

Circulating vaccine-derived poliovirus outbreak in Chiengi District, Zambia, September 2019

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ABSTRACT

Introduction: Africa has seen a significant rise in circulating vaccine-derived polioviruses type 2 (cVDPV2) outbreaks following the switch to the bivalent oral polio vaccine (bOPV) in 2016. In September 2019, the World Health Organisation (WHO) country office notified the Zambian Ministry of Health of a case of cVDPV-2 in Chienge District, Luapula Province. This paper describes the investigation and public health response to the outbreak. **Methods:** Investigation and response were conducted per WHO standards. cVDPV was defined as VDPV demonstrating person-to-person transmission in the community. The patient's guardians were interviewed, blood samples collected from the patient, and 34 stool samples collected from direct and community contacts aged <5 years. Genetic sequencing intratypic differentiation was used to determine the relatedness of poliovirus samples. The WHO immunisation coverage cluster survey design was used to assess polio immunisation coverage in children aged 6-59 months in the catchment area surrounding Chipungu Rural Health Centre (RHC) and Lunchinda RHC. Population data was collected and documented at the health facility level and from community surveys to triangulate population size in preparation for the monovalent oral polio vaccination campaign. Results: The index case patient was an unvaccinated two-year-old boy from Chienge District bordering the Democratic Republic of Congo (DRC). History indicated he developed sudden weakness in his lower limbs following a fever of < 24 hours in July 2019. Sequencing results of the case showed VDPV2 with nine nucleotide differentiation from Sabin-2. Of the 36 stool samples, five (15%) were non-polio enteroviruses and three (9%) were suspected poliovirus (2 type-2 polioviruses (PV2)) and one SabinLike1. PV2 sequencing results confirmed cVDPV with genetic linkage to the index case but no established link to ongoing outbreaks in the DRC. The vaccination coverage survey found less than 80% (47/60) fully immunised children, OPV 3 coverage was 80% (48/60) while that of IPV was just 23% (14/60). A total of 323,936 children were reached in 11 districts with a vaccination coverage of 101% during 3 rounds of mOPV2 supplementary immunization activity (SIA). A countrywide catch-up vaccination campaign with inactivated polio vaccine (children 3-59 months) was also conducted in 2020. **Conclusion:** The confirmed presence of a cVDPV outbreak in Zambia represents another novel emergence of a cVDPV since the switch from tOPV to bOPV. This emergence highlights a gap in the immunity profile of the Zambian child and the continued risk of Polio outbreaks. Country boarders remain areas of high concern and there is need to strengthen cross-border collaboration with neighbouring countries through synchronized vaccination campaigns, and strategies to strengthen routine immunization and active surveillance.

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Poliomyelitis is a highly infectious disease that is often spread from person to person through contact with an infected person's faecal matter. On rare occasions, infection can occur through ingestion of contaminated water or food. The majority of the population infected with poliovirus asymptomatic thus completely unaware of their infection and potentially infect thousands by the time the first case of paralytic polio appears[1]. Only humans can contract polio and there are no known animal reservoirs. There are two forms of Polioviruses, the wild type (WPV) which is the most common type and the circulating vaccine-derived polioviruses (cVDPV)[2].

The disease is caused by three polioviruses that are part of the human enterovirus C group (types 1, 2 and 3) and can present with acute flaccid paralysis (AFP) in those affected. Children under 5 years of age are most susceptible to Polio infection with 1 in 200 infections leading to irreversible paralysis; 5% to 10% of those with central nervous system involvement die when their breathing muscles become immobilized [1].

Once AFP occurs, there are no effective treatments that can reverse or restore motor neuron function after paralytic poliomyelitis. Vaccination is the primary tool for polio prevention, control and eradication [3-5]. However, vaccination can rarely lead to AFP in the form of either vaccine-associated paralytic poliomyelitis (VAPP) or vaccine-derived poliovirus (VDPV) [6, 7]. cVDPV cases remain a major barrier to polio eradication, with the number of cVDPV type 2 outbreaks increasing in recent years [8,9]. The increase in cVDPV cases has been attributed to low population immunity communities resulting in the oral polio vaccine virus mutating as it spreads among unvaccinated children, the mutated vaccine virus is thus able to cause paralysis [2].

