

# Type 1 wild poliovirus and putative enterovirus 109 in an outbreak of acute flaccid paralysis in Congo, October–November 2010

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**An outbreak of flaccid paralysis syndrome in adults is ongoing in Congo. Molecular analysis of faecal, throat and cerebrospinal samples identified wildtype 1 poliovirus and an additional enterovirus C strain related to enterovirus 109 as the cause. As of 22 November, the cumulative number of cases was 409, of which 169 (41.3%) were fatal. This is one of the largest wild type 1 poliovirus outbreaks ever described associated with an unusually high case fatality rate.**

## Background

Following mass vaccination campaigns organised through the Global Polio Eradication Initiative, the World Health Organization (WHO) declared that poliomyelitis had been eradicated from many regions of the world. However, although transmission of wild poliovirus type 2 (WPV2) has been interrupted, isolated human cases and outbreaks of WPV1 and WPV3 are still being reported in many countries [1]. Epidemiological investigations showed that all these clinical cases during the last decade were due to importation of WPV from one of the four countries where indigenous WPV transmission is still ongoing, namely Afghanistan, India, Nigeria and Pakistan [1]. In the past decade, several outbreaks and isolated clinical cases resulting from WPV1 and/or WPV3 importation from Nigeria or India were reported in 15 polio-free countries in Africa. Two small WPV1 outbreaks occurred recently, in Namibia in 2006 and Angola in 2010, after importation of WPV1 of Indian origin [2]. In order to prevent these episodic cases of imported and indigenous WPV transmission, polio immunisation campaigns were conducted that targeted some 72 million children in 15 countries across western and central Africa.

## Outbreak description

Congo had recorded its last official case of indigenous polio in 2000 [3]. On 5 November 2010 the Ministry

of Health of Congo declared an outbreak of poliovirus centred in the second largest town, Pointe-Noire. The outbreak presumably started in mid-October 2010, with an unusual accumulation of cases of acute flaccid paralysis (AFP) syndrome in patients between 15 and 72 years-old. Most cases occurred in Pointe-Noire and some in Cabinda province. Cases exported from Pointe-Noire were then reported in several towns and villages of Congo. As of 22 November the cumulative number of cases was 409, of which 169 (41.3%) were fatal. Direct contact between cases was rare, and there was no apparent spatial pattern. Likewise, there was no evidence of a common source such as food or water. In most hospitalised patients the disease started with influenza-like symptoms four to seven days before the onset of AFP of the legs. AFP then ascended rapidly (within a day), frequently leading to death from cardiac and/or respiratory failure. The large number of severe cases and the high case fatality rate contrast sharply with previous WPV outbreaks and point to a role of unknown viral/host features or to the existence of massive numbers of mild and therefore unreported additional cases.

## Laboratory investigations

The Centre International de Recherches Médicales de Franceville (CIRMF), Gabon, received three plasma samples for aetiologic investigation on 29 October. Real-time and conventional reverse transcription (RT)-PCR testing was negative for neurologic, enteric and respiratory viral pathogens, namely the genera *Enterovirus*, *Flavivirus*, *Alphavirus*, *Phlebovirus*, human mastadenoviruses, the family *Paramyxoviridae* (mumps virus, measles virus, parainfluenza viruses 1–4, respiratory syncytial virus, human metapneumovirus) and the sub-family *Coronavirinae* (human coronavirus NL63, HKU1, OC43 and 229E). Also negative were species-specific real-time RT-PCR tests for West Nile virus, tick-borne

encephalitis virus, cytomegalovirus, human herpesvirus type 6, herpes simplex virus type 1, varicella zoster virus, rotavirus serogroup A, norovirus genogroups 1 and 2, sapovirus, astrovirus, influenza viruses A and B and rhinovirus.

CIRMF then received 15 rectal swabs, 14 throat swabs, and five cerebrospinal fluid (CSF) samples on 2 November of which thirteen rectal swab specimens (86.7%), five throat specimens (35.7%) and one CSF specimen (20.0%) were positive for enterovirus in a real-time RT-PCR targeting the 5'-noncoding region [4]. The faecal and throat samples had threshold cycles ranging from 24 to 38 in real-time RT-PCR, indicating medium to high virus concentrations, while the concentration in the positive CSF sample was low (cycle threshold (CT) 38). The genome was studied by the Institute of Virology at the University of Bonn Medical Centre in Germany, based on partial VP1 sequencing [5], 3D sequencing (unpublished in-house assay) and 5'-UTR sequencing [6].

