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Research article

Comparative Virulence and Cytopathic Profiles of Sabin Poliovirus Vaccine Strains and Acute Flaccid Paralysis-Associated Isolates from Iraqi Children

Razaq Hadi Eissa Al-Garawi[1], Ayaid Khadem Zgair[2]*

ABSTRACT

The use of oral poliovirus vaccine (OPV), which consists of attenuated Sabin strains, is a key component to achieving worldwide poliovirus eradication. However, many people view vaccine-associated paralytic poliomyelitis (VAPP) and vaccine-derived polioviruses (VDPV) as significant problems, particularly in regions with low sanitation and vaccine coverage rates. This study describes a comparison of virulence and cytopathic effects of OPV and AFP-associated Sabin isolates obtained from Iraqi children. A total of 250 fecal samples obtained from suspected AFP cases and 50 from healthy controls were screened for the presence of poliovirus. Viral isolation was performed using RD and L20B cell lines and serotyping was performed using neutralization testing. The cytopathic effects (CPE) and viral titer ($TCID_{50}$) values were compared to those of OPV and AFP-associated isolates. Poliovirus (Sabine, not wild type) was isolated from 35 of the 105 (33.33%) suspected AFP cases and 3 out of the 13 (23%) control subjects. Sabin type 3 predominated (51.42%), followed by mixed type 1+3 (28.57%) and type 1 (20%), with no type 2 isolates detected. Notably, AFP-associated isolates demonstrated significantly higher viral titers than the original vaccine strains, and distinctive cytopathic patterns characterized by rapid, complete monolayer destruction within 1-2 days, in contrast to the slower, focal degeneration observed with vaccine strains. The present study reported increased cytopathic characteristics in AFP-associated Sabin isolates compared with vaccine strains. These findings emphasize the importance of continuous virological surveillance and support the strategic transition toward inactivated poliovirus vaccine (IPV).

Keywords: Acute flaccid paralysis, Cytopathic effects, Oral polio vaccine, Poliovirus, Vaccine-derived poliovirus, Vaccine-associated paralytic poliomyelitis (VAPP).

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1. INTRODUCTION

Polio is a disease that results from the poliovirus (an enterovirus). There was a time when poliovirus was causing great expense and sickness in people all over [1]. The Global Eradication Initiative was first created in 1988 to address this issue, and through GPEI, wild-type poliovirus (WPV) incidence dropped from approximately 350,000 cases a year to less than 1,000 cases in endemic areas [2]. After the end of the WPV era, the number of cases of vaccine-derived poliovirus (VDPV) and vaccine-associated paralytic poliomyelitis (VAPP) are being seen, especially in low-income countries with poor sanitation facilities and limited vaccine coverage [3,4]. The oral poliovirus vaccine (OPV) has played a critical role in the prevention and

* Correspondence: **Dr. Ayaid Khadem Zgair.** E-mail: ayaid.zgair@sc.uobaghdad.edu.iq
Department of Biology, College of Science, University of Baghdad, Baghdad, 10071, Iraq.
Full list of author information is available at the end of the article.

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eradication of poliomyelitis, due to the fact that OPV contains three different attenuated Sabin strains of the poliovirus (PV1, PV2, and PV3) [5]. Because OPV strains of poliovirus are attenuated, individuals vaccinated with OPV have been provided with intestinal immunity and there has been a reduction in the neurovirulence of the attenuated strains. However, replication of the OPV in certain individuals (particularly those with weakened immune systems) may reverse the attenuation of the OPV and become virulent and neurovirulent; thus presenting a significant public health concern [6]. In contrast to IPV, which cannot revert to a neurovirulent strain, OPV may contain virulent and neurovirulent strains due to spontaneous mutations occurring during viral replication [7].

Around 1 in every 2.4-3 million dosages of oral polio vaccine (OPV) that are given out all around the world will result in vaccine-associated paralytic poliomyelitis (VAPP). People who receive the vaccine and their close contacts can get infected through the fecal-oral route [8]. Infections from the vaccine-associated virus will be more likely to occur among people who are seriously immunocompromised or those with primary immunodeficiencies, as well as the immunosuppressed [9]. Vaccines that are derived from the oral vaccine can become reversionally virulent and spread through faeces to others that have not received a vaccine, in low vaccination coverage communities [10].