In 1995, Zambia through Kafue district of Lusaka Province reported its final two indigenous WPV cases [10]. In 2001/2002, the surveillance system detected and investigated five WPV cases imported into the Kalabo and Shangombo districts of Western Province from Angola. Through two rounds of Polio mop-up activities conducted in 14 districts in the Western and North-Western provinces, the outbreak was successfully controlled in 2002. As part of the

overall country polio eradication initiative (PEI), Zambia instituted a surveillance system for AFP cases in August 1998 [11]. AFP case-based surveillance is implemented through the Integrated Disease Surveillance and Response (IDSR) system, and notifications of all cases are recorded weekly. IDSR reported AFP cases are routinely tested for polioviruses at the WHO-accredited National Polio Reference Laboratory at the University Teaching Hospital (UTH)[12].

With these measures in place, the country successfully presented evidence that there was no indigenous poliovirus in circulation and that the surveillance system was sensitive enough to detect any imported cases of WPV. Zambia was declared polio-free by the African Regional Certification Commission in October 2005 [13].

On the 13 September 2019, the Ministry of Health in Zambia was notified of a case of laboratory-confirmed vaccine-derived poliovirus type 2 (VDPV2) through the WHO Country Office. The reporting facility was Katabe Private Hospital in Pweto District of the Democratic Republic of Congo (DRC). The case was a male aged 2 years and 8 months from Kalima 2/Pilliashi villages in Chiengi District, Zambia. The notification triggered an immediate response by Zambia National Public Health Institute (ZNPHI). We describe the investigation of the case and public health responses that were undertaken following the notification.

Methods

Study Setting and Population

We conducted an outbreak investigation in Chienge District of Luapula Province, Zambia from 18 to 23 September 2019. Chienge District is approximately 1,110 km from Lusaka, the capital city of Zambia. It is one of 10 Districts located in Luapula Province and it covers an estimated 3,765 square kilometres. It is bordered by Nchelenge District, Lake Mweru, Kaputa District and the DRC. The projected population of Chienge District for 2019 was estimated at 144,557. However, headcount data collected in early 2019 under the Malaria Elimination Program for Indoor Residual Spraying (IRS) estimated the population count at 295,000.

Chienge District is a predominantly rural community and the main economic activities are fishing and cross-border trade. The district sees an influx of population movement through both official and unofficial border points, Chiengi also hosts a refugee camp which mostly houses refugees from the neighbouring DRC. The district has 13 public health facilities and no registered private health facilities. Long distances from health facilities to the villages is a major deterrent in accessing vaccination services with certain areas located as far as 10km from the nearest health facility. The road network is mostly gravel but well-marked which facilitates easy movement within the district except in some seasons when some of the roads become inaccessible, such as the rainy season which begins in late October up until April. There is no developed municipal sewage system and few areas in the district have access to electricity whereas the majority rely on solar energy to generate power. The main sources of water are Lake Mweru and unprotected private shallow wells. The rest have access to a few functional hand pumps. The water table is generally high, between 1.5 and six meters. At such depths, water is largely subsurface flow making it susceptible to contamination in the absence of a developed municipal sewage system in the district.

Outbreak Investigation

An outbreak investigation led by the district through the office of the District Health Director was conducted on 14 September 2019. The scope of this initial investigation was limited due to resource and technical constraints. However, we conducted a second investigation from 18 to 23 September 2019 with the support of members from the Ministry of Health (MOH), Zambia National Public Health Institute (ZNPHI), World Health Organisation (WHO), U.S. Centers for Disease Control and Prevention (CDC), and United Nations Children's Fund (UNICEF). The team established a timeline of events and conducted contact tracing of direct and community contacts in accordance with the GPEI SOP Version 3[9].

Laboratory Investigation

A blood sample was collected from the index case to investigate for possible primary immunodeficiency. Stool samples of close and community contacts were collected to test for cVDPV-2 from the two villages

of Pilliashi and Kalima 2 where the index case resided. Specimen bottles were distributed to 40 households in the community with healthy children below the age of 6. Instructions were given to their guardians to collect the stool specimens in the morning. Community-based volunteers (CBV) within the areas were provided with sample collection cooler boxes with frozen ice packs and guardians were asked to submit samples to the CBVs immediately after collection of specimens to ensure samples were kept at 2-8°C until the teams collected the filled bottles. Out of the 40 distributed bottles, 34 samples were successfully collected and ready for submission. These samples included 2 from direct contacts and 32 randomly sampled from the community. The samples were then transported to the National Polio Laboratory at the University Teaching Hospital in Lusaka for analysis.

