Molecular Analysis and Implications of Neurovirulent Circulating Vaccine-Derived Poliovirus in Jamaica

A Case Report and Review of Literature

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ABSTRACT

As the goal to eradicate wild polio virus (WPV) is approached, outbreaks associated with vaccine derived polioviruses (VDPV) with neurovirulent properties have emerged. The relevance for the spread of infection by nonparalytic cVDPV cases, with mutations associated with neurovirulence, is discussed with reference to the molecular analysis of a VDPV isolated from a Jamaican child who presented with aseptic meningitis. Potential risks to the Jamaican community resulting from circulation of cVDPV, and critical factors defined by the World Health Organization (WHO) in the global eradication of Polio are analyzed in the context of immunization coverage, and the need to stop all Oral Polio Vaccine (OPV) use once wild polioviruses (WPVs) have been eradicated.

Análisis Molecular e Implicaciones del Poliovirus Derivado de las Vacunas Neurovirulentas Circulantes (Reporte de un Caso y Revisión de la Literatura)

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RESUMEN

A medida que nos hemos acercado a la meta de erradicar el virus de la polio salvaje (VPS), se han producido brotes asociados con los poliovirus derivados de la vacuna (VDPV) con propiedades neurovirulentas. El presente trabajo discute la importancia de estos en la diseminación de la infección por casos no paralíticos de cVDPV, en relación con el análisis molecular de un VDPV aislado a partir de un niño jamaicano que presentaba meningitis aséptica. Los riesgo potenciales para la comunidad jamaicana como resultado de la circulación de cVDPV, y los factores críticos definidos por la Organización de Mundial de la Salud (OMS) en la erradicación global de la polio, se analizan en el contexto de la cobertura de la inmunización, y la necesidad de detener todo uso de la Vacuna Oral de la Polio (VOP), una vez que los poliovirus salvajes (PVS) hayan sido erradicados.

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INTRODUCTION

Since the last reported indigenous wild polio virus (WPV) case reported by the Caribbean Epidemiology Research Centre (CAREC) in 1982, Jamaica has continued collaborative surveillance with the World Health Organization (WHO) in its goal to achieve the global eradication of Polio (1). Some countries including Jamaica have achieved Polio eradication status but have experienced delays in the WHO recommended transition from the use of Sabin Oral Polio Vaccine (OPV) to the Inactivated Polio Vaccine (IPV). This delayed transition has resulted in the continued use of the OPV which has occasionally been associated with the isolation of vaccine-derived polioviruses (VDPV's) from asymptomatic children (1–8). The circulation of VDPV will continue as long as oral polio vaccine (OPV) is in use (2–4, 9, 10). One of the critical factors defined by the WHO in the global eradication of Polio is the need to stop all Oral Polio Vaccine (OPV) use once wild polioviruses (WPVs) have been eradicated (2). If surveillance emphasis does not greatly enhance the observation of cVDPV, detection of emerging neurovirulent cVDPV associated with non-paralytic and paralytic polio cases may be overlooked, thus prolonging the goal of global eradication and creating hidden reservoirs of potential mutant strains (2, 11).

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This report describes a case of symptomatic VDPV infection in a child presenting with meningitis, reviews the significance of molecular analysis of this isolate and discusses the need to stop all OPV once wild poliovirus has been eradicated (2– 4, 12).

Case Report

A three-year-old male was admitted to hospital with an abrupt onset of a two-day history of headache, photophobia and fever and a one-day history of nausea and vomiting. There was no history of similar illness in any other family members and the child lived in a lower income home with modern sanitary facilities. The child's immunization status was in-complete as indicated by hospital records and a history from the mother. The last immunization (including OPV) had been given when the child was one year of age. On examination, the child was febrile and irritable. No abnormality was de-tected in the cardiovascular, respiratory or gastrointestinal system. There were signs of neck stiffness, although the Brudzinski's sign was negative. No evidence of focal neuro-logical involvement, papilleodema or motor weakness was detected. Cerebrospinal fluid (CSF), blood, throat and rectal swabs were taken for laboratory investigations where stand-ard virologic procedures were followed. A provisional diag-nosis of meningitis was made and the child was placed on empirical intravenous penicillin and chloramphenicol. Hav-ing received results that all bacterial laboratory investigations were negative, the diagnosis was subsequently changed to that of aseptic meningitis.