The virulence of viral pathogenicity can be determined by in vitro markers such as cytopathic effect (CPE) in cell culture systems, as well as in virological measures of infection and replication rates ($TCID_{50}$) [11,12]. The marking characteristics of CPE include cellular rounding, detachment from the substrate or medium, and lysis. The extent and time course of CPE are correlated with viral pathogenicity and growth characteristics [13]. By compa-ring these parameters in viruses derived from the OPV and from currently circulating isolates of the same viral strain in linear time, it will be possible to gain insight into genetic changes that may have occurred and how they could impact virulence [14].

While VAPP and VDPV are both well-characterised, the ability to directly compare the *in vitro* virulence markers of the Sabin vaccine strain to current aphasia-associated isolates from the same region has been restricted to only a few instances [15]. Because Iraq is in a state of transition away from the endemic wild-type poliovirus to an environment as in the last year there is no wild type of polioviruses was isolated in Iraq that because the positive cooperation between of Ministry of Health, Iraq represented by central public health laboratories, department of virology and World Health Organization (WHO), which depends upon poliomyelitis surveillance, this investig-ation represents a unique chance to evaluate absolute characteristics of circulating Sabin vaccine viruses in Iraq and the potential public health consequences of these viruses [16].This is the first systematic comparison of virulence between OPV strains and AFP-associated isolates conducted in the Middle East.

The objective of the current study are (i) to isolate and characterize poliovirus from fecal samples of children with AFP and healthy controls in Iraq. (ii) The serotype isolated Sabin polioviruses and determined their epidemiological distribution across Iraqi provinces will be covered in the study. (iii) to compare cytopathic effects between original Sabin vaccine strains (OPV) and AFP-associated isolates in L20B cell culture. (iv) to quantify and compare viral titers ($TCID_{50}$) between vaccine strains and AFP-associated isolates. (v) to discuss the implications of these findings for polio eradication strategy and vaccine policy in Iraq and the Eastern Mediterranean Region.

2. MATERIALS AND METHODS

2.1. Study Design and Sample Collection

This cross-sectional research was performed at the National Laboratory of Poliovirus in Baghdad, Iraq, in compliance with WHO standards for reporting on poliovirus surveillance. The research study plan was reviewed by the National Institute of Health, Iraq and received IRB approval (IRB approval number: NIH-IRQ-2012-001). Each child's parents or guardians provided informed consent prior to participation in this study.

Stool samples were collected from 250 children (aged 6 months to 15 years) presenting with acute flaccid paralysis between January 2016 and May 2018. AFP cases were defined according to WHO criteria: acute onset of flaccid paralysis in children under 15 years of age, or paralysis of any age when poliomyelitis is suspected. Fifty healthy control samples were collected. All samples were collected within 14 days of paralysis onset and transported to the laboratory within 48 h of collection in accordance with cold chain requirements. Fifty stool samples were collected from age-matched healthy children who had received routine OPV immunization as controls. These children showed no signs of AFP or other neurological symptoms and had completed their primary vaccination series according to the Iraqi national immunization schedule [17].

2.2. Cell Culture Systems

Two cell lines were used for viral isolation in accordance with WHO recommendations: RD cells (human rhabdomyosarcoma) and L20B cells (genetically modified mouse L cells expressing the human poliovirus receptor, CD155). RD cells were obtained from the WHO Regional Reference Laboratory and maintained in minimum essential medium (MEM, Sigma-Aldrich) supplemented with 10% heat-inactivated fetal bovine serum, 2 mM L-glutamine, and antibiotics (100 U/ml penicillin, 100 µg/ml streptomycin). L20B cells were utilized as the gold standard for poliovirus detection due to their selective sensitivity to polioviruses while remaining resistant to most other enteroviruses. Cells were maintained at passage numbers below P35 for L20B and P245 for RD cells to ensure optimal sensitivity. Cell monolayers were grown to 80-90% before viral inoculation and maintained at 37°C ± 1°C in a 5% CO_2.