At the laboratory, the presence of the virus was analyzed through two-phase separation, virus isolation, intratypic differentiation, and sequencing. Additionally, genetic sequencing was performed at the National Institute for Communicable Diseases (NICD) reference laboratory in South Africa on the sample from the index case to determine if there was any linkage to the VDPV outbreaks recorded in the DRC.

Immunisation Coverage Survey

The WHO immunisation coverage cluster survey design was used to assess polio immunisation coverage in children aged 6-59 months in the catchment area communities served by the two facilities, Chipungu Rural Health Centre (RHC) and Lunchinda RHC. The 2010 Central Statistics Office Standard Enumeration Areas (SEAs) were used for the survey. Each SEA is associated with a unique GeoID, which was used to randomly select three community clusters for inclusion in the survey for each catchment area. Ten households in each community cluster were randomly selected. A total of 30 households were sampled in each of the catchment areas, giving a total of 60 sampled households. The community immunisation coverage questionnaires were uploaded onto Open Data Kit (ODK). At each household, investigators conducted a survey to assess the immunisation status of all children physically present aged 6 to 59 months by immunisation card or oral history. Children were excluded if they were visitors or if their guardian did not reside in the household for the last 6 months. Investigators asked questions about the family demographics, the child's polio immunisation records, and the birth date was used to determine if a child had received tOPV or bOPV. Children who completed their vaccinations before April 2016 were considered to have received tOPV, and those after were counted as receiving bOPV.

Response Interventions

In response to the outbreak, Zambia conducted three main activities which included: three rounds of supplementary immunization activity (SIA) using mOPV2, round 0 was conducted from 6 to 12 November 2019; strengthening of AFP surveillance at all levels was initiated from November 2019 and surveillance enhancement activities were supported for the following two years; and increasing population immunity through strengthening of routine immunization services was initiated in November 2019.

The thematic areas in the coordination of the vaccination campaign were: Advocacy, Communication and Social Mobilization; Monitoring & Evaluation; Service Delivery; Vaccine & Cold Chain; Planning & Coordination; and surveillance teams.

Three SIA rounds were conducted with Round 0 (R0) conducted in 3 districts and the scope later expanding to 11 districts in R3. These included all nine Districts in Luapula and two Districts in the neighbouring Northern province. A total of 323,936 children were reached with a vaccination coverage of 101% to prevent further spread of virus by increasing population immunity. Independent monitors (IM) and Lot Quality Assessment Survey (LQAS) were used to assess the quality of the campaign. Results of the IM improved with each round with only one district performing below 90% in R2. However, no significant improvement was noted in the LQAS across all three rounds, with a notable 63% of districts failing the LQAS in R2. Although the mOPV campaigns were not synchronized with neighbouring DRC, transit teams were stationed at formal and informal border crossing areas to vaccinate under 5 children travelling between the two countries.

Several measures were put in place to improve surveillance indicators in the country. A desk review of indicators was conducted, and a national training of polio surge staff was conducted to ensure onsite orientations at the facility level for all surveillance focal point persons. At the close of the year, Zambia had attained a non-Polio AFP rate of 3.6 above the 3/100,000 population >15 and a stool adequacy of 86% which was above the 80% recommended threshold.

The expanded program for immunization (EPI) in Zambia adopted the Reach Every Child (REC) strategy to improve planning, resource management, service delivery, and monitoring to maintain routine EPI coverages. Strategizes developed also focused on equity in service provision of immunization services, thus improving access and overall coverage of vaccines. Additionally, in 2020, the country integrated the inactivated Polio vaccine (IPV) into the Child Health Week as a platform for supplementary immunization. This initiative aimed to catch up on IPV vaccinations for children aged 3 to 59 months who missed receiving a type 2containing polio vaccine during the transition period from March 2015 to March 2020. This transition involved a shift from using the trivalent oral polio vaccine (tOPV) to the bivalent oral polio vaccine (bOPV) and the introduction of IPV.

Data Analysis

We used Epi Info version 3.5.4 for analyzing data collected during the household survey. Descriptive statistics were conducted by calculating mean of the variable age and calculating frequencies of the following variables: sex, education of caregiver, vaccination status, under 5 vaccination card and Polio vaccination status.