Haematological results for CSF showed an elevated white blood cell (WBC) count of 48/mm^3 but no differential was done. Other results included a normal CSF/blood sugar ratio of 0.6 (3.7 mmol, CSF/6.1mmol blood glucose) and a CSF protein value of 0.1g/L. The whole blood WBC count was elevated at 17 900/mm^3 with a differential of 62% neutrophils, 24.5% lymphocytes, 10.9% monocytes and 1% basophils. Viral cultures were positive for enterovirus in the CSF, throat and rectal swab. The isolates were initially identified by an indirect immunofluorescence assay (IFA) by Chemicon International®. Further typing by IFA confirmed the isolate as Polio type 2 virus. The isolate was subsequently characterized by molecular analysis as a derivative of the Sabin OPV vaccine. The child remained in hospital for seven days and recovered completely without complications at the time of discharge. Follow-up of this case, however, was not completed as a result of non-attendance to clinic appointments.

Sequence Analysis

Sequence analysis of the VP1 region of the isolate identified the isolate as an oral vaccine-derived polio strain originating from Sabin 2. The sequence revealed 12 base changes from published Sabin 2 sequences. One of these changes at base 481 in the NTR was in keeping with reversion back to the wild-type sequence. Within the VP1 region, 9 base changes were seen resulting in 4 amino acid changes. One of these was identified at base 2909 (amino acid 143) which translates as an *Asn* instead of *Ile* in the reference Sabin 2 (Table 1). The nucleotide sequences have been deposited in the Genbank data library under accession numbers EU004574 and EU004575 and Jamaica 2005, EV55-05Y7-Q8con

Table 1: Comparison of mutational changes in the Jamaican Sabin 2-like isolate with Sabin 2 vaccine and wild type polio 2 virus reference

Genome nt #	Jamaican isolate (EV55-05Y7-Q8con)**	Sabin 2 ref. (AY184220) **	Wild type Polio 2 (M12197)**
Changes within partial 5'NTR (nt#175 to 635):			
481*	G	A	G
Changes within VP1 region (nt#2482 to 3384):			
2644	C	T	T
2909*	A	T	C
3275	G	A	A

*major reversion sites associated with neurovirulence
nt = nucleotide
** Accession number of reference virus

Phylogenetic analysis of the VP1 sequences

Using data from the VP1 sequences, MEGA 3.1 software was used to perform Clustal W alignments of nucleotide sequences (13). Phylogenetic analysis was done using the neighbour-joining method with a Kimura 2-parameter model to compare the Jamaica strain with the Sabin 2 vaccine strain, a Sabin 2 related strain, and Beni Suef 98 Egyptian isolate (14). Respective accession numbers were X00595, AY184220 and AF551838.

DISCUSSION

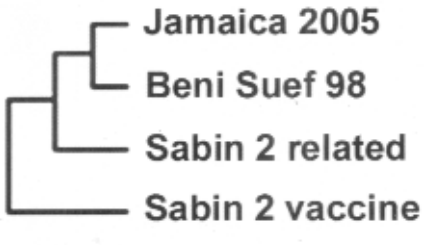
In the WHO immunization surveillance assessment and monitoring system, the word "Polio", refers to all polio cases (indigenous or imported) including polio cases caused by vaccine-derived polioviruses (VDPV). This assessment and

monitoring system however does not include cases of vaccine-associated paralytic polio (VAPP) and cases of non-polio acute flaccid paralysis [AFP] (15). The Centers for Disease Control and Prevention (CDC) case definition of nonparalytic polio (NPP) applies to poliovirus infections found in asymptomatic persons or those with mild, non-paralytic disease (*eg* those with a nonspecific febrile illness, diarrhoea or aseptic meningitis] (16).

The role of surveillance in the early detection of vaccine-derived polioviruses (VDPVs) has gained increased importance as the global eradication of Polio is approached. Today we know that the clinical manifestations of poliovirus may be inapparent (asymptomatic) in 90–95%, abortive or minor in 4 to 8%, non-paralytic poliomyelitis (NPP) in 1 to 2% and paralytic in 0.1–2% (5). Several authors have discussed the significance of the recognition of the large parentage of abortive and nonparalytic cases of polio and their relevance for the spread of the infection (11, 12). Others have referred to the frequency of meningism associated with characteristic headache and the difficulty in identifying the aetiology of such cases as these features are not pathognomic for polio, though very significant in children (17).