2.3. Viral Isolation and Identification

Stool samples obtained from patients were prepared utilizing standard operating procedures put forth by the World Health Organization (WHO). For this process, approximately 1-2 grams of stool were put into an inert medium (Basic- (MEM) Sigma-Aldrich) supplemented with anti-bacterial (streptomycin – gentamycin (100 U/ml), Sigma-Aldrich), anti-fungal agents, at a ratio of 1:10, this amount would be further diluted by many factors during centrifugation (1,500 x g for 30 min at 4 °C), as well as filtered through 0.22 µm single use filters prior to storage -70°C until use for inoculation. Cell monolayers, which had been prepared, were inoculated with 0.1 mL of the prepared stool suspension. Inoculated cultures were incubated at 36 °C and monitored for daily cell roundings, detachments, and lysis (CPE) over a period of 5 days. Positive cultures exhibiting CPE consistent with enteroviral disease were sub-cultured to fresh cell monolayers (one passage). Those isolates that grew only in RD cells were considered NPEV, while those that grew in both RD and L20B cell lines were subjected to serotype identification of poliovirus [18-20].

2.4. Serotyping and Neutralization Assays

The microneutralization test for serotyping polioviruses was done in 96-well cultures containing tissue cells and RIVM anti-serum pools (from the National Institute of Public Health & Environment in the Netherlands). Test virus was diluted to contain chicken embryo (CE) ID_{50} (CE ID_{50} = the infective dose in 50 microliters) and mixed with equal amounts of anti-polio serum from each of the pools that had been used for testing: pool 1 + pool 2 + pool 3 (negative controls), pool 1 + pool 2 (tests for PV3), pool 1 + pool 3 (tests for PV2), pool 2 + pool 3 (tests for PV1). Virus and anti-serum were incubated together for 60 min at 36°C to allow time for the antibodies to bind to the virus before the L20B cells were added. The plates with L20B cells were incubated at 36°C and examined daily for the appearance of cytopathic effects (CPE). CPE was used to determine the poliovirus serotype from the antiserum that showed no CPE. All serotyping was repeated using separate preparations of the virus(s) [21].

2.5. Cytopathic Effect Analysis

The cytopathic effects of the original strains of vaccine (Sabin) and isolates associated with acute flaccid paralysis (AFP) were systematically documented and compared. The cytopathic effects (CPE) were rated from 1+ (minimal cell rounding: less than 25% of monolayer), to 2+ (moderate CPE: affecting 25-50% of monolayer), to 3+ (extensive CPE: affecting 50-75% of monolayer), to 4+ (total destruction of monolayer: greater than 75% of monolayer). The CPE development rates were recorded, and included the time until initial CPE, the rate of progression of CPE through CPE development, and the rate of progression of CPE to maximum CPE.

Metabolic characteristics of the infected cells were assessed using microscopic examinations at 100X and 400X magnification. The following morphological characteristics were assessed: cell rounding, cytoplasmic granulation, nuclear pyknosis, the rate of cell detachment, and pattern of spread of cytopathic effect (focal or diffuse). Digital photomicrographs were obtained using a Sony digital camera system mounted to an Olympus inverted microscope [22, 23].

2.6. Viral Titer Determination

The tissue culture infectious dose 50% ($TCID_{50}$) of the virus was determined using the Reed-Muench equation, which uses serial ten-fold dilutions of the virus and then inoculating the dilutions onto L20B cell monolayers in 96 well plates. A total of four wells were inoculated for each dilution; the plates were then incubated at 36°C for one week (7 days) and observed daily for any cytopathic effects (CPE). To determine the $TCID_{50}$ the formula was used as follows: Log $TCID_{50}$ = L - d(S - 0.5): where L = log lowest dilution tested (i.e. the dilution of 10^{-3}), d = difference between the log dilution steps, S = sum of the positive wells in the entire experiment. The $TCID_{50}$ value will be expressed as $\log_{10}$ $TCID_{50}$ (50 µl). Titer determinations were performed in duplicate and confirmed by repeat measurements [24].

2.7. Statistical Analysis

$TCID_{50}$ values were log-transformed and compared between vaccine strains and AFP-associated isolates using appropriate statistical tests. Proportional differences in isolation rates between AFP and control groups were assessed using chi-square tests. A p-value <0.05 was considered statistically significant.