Results

Outbreak investigation

We interviewed the guardians of the case who narrated that the child was born in the DRC to a Congolese father and Zambian mother from Chienge District in February 2017. He returned to Zambia a week after birth with his mother and resided between the two villages of Pilliashi and Kalima. Vaccination history revealed that the child

had not received any vaccines since birth as the mother reported having been ill in the early years of the child's life. The child was noted to have travelled to the DRC several times since birth, the most recent being in April 2018 (over a year before the onset of symptoms). He had been admitted in the DRC on two separate occasions before the current illness.

On unknown dates in July 2019, he was reported to have developed a fever and less than 24 hours from the onset of the fever, he developed weakness in the left lower limb which progressed to weakness in the right lower limb. This was followed by pain in the neck and later progressed to the inability to support the head. He also had pain in the lower limbs and back. The child was taken to Katabe Private Hospital in Pweto District of Haut Katanga Province in the DRC by his father where he was hospitalised and samples collected to investigate for poliomyelitis.

We examined a well-nourished and fully conscious male child, who presented with paralysis of the left and right lower limbs. On physical examination, he had a right-sided cervical lymphadenopathy which was non-tender measuring: 2x3cm, 0.5x1cm and 0.5x0.5cm. The nasal cavity showed dried mucus discharge bilaterally. He had multiple traditional tattoo marks on both lower limbs. His temperature was 36.7 degrees Celsius; heart rate 94 beats per minute (bpm); pulse 102 beats per minute; and respiratory rate 27 breaths per minute. The child had no obvious spinal deformities, muscle power was 3/5 on the right lower limb and 0/5 on the left lower limb. Both upper limbs scored 5/5. He had no loss of sensation. Reduced tone was noted in the right lower limb and he had a floppy hypotonic left lower limb. His reflexes were brisk in the right lower limb but absent in the left lower limb. Reflexes were present and normal in the upper limbs.

Laboratory Investigation

Following the initial confirmation of the index case as a type two poliovirus, sequencing results showed a VDPV with nine nucleotide (nt) differentiation from Sabin 2. Primary serum immunological deficiency results did not suggest any hypogammaglobinemia or agammaglobinaemia states and thus it was concluded there was no primary immunodeficiency syndrome. However, a full blood count and white cell population analysis were not done. Primary isolation from the thirty-four

community stool samples included: 26 negative results, five non-polio enterovirus (NPENT) and three suspected poliovirus. Intratypic differentiation (ITD) results for the three suspected cases showed two polioviruses type 2 (PV2) and one Sabin-Like1 (SL1) while sequencing results for the two PV2 were confirmed cVDPV with 9 and 10 nt-differentiation with linkage to the index case. The Poliovirus Type 2 samples were sent to the NICD reference laboratory in South Africa for sequencing, in accordance with the Global Polio Eradication Initiative guidelines on responding to a polio event or outbreak. Results of the genetic sequencing found that there was no linkage to the recent cVDPV outbreaks recorded in DRC.

Immunisation coverage survey

The male:female ratio of children under five years was 3:2 in the sampled communities. Majority of caregivers (56.7%, 34/60) had primary education while 38.3% (23/60) had no formal education. Fully immunised children were less than 80% (47/60), card retention was at 82% (49/60). The coverage for OPV 3 was 80% (48/60) while that of IPV was just 23% (14/60) (Table 1).

Discussion

We investigated a case of cVDPV-2 in Chienge District, Luapula province, Zambia. Based on the available epidemiological and laboratory data, the index case represents a novel emergence of cVDPV2 in Zambia. This was the first polio case in the country in 17 years. Two other cases of polio infection were detected among contacts of this case, proving that the virus was circulating in this community.

Zambia responded in accordance with the guidance provided by WHO's standard operating procedure to any nation that detects any form of poliovirus outbreak. Investigation and response operations were carried out with the explicit goal of interrupting polio transmission within 120 days [14]. Despite achieving 101% vaccination coverage achieved during the SIA, there was a notable lack of significant improvement in the LQAS results across all three rounds of vaccination campaigns, with 63% of districts failing in Round two.