Poliovirus isolates are classified as vaccine, vaccine-derived (VDPV) or wild type poliovirus based on the per cent nucleotide sequence homology between its capsid protein VP1 and that of the corresponding OPV vaccine serotype. VP1 homology of 99–100% is classified as vaccine virus, 85% to 99% as VDPV and < 85% as wild type poliovirus. VDPVs are further subdivided by the letter "i" or "c" preceding the word "VDPV". VDPVs that arise during persistent infection of immunodeficient individuals are termed iVDPV. VDPVs that evolve during continuous transmissions of vaccine virus among unvaccinated individuals in populations with low vaccination coverage or when vaccination programmes are interrupted and sufficient numbers of unimmunized infants accumulate are termed "circulating VDPV" [cVDPV] (3, 10). Specific nucleotide positions in the genomes of VAPP strains have been associated with reversion to neurovirulence (19). Sequence analysis of the VP1 region of the isolate in this case identified the isolate as an oral vaccine-derived polio strain originating from Sabin 2, which appeared to have evolved significantly in keeping with similar VDPVs that have had a history of circulating from person to person over a period of time (2, 3). Both mutations in this isolate at sites 481 and 2909 implicate reversion to neurovirulence as seen both in humans and the mouse model for polio neuro-invasiveness (9). These changes could account for the meningitis symptoms seen in this patient and its role as the aetiological agent in this case is supported by the repeated recovery of this isolate from the throat, rectal swab and CSF of this patient. Moreover, its discovery has implications for public health, since these mutants appear in areas where OPV vaccination coverage of the population has been incomplete. Immunization to polio in this case was incomplete, the child having received OPV two years prior to his presentation resulting in this isolate being classified as a cVDPV.

VDPVs associated with a high VP1 sequence drift have been described in healthy children and like WPV may be asymptomatic in the majority of cases (2, 3, 5). The cVDPV isolate identified most closely resembled similar isolates identified in outbreaks in Egypt from 1988 to 1993 and which were associated with poliovirus type 2 vaccine derivatives (Figure). The rate and pattern of VP1 divergence among the



** *Accession number of reference virus*

Figure: Phylogram showing the Jamaica vaccine-like isolate (Jamaica 2005, EV55-05Y7-Q8con) ** Beni suef 98 (AF551838 BeniSuef98) **, Sabin 2 related (AY184220) ** and Sabin 2 vaccine (X00595) **

Egyptian circulating vaccine-derived poliovirus (cVDPV) isolates suggested that all lineages were derived from a single OPV infection that occurred around 1983 and that progeny from the initiating infection circulated for approximately a decade within Egypt along several independent chains of transmission. Complete genomic sequences of an early (1988) and a late (1993) cVDPV isolate revealed that their 5' untranslated region (5' UTR) and noncapsid- 3' UTR sequences were derived from other species C enteroviruses (9).

The potential risk of cVDPV poliomyelitis has increased dramatically and is reflected in outbreaks which have occurred in Hispaniola (Type 1) 2000-01, the Philippines (Type-1) 2001, and Madagascar (Type-2) (2001-02). Occurrences of these cases have been associated with Sabin OPV subtypes 1, 2 and 3. The greatest risk of infection has been associated with OPV subtype- 2 and is related to its increased transmission within communities (3).

Jamaica immunization coverage for polio was 84% as reported by the WHO in 2005 (11). Prior immunization survey results in 1985 showed seropositive coverage of 81.4%, 94.7% and 72.3% for Polio types 1, 2 and 3 respectively in Jamaican children (20). Studies have confirmed the role of environmental surveillance of sewage from populations with high (>95%) immunity and have identified evolutionary clusters of VDPVs. These findings indicate the need to incorporate environmental surveillance in the overall polio surveillance system, particularly in countries in which the OPV vaccine is still in use (8).

The tropical climate of Jamaica, deficiencies in the hygiene/sanitation environment, the continued use of the OPV and immunization coverage to polio less than 95% are critical risk factors for the evolution and circulation of cVDPV (3). The significance of the circulation of neurovirulent cVDPVs in Jamaica must be addressed urgently. The finding of cVDPV as documented is indicative of possible gaps in the immunization coverage (2, 3, 9). Continued assessment of immunization coverage and immunization campaigns are essential if a goal of 95% polio vaccine coverage is to be achieved. Health Policy makers must aim to replace the OPV vaccine with the use of inactivated polio vaccine (21). Necessary economic and health budget adjustments need to be reviewed to ensure availability and accessibility of the IPV vaccine. Environmental surveillance for possible reservoirs of cVDPV need to be identified and continued community education, encouragement and rewards for compliance with health regulations may create a favourable working community response.

Polio developed into a pandemic over a period of four hundred years, reaching its peak in the 1950s (11). Paralysis was the most significant feature of this pandemic, although it is known that paralytic poliomyelitis occurs in 0.1 – 2% of infected persons. Asymptomatic and non-paralytic poliomyelitis must have contributed significantly to the perpetuation of this disease. Achievement of the global eradication of Polio will necessitate increased consideration of risks associated with the potential transmission of non-paralytic neurovirulent cVDPV and the surveillance of such viruses in Jamaica and worldwide.

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