Poliovirus type 1 was identified in one sample (100% amino acid identity to recent poliovirus strains of Indian genotype in 'typing' VP1 PCR). The amplified 327 nt sequence of this sample (corresponding to genome positions 2,631 to 2,957 in WPV1 strain Brunhilde) shared 94.8-96.3% identity with poliovirus type 1 sampled in Africa (Angola and Democratic Republic of the Congo) in 2006 and 2007 (isolates ANG-LUA-KIL-07-003 and RDC-BCG-SEK-06-004) and 95.1% identity with a strain recovered during a polio outbreak in Tajikistan in 2010 (GenBank accession number HQ317702). A more sensitive strain-specific nested PCR assay amplifying a 201 bp VP1 fragment was developed from the initial sequencing data, and all but two of the 19 samples positive in the enterovirus real-time RT-PCR were typed as wildtype 1 polio virus. Partial sequences of the 3D genomic region encoding the viral polymerase (181 nt corresponding to genome positions 6869-7049 in WPV1 strain Brunhilde) were retrieved from five samples. The maximum nucleotide identity of all these samples was 96.1% with two poliovirus type 1 strains recovered in Russia and the Philippines after the year 2000 (isolates P1W/Bar65 and Mindanao-01-1, respectively). Partial 5'-UTR sequencing yielded positive results for all the specimens tested. The 115 nt thus obtained (corresponding to genome positions 466 to 580 in WPV1 strain Brunhilde) were 96.5% identical to recent WPV1 strains from China (isolate CHN-Hainan/93-2). Therefore, all analysed genomic regions were identified as WPV1, indicating absence of putative intra- or interspecies recombination. An additional enterovirus C strain distantly related to enterovirus 109 (EV109) in the VP1 region was retrieved from a rectal swab of a deceased patient. In the 322 nt VP1 sequence fragment that could be retrieved, the virus showed 75% to 77% nt sequence identity with the five EV109 sequences available in GenBank, and 90.5% nt identity in the 3D genome region with EV109 isolate NICA08-4327 recovered in Nicaragua in 2010 [7]. Of note, the corresponding sample contained one of the highest enterovirus

RNA copy numbers (CT value 24). Polio virus was not detected in this sample with the broad-range typing assays described above, nor with strain-specific VP1 nested and 3D real-time PCR assays. No other sample was positive for the EV109-related virus in a strain-specific 3D real-time PCR assay. The presence of other enteric viruses (adenovirus, astrovirus, enterovirus, rotavirus A, sapovirus and norovirus genogroups 1 and 2) was ruled out in all the faecal samples except for one WPV-positive sample which also contained norovirus RNA. Genome sequencing and further typing of samples containing poliovirus and enterovirus, targeting the complete VP1 genomic region, are ongoing. Additional samples have been sent to CIRMF for analysis.

## Conclusions

The preliminary sequencing data and clinical picture are compatible with a wildtype poliovirus outbreak. Further epidemiological and serological studies are required to explain the unusually high case fatality rate and the patients' relatively advanced age. One possible explanation is that only severe cases may be reported. Alternatively, the population may be immunologically naïve and highly susceptible, although this is unlikely given the claimed success of vaccination campaigns. Epidemiological investigations have just begun. At this time, no direct contact between cases has been observed, and no apparent spatial pattern was identified, with neither domestic dissemination nor within the same subdivisions. Together, these observations suggest a diffuse source of contamination, e.g. from water drawn from wells in the poor neighbourhoods of the city. Involvement of a more virulent virus, or potentially other viruses, is another possibility. More information on non-hospitalised patients and mild cases is needed. Information on predisposing conditions of the fatal cases as well as full-length sequencing of VP1 and the full genome are currently ongoing.

The WHO Country Office, Regional Office, and Headquarters are supporting the Ministry of Health in Pointe-Noire, and the WHO Country Office is supporting the operational costs of the investigation and response teams. At least 1.1 million people are to be vaccinated in the epicentre of the outbreak (Pointe-Noire region), and further 600,000 people will be vaccinated simultaneously in the neighbouring regions of Congo near Angola, where the last cases of WPV1 occurred in 2010.

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