The failure to improve LQAS results suggested that a large number of children remained unvaccinated, increasing the risk of poliovirus transmission and outbreaks. Public health implications of the failed LQAS ranged from straining health system resources to posing a significant risk for the importation and transmission of poliovirus due to high mobility and cross-border activities with the DRC. This situation underscored the need for policy adjustments to strengthen routine immunization, surveillance, improve community engagement and education, and coordinate international response It highlighted importance the synchronizing vaccination efforts across borders and improving logistical support for remote areas. Addressing these challenges requires comprehensive approach involving community engagement, improved infrastructure, coordinated international efforts to enhance vaccination coverage and prevent future outbreaks.

Several factors contributed to the challenges faced during the vaccination campaign. Luapula Province, being predominantly rural, presented with limited access to health facilities, long distances between villages, and a lack of reliable infrastructure such as road networks, which made it difficult to reach all children. Additionally, the presence of refugee camps and frequent cross-border movement with the Democratic Republic of Congo (DRC) further complicated vaccination efforts due to high population mobility. A significant portion of caregivers in the district had only primary education or no formal education, which impacted their understanding and compliance with vaccination efforts. Moreover, economic activities such as fishing and trade limited the availability of caregivers to provide consent for children to be vaccinated.

According to recommendations by the Strategic Advisory Group of Experts on Immunization (SAGE), countries that were solely using OPV in their routine vaccination programs at the time of the switch were required to introduce at least a single dose of IPV by the end of 2015. This introduction was intended to reduce the risk of type 2 PV outbreaks in the event of reintroduction or emergence of the virus. However, a cohort of Zambian children remained susceptible to PV2 due to a gap of two years and two months that elapsed between the transition from tOPV to bOPV in April 2016 and the introduction of a single dose of IPV in

June 2018. This outbreak highlighted the risks of polio in this cohort and the urgent need to intensify polio vaccination efforts in Zambia. As a result, a catch-up SIA for IPV was conducted in June 2020 to provide IPV to children aged 3 to 59 months who missed type 2-containing polio vaccines from 2016 to March 2020.

According to recommendation by strategic advisory group of experts on immunization (SAGE), countries that were solely using OPV in their routine vaccination programs at the time of the switch were required to introduce at least a single dose of IPV in their vaccination program by the end of 2015. The importance of this introduction prior to the withdrawal of tOPV would be to reduce the risk of type 2 PV outbreaks in case of reintroduction or emergence of the virus [15]. As a result, a cohort of Zambian children remained susceptible to PV2 as there was a two years two months' gap that elapsed following the transition from tOPV to bOPV transition in April 2016 to the introduction of a single dose of inactivated polio vaccine (IPV) in June 2018. This outbreak highlights the risks of polio in this cohort, and the urgent need to redouble polio vaccination efforts in Zambia. As a result, a catch-up supplemental immunization activity (SIA) for IPV was conducted in June 2020 to provide IPV catch up to 3 to 59 months old children who missed type 2 containing polio vaccine. Prior to the 2019 cVDPV outbreak, majority of the health facilities only conducted passive surveillance for polio. This involved the surveillance focal point persons reviewing the inpatient and outpatient department registers for cases of AFP and ensuring that they were followed up and investigated for Polio. The district had to maintain a non-Polio AFP rate of two cases per 100,000 populations. In light of the heightened risk due to the isolation of PV2, all silent Districts in the country were instructed to conduct surveillance of AFP cases in all IDSR high priority reporting sites, including all District, secondary, and tertiary hospitals. AFP surveillance of children under 15 years in Chiengi District was heightened with a target to maintain a non-Polio AFP rate of four cases per 100,000 populations. The herd immunity of the catchment populations of the two facilities (Chipungu RHC and Lunchinda RHC) assessment identified a gap in PV2 immunity in the area. An SIA was conducted in Luapula Province providing three rounds of monovalent polio vaccine for PV2 (mOPV2) to all children <5yrs.

However, there were issues that may have compromised the findings of this research, including limited information on population movement within the province as well as cross-border movement with the neighboring DRC. Additionally, there was a lack of reliable data on case files to corroborate the reported history used to develop a timeline of events, which is subject to recall bias. The patient's file reviewed at the local hospital was not comprehensive, and the case file used in the DRC was not available for review.

Conclusion

The investigation has unequivocally identified the presence of poliovirus within the stool samples collected from the index case's community, marking the confirmation of a cVDPV outbreak within Zambia. This occurrence signifies a noteworthy emergence of cVDPV since the transition from tOPV to bOPV, underscoring the persisting vulnerability of the population to Polio outbreaks.