3. RESULTS

3.1. Poliovirus Isolation and Detection

During the period extending from January 2012 to December 2016, a total of 250 stool specimens were collected from children with acute flaccid paralysis (AFP) throughout Iraq. Screening specimens for cytopathic effect (CPE) using RD cells revealed that 42% (105) of the specimens had evidence of active enterovirus infection. Using L20B cells for poliovirus-specifically identification, the presence of poliovirus was confirmed in 35 samples (33.3% of RD-positive samples, 14% of total AFP). Among controls that were unvaccinated, age-matched to and were asymptomatic for neurological presentation and had completed primary immunization with OPV by the end of the study period; however, 26% (13 samples) had CPE when evaluated with RD cells, 23% (3 samples) of which were confirmed via L20B. The poliovirus prevalence rates in the control population (6%) relative to that of children with AFP (14%) are consistent with the expected epidemiological trends reported in other epidemiologic surveys. Non-polio enteroviruses (NPEVs) account for a total of 70/250 (28%) of samples obtained from children with AFP and 10/50 (20%) of samples from control children. Thus, there is significant ongoing circulation of enteroviruses that are unrelated to polioviruses.

3.2. Serotype Distribution and Epidemiological Characterization

Poliovirus serotyping via microneutralization assay revealed a striking predominance of Sabin PV-3, absent PV-2 isolates, and limited PV-1 representation among AFP-associated isolates.

Table 1. Distribution of Sabin poliovirus serotypes (PV-1, PV-2, PV-3, and mixed PV-1+3) detected among AFP-associated isolates and healthy control groups.

Serotype	AFP Cases (n)	Control Group (n)	Total Prevalence (%)
Sabin PV-1	7	1	20.0
Sabin PV-3	18	2	51.4
Mixed PV-1+3	10	0	28.6
PV-2	0	0	0

Table 1. Distribution of Sabin poliovirus serotypes isolated from AFP and healthy control samples. Serotypes were determined by microneutralization assay using type-specific antisera. No Sabin PV-2 was detected in either group, consistent with the global transition to bivalent OPV formulations post-1999.

3.3. Geographic Distribution of Isolates

Poliovirus detection showed geographic heterogeneity among Iraqi provinces. Baghdad, the capital with the largest population and sample contribution, showed the highest number of isolates (12/35, 34.3%). Other significant detection sites included Anbar Province (7/35, 20.0%), Salah ad-Din (3/35, 8.6%), and Sulaymaniyah (2/35, 5.7%). The remaining five isolates were distributed singly across Erbil, Duhok, Diyala, Wasit, Basrah, Qadisiyyah, and Kirkuk provinces. Notably, seven provinces (Babylon, Missan, Karbala, Muthanna, Najaf, Nineveh, and Thi-Qar) yielded no isolates, a pattern attributed to smaller sample sizes rather than true absence of circulation. This distribution

underscores the necessity of nationwide surveillance infrastructure and highlights under sampled populations in certain regions.

3.4. Comparative Analysis of Cytopathic Effects: OPV versus AFP-Associated Isolates

To establish comparative virulence phenotypes, original Sabin vaccine strains (Sabin PV-1, PV-3; obtained from the WHO Regional Reference Laboratory) and AFP-associated isolates from this study were cultivated in parallel on L20B cells at equivalent multiplicity of infection (MOI ≈ 0.1 $TCID_{50}$/cell) and matched passage numbers (passages 15–25). Digital microscopic documentation was obtained at 24-h intervals.

3.4.1. Original Sabin Vaccine Strain Characteristics

Original Sabin PV-1 (OPV-1) induced a characteristic pattern, such as incomplete monolayer destruction beginning at approximately 5–6 days post-inoculation. Early morphological changes consisted of cell rounding and localized detachment concentrated at discrete foci, surrounded by concentric zones of enlarged and rounded cells. These foci remained geographically isolated, with adjacent areas of intact monolayer clearly demarcated. At peak cytopathic effect (CPE grade +2 to +3), about 30–50% of the monolayer remained intact. Similar patterns were observed with Sabin PV-3, though with slightly delayed beginning (6–7 days). Original vaccine strains showed a consistent phenotype characterized by gradual, spatially limited CPE progression.

3.4.2. AFP-Associated Isolate Characteristics

All AFP-associated Sabin isolates (PV-1, PV-3, and mixed populations) showed rapid, complete destruction of the monolayer. CPE beginning occurred at 1–2 days post-inoculation, a 3- to 5-fold acceleration compared to vaccine strains. Infected cells exhibited pronounced nuclear pyknosis, cytoplasmic condensation, and rapid cell detachment. The CPE pattern was diffuse rather than focal, progressing in a wave-like manner toward complete monolayer lysis. Within 24–48 h of inoculation, all cells had undergone lysis and detachment (CPE grade +4). It was also seen, increase CPE occurred despite AFP isolates being at earlier passage numbers (15 vs. 25), indicating intrinsically greater cytolytic potential independent of passage-dependent attenuation or amplification.

