraction. The extraction was performed using QIAamp viral RNA extraction kit (Hilden, Germany) according to the manufacturer's recommendations. Reverse transcription was carried out at 42°C for 60 min in a GeneAmp 9700 thermocycler (CA) followed by denaturation at 95°C for 3 min. Amplification consisted of 30 cycles (95°C for 30 sec, 42°C for 30 sec, and 60°C for 2 min) using primer EV2S and EV1A for the 5′UTR. Before sequencing, the RT-PCR products were purified with the QIAquick PCR purification kit and 5′UTR gene (nucleotides 500) was sequenced using cycle sequencing with the Big Dye Terminator Cycle sequencing kit ver. 3.1 (CA). The DNA sequence was determined using the ABI 3100 Genetic Analyzer, version 3.1.

RESULTS

One hundred and six (106) AFP cases from whom the Sabin *Poliovirus* was isolated previously were identified within the 9 year study period. Eleven (11) out of the 106 cases (10.4%) were characterized as VAPP. The estimation of the VAPP rates in Uganda is shown in Table I. The rate of VAPP over the study period ranged from 0 to 3.39 per a million birth cohorts with a peak in 2009, and no case identified for 2003, 2004, 2005, and 2006. A greater proportion of the AFP cases: 37 of the 106 AFP (34.9%) and 5 of the 11 VAPPs (45.5%) occurred in 2009. Twenty-five percent of the VAPP cases (25%) occurred after the administration of the second trivalent OPV dose (OPV2). See the list below:

Last OPV dose	no. of OPV doses	no. of AFPs	no of VAPPs	percentage of VAPPs
OPV0	1	7	0	0.0
OPV1	2	13	0	0.0
OPV2	3	12	3	25.0
OPV3	4	36	4	11.1
OPV3 +1S	5	24	4	16.7
OPV3 +2S	6	3	0	0.0
OPV3 +3S	7	4	0	0.0
OPV3 +4S	8	-	-	-
OPV3 +5S	9	-	-	-
OPV3 +6S	10	1	0	0.0
Unknown	?	6	0	0.0

-: no AFP case, S: Supplemental OPV dose(s)

TABLE I. VAPP rates from 2003 to 2011 in Uganda:

Year	AFP cases with Sabin isolation	Proportion of AFPs (%)	Proportion of AFP cases that were clinically classified (%)	VAPP cases	Proportion of VAPP cases (%)	VAPP rate (cases per 1,000,000 birth cohorts)
2011	24	22.6	75	2	18.2	1.25
2010	10	9.4	90	1	9.1	0.65
2009	37	34.9	100	5	45.5	3.39
2008	8	7.5	100	1	9.1	0.70
2007	3	2.8	100	2	18.2	1.46
2006	9	8.5	100	0	0	0
2005	7	6.6	100	0	0	0
2004	5	4.7	100	0	0	0
2003	3	2.8	100	0	0	0
Total	106			11		

AFP, Acute Flaccid paralysis; VAPP, Vaccine Associated Paralytic Poliomyelitis.

Furthermore most VAPP cases (63.6%) manifested after the age of 1 year (Table II). Five VAPP cases were categorized as contact cases. The other five VAPP cases; V002, V005, V008, V009, and V010 fulfilled the criterion of recipient VAPP. Two of the recipient cases; V002 and V010 manifested after the first dose of OPV. The eleventh case (V011) was a 14 year old child who could not be categorized but has been considered to be a 'VAPP' case because the patient had a history of AFP with Sabin isolation and further manifested with residual paralysis at 60 days following the onset of AFP. Four VAPP cases with viral isolates were sequenced in the 5′UTR. Two harbored the 'typical' revertant-mutations yielding Sabin *Poliovirus* 1 and 2; G480A and A480G mutants and the other two cases did not harbor the 'typical' 5′UTR revertant-mutations.

DISCUSSION

VAPP has been reported to most frequently occur with the initial exposures to OPV. The Uganda data are interesting because many of the VAPP cases appear to be associated with subsequent exposures. Birth cohorts were used to estimate the VAPP rate because VAPP is common with the routine OPV doses before children develop the immunity to OPV. The reports of VAPP cases available are from the developed and developing countries however VAPP cases are described in Uganda for the first time. From 2003 to 2011 eleven (11) cases of VAPP were identified in Uganda.