In light of these findings, it is evident that addressing the issue of cross-border transmission is of paramount importance. We recommend the establishment of cross-border committees tasked with overseeing information sharing, coordination of joint planning and investigative activities, and devising strategies to maximize vaccination coverage among mobile populations. These proactive measures will not only minimize the risk of virus importation but also facilitate the timely detection of Polio cases. Such measures align with the International Health Regulations (IHR) of 2005, which emphasize the need for coordinated international responses to public health threats.

In the pursuit of a Polio-free Zambia, it is imperative to continue strengthening Acute Flaccid Paralysis (AFP) surveillance systems, especially in rural-border towns and silent districts. Additionally, efforts should focus on improving vaccination coverage for both bOPV and IPV throughout the country. A critical step in this direction was the mass vaccination campaign with mOPV2 across Luapula province and the IPV catch-up campaign conducted in June 2020, aimed at reaching children aged 3 to 59 months who missed type 2-containing polio vaccines from 2015 to March 2020.

Furthermore, the expansion of environmental surveillance beyond the current two provinces to include additional regions is essential. This broader scope will enhance our ability to identify any polioviruses that may be silently circulating in the population, enabling more proactive and effective response strategies. In conclusion, our findings highlight the urgency of collective action, both within Zambia and in collaboration with neighboring countries to strengthen immunization efforts, surveillance systems, and cross-border cooperation. Only through these concerted efforts can we strive towards a Polio-free future.

What is known about this topic

- Poliomyelitis is a highly infectious disease that is transmitted via the faecal oral route;
- There is no treatment for poliovirus and prevention via vaccination remains the only established method of preventing infection;
- Areas with populations that have low population immunity are at increased risk of having circulating vaccine derived polio viruses which occurs when the vaccine-virus is able to circulate for an extended period of time uninterrupted resulting into the virus mutating to reacquire neurovirulence.

What this study adds

- This study identifies the first polio case in Zambia in 17 years following the last case in 2002
- The polio case like many other Zambian children born between 2016-2018 remained susceptible to type 2 polio virus due to a two-year plus gap between withdrawal of trivalent oral polio vaccine (tOPV) until the introduction of inactivated Polio vaccine (IPV) in 2018
- The study identifies the need to intensify acute flaccid paralysis (AFP) surveillance and strengthen sensitization of health worker on AFP surveillance in order to detect and interrupt transmission in a timely manner.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

Musole Chipoya: responsible for the initial idea and design of the study, developed the methodology and protocols used in the study. Albertina Ngomah: generated the original draft and reviewed and revised the manuscript, including any critical revisions. Kennedy Matanda and Dien Francis Mwansa: managed and coordinated the project. Belem Matapo: developed the data collection tools and conducted data analysis for the study. Lwara Kalembe Musa: took medical history and conducted physical examination of the patient. Mwaka Monze: provided the laboratory resource necessary for the research. Mwangala Situmbeko: Conducted data collection in the field. Davis Simwaba and Nkomba Kayeyi: reviewed and revised the manuscript, including any critical revisions. Danielle Barradas: Conducted data collection and was one of the team leads for the field activities. Muzala Kapina, Nyambe Sinyange and Jonas Hines: supervised the project.

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Table

Table 1: Community Coverage Survey

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Table 1: Community Coverage Survey Characteristics	(n=60)
Age	(* 55)
Mean (range)	2.3 (3-58)
Sex	<u> </u>
Male	36 (60.0%)
Female	24 (40.0%)
Education of care giver	<u> </u>
None	23 (38.3%)
Primary	34 (56.7%)
Secondary	3 (5.0%)
Tertiary	0 (0.0%)
Vaccination	<u> </u>
Fully vaccinated	47 (78.3%)
Partially vaccinated	11 (18.3%)
Not vaccinated	1 (1.7%)
Does not know	1 (1.7%)
Card	•
Yes	49 (81.7%)
No	11 (18.3%)
Polio vaccination	
OPV0	33 (55.0%)
OPV1	57 (95.0%)
OPV2	54 (90.0%)
OPV3	48 (80.0%)
OPV4	11 (18.4%)
OPV3 + IPV	14 (23.3%)