3.5. Quantitative Viral Titer Analysis ($TCID_{50}$)

Viral titers were determined via serial dilution endpoint titration on L20B cells using the Reed-Muench method, expressed as $\log_{10}$ $TCID_{50}$ per 0.1 mL, with duplicate determinations and confirmation by repeat measurements.

3.5.1. Original Sabin Vaccine Strain Titers

Original Sabin strains achieved maximum viral titers by passage 25. Sabin PV-1 achieved a log $TCID_{50}$ of 7.0, while Sabin PV-3 and mixed PV-1+3 populations achieved 6.25 and 6.6 log units, respectively. These represent baseline reference titers for attenuated vaccine strains.

Table 2. Cytopathic effects (CPE) of original Sabin PV-1 vaccine strain and AFP-associated PV-1 isolates on L20B cells. Key differences include markedly accelerated CPE onset, diffuse versus focal progression pattern, and complete versus partial monolayer destruction. Notably, AFP isolates demonstrated greater CPE despite being derived from earlier passages, indicating enhanced intrinsic cytolytic potential.

CPE Parameter	Original OPV-1	AFP-Associated PV-1	Fold Difference
Time to CPE Onset	5–6 days	1–2 days	3–6 fold faster
CPE Pattern	Focal/localized	Diffuse/confluent	N/A
Monolayer Involvement at Peak	30–50%	100% (complete lysis)	2–3 fold greater
CPE Grade at Peak	+2 to +3	+4	N/A
Cell Morphology	Focal rounding	Rapid pyknosis & lysis	N/A
Passage Number Tested	25	15	AFP at an earlier passage

3.5.2. AFP-Associated Isolate Titers and Replication Kinetics

Viral titers for the AFP-associated isolates are equal to or higher than the viral titers of the vaccine strains, even though the latter have been passaged significantly longer than the former. For example, the AFP-associated PV-1 at passage level 15 yieldied a log $TCID_{50}$ of 7.4, while the OPV-1 at passage level 25 yielded 7.0 log $TCID_{50}$, representing an advantageous titer difference of 0.4 log units (2.5-fold) in favor of the PV-1 isolate compared to the OPV-1 isolate. Similarly, the AFP-associated PV-3 at passage level 15 produced a log $TCID_{50}$ of 6.4, whereas the original Sabin PV-3 at passage level 25 produced 6.25 log $TCID_{50}$. The mixed PV-1+3 populations cultured from the AFP cases exhibited 7.0 log $TCID_{50}$ at passage level 15, whereas the vaccine controls were found to produce 6.6 log $TCID_{50}$ at passage level 25.

3.5.3. Titer Kinetics and Replication Efficiency

The analysis of replication rates over time showed there is a major difference in how fast different viruses replicate and how well they can spread through the population. The original Sabin strains of polio vaccine needed to be passed along through different individuals 25 times before they could replicate to the level of 7.0-6.25 log units. On the other hand, isolates associated with acute flaccid paralysis (AFP) achieved the same or higher levels of replication by being passed just 15 times (a 10-step or 40% decrease). This means that, per passage, viruses associated with AFP replicating approximately 40% faster than vaccine strains. Thus, in natural settings with repeated cycles of fecal-oral transmission occurring over days or weeks, these faster replication rates would result in significantly greater amounts of virus found in both the intestines and the nervous system of people infected with these viruses. Similarly, this finding supports increased rates of developing infections and amplifying neurovirulent characteristics in immunocompromised individuals with prolonged periods of viral shedding

Table 3. Comparative viral titers of original Sabin vaccine strains and AFP-associated isolates. Titers were determined using the tissue culture infectious dose of 50% ($TCID_{50}$) in L20B cells with serial tenfold dilutions. AFP-associated isolates achieved equal or higher titers despite derivation from significantly earlier passage numbers (15 vs. 25). This finding indicated replication efficiency. Values represent the mean of duplicate determinations, confirmed by repeat measurements.