In the developing countries most children present with VAPP at 1–4 years of age. This occurred in four of the VAPP cases in this study. Three occurred after the age of 4 years. Sabin *Poliovirus* 1, 2, and 3 were equally distributed among the VAPP cases. Three cases had mixed infections of Sabin *Poliovirus* 2 and 3 among the recipient cases which could be explained by the administration of a trivalent vaccine. In this study the immune status of the subjects was more critical for the outcome of VAPP than the number of OPV doses received. This is in agreement with the existing data that VAPP portrays the immune status of the population. There were two 5′UTR revertant-mutants and these have been classified as Sabin *Poliovirus* 1 and 2. Attenuating sites of the *Poliovirus* have been characterized in other regions of Sabin *Poliovirus* 1, 2, and 3 however most cases of the OPV genetic instability occur in the 5'UTR. Sabin *Poliovirus* 2 viruses revert with most ease (Kew O et al., 2005). It was not possible to investigate the proportion of Sabin 2 mutants in this study because of specimen limitations.

Cases V002 and V010 manifested with VAPP after the first dose of OPV. Such event is common in the high-income settings whereas VAPP cases after the second and subsequent OPV doses have been observed especially in low-income settings (Platt and Estívariz, 2014). In this study 8 out of 11 VAPP cases

TABLE II. Characteristics of VAPP cases in Uganda: 2007–2011

Study no.	Year of report	Age in month	No. of OPV doses given since birth	OPV dose before AFP onset	Time to develop AFP (days)	SABIN Type	5′UTR	Recipient (R) OR contact (C) VAPP
V001	2011	19	3	+–	28	2	NP	C
V002	2011	14	2	tOPV1	17	2 & 3	NP	R
V003	2010	36	4	–	–	1	NP	‘C’
V004	2009	60	4	–	–	1	NP	‘C’
V005	2009	3	3	tOPV2	4	3	NM	R
V006	2009	4	2	tOPV2	35	2	A481G	‘C’
V007	2009	24	4	–	–	1	G480A	‘C’
V008	2009	60	4	×	4	3	#G537A	R
V009	2008	5	3	tOPV2	30	2 & 3	NP	R
V010	2007	5	2	tOPV1	18	2 & 3	NP	R
V011	2007	166	3	–	–	1	NP	?

L+, had contact with a recently immunized sibling; ‘C’, probable contact case; e?, not categorized; ×, record missed out; g–, not known; #, non attenuating mutation; eNP, not performed; AFP, Acute Flaccid Paralysis; nVAPP, Vaccine Associated Paralytic Poliomyelitis; tOPV, trivalent Oral Polio Vaccine; dNM, no ‘typical’ revertant-mutation was identified in the 5′UTR.

(72.7%) were in this category. The outcome of VAPP at an older age could be explained by the low sanitation levels in the low-income settings which are associated with high maternal anti-*Poliovirus* antibodies in the community. The maternal antibodies compromise on the immunogenicity of the vaccine and result in low OPV uptake and thus the vaccine recipients remain at a risk of manifesting with VAPP at a later stage.

The VAPP rate during the study period ranged from 0 to 3.39 per a million birth cohorts, which falls within the projected rate of 2 to 4 cases per million birth cohorts (WHO - Weekly Epidemiological Record, 2014). There was an importation of wild *Poliovirus* in the country in 2009 which triggered increased awareness and scaling up of immunization activities, and these were associated with a high number of AFPs and VAPPs in same year.

We describe a considerable prevalence of VAPP cases in our setting that has used OPV for both routine and supplementary immunization activities. With the continued use of OPV vaccine-associated paralysis is becoming more frequent than wild polio paralysis. This study supports the current Polio Eradication Strategy to introduce one dose of IPV while still giving OPV3 in preparation for the eventual total withdrawal of OPV from routine immunization program (GPEI, 2013). The outcome of VAPP and 5′UTR mutations could not be investigated for all the VAPP cases because of specimen limitations.

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