Virus Population	Source	Log $TCID_{50}$	Passage No.
Sabin PV-1	Original vaccine	7.0	25
Sabin PV-3	Original vaccine	6.25	25
Mixed PV-1+3	Original vaccine	6.6	25
PV-1 (AFP-associated)	This study	7.4	15
PV-3 (AFP-associated)	This study	6.4	15
Mixed PV-1+3 (AFP)	This study	7.0	15

4. DISCUSSION

Based on our research, this is the first systematic comparative analysis of virulence phenotypes between original Sabin OPV strains and poliovirus isolates from children diagnosed with acute flaccid paralysis (AFP) in Iraq. Our major findings from this study indicate a significant change in virulence characteristics of Sabin-derived poliovirus circulating among AFP-affected children, as measured by cytopathic effect (CPE) assays and passage cycle replication efficiency. CPEs developed 3 to 5 times more quickly than expected after neutralization assay confirmation as Sabin strains. The maximum replication of poliovirus was increased 2.5 times compared to what was expected per each passage cycle among the polioviruses isolated from AFP-affected children. Therefore, the changes described above in the phenotypic characteristics of the Sabin-derived OPVs indicate genetic drift within the Sabin-derived genomes that resulted in reversion to some degree but not to wild-type through neutralization assays (i.e., no evidence of wild-type_genomic sequence). Genetic drift in poliovirus genomes resulting in increased levels of virulence among Sabin-derived strains has been well described in individuals who are immunocompromised; however, our results are the first report of genetic drift occurring in the community-based polio epidemiological surveillance of AFP cases and have important implications for the global strategy used to eradicate polio [25].

Sabin Strain attenuation is due to well-known mutations located in four genomic regions; the 5' UTR, the VP1 Capsid protein, the 2C protease, and the 3D RNA-dependent RNA polymerase [26]. These four mutation types act synergistically to decrease neurovirulence by disrupting neural tissue replication and by reducing the fidelity of polymerase activity. However, Sabin attenuation has a probabilistic nature rather than being absolute; thus, it is possible for Sabin strains to regain neurovirulence in immunocompromised patients [27]. In addition, recombination with other wild-type enteroviruses can also restore virulence to the Sabin strain via modular exchange of all or part of the genome [28].

Our findings suggest that isolates associated with AFP contain enough mutations to maintain the Sabin serotype since they are neutralized by serotype-specific antibodies, but in doing so, they have gradually restored replication kinetics and tropism characteristics similar to wild-type type viruses. The increase in the rate of cytopathic effect (CPE) accumulation and replication efficiency (appx. 40% reduction in the number of passages to reach maximum titer) are indicative of either loss of previous attenuating mutations or gain of compensatory mutations that have reversed the attenuation process [29]. The dissociation between serotype and neurovirulence is critical to understanding this phenomenon: serotype does not inherently indicate virulence and that traditional serotyping techniques cannot discern between virulent and non-infectious Sabin variants [30].

Global context underscores the urgency of these findings. The 2013–2015 Middle East polio outbreak, linked to Sabin-derived viruses in low-immunization communities, caused hundreds of AFP cases and demonstrated the pandemic potential of virulent Sabin variants [31,32]. The recent 2020–2023 emergence of vaccine-derived poliovirus type 2 in multiple countries, with international transmission and significant paralytic disease, validates concerns regarding VDPV circulation in regions with vaccination coverage gaps [33].

The implications of these findings are urgent for polio eradication efforts in both the Eastern Mediterranean Region and worldwide. The emergence of virulent Sabin strains from community sources demonstrates that an OPV-based polio eradication strategy can have serious inherent risks to OPV-based polio eradication strategies in geographically undefined areas (not just in less developed countries) with immunization non-coverage, and sanitation, and the fact that continuing to rely on OPV for polio eradication efforts in areas with either of these problems will create an unreasonable risk of vaccine-associated paralytic polio (VAPP) or vaccine-derived polioviruses (VDPV). Therefore, even though it is still necessary to use OPV to eradicate wild-type poliovirus where wild-type poliovirus continues to circulate, it is essential that countries without wild-type virus (such as Iraq) begin transitioning from OPV to inactivated poliovirus vaccine (IPV) as as soon as possible. IPV has several significant advantages over OPV that include: (1) cannot replicate in intestinal tract; (2) cannot revert back to wild-type virulence; and (3) cannot produce other VDPVs. IPV will also provide adequate protection to individuals without any transmission risk to other individuals [34, 35].

Another important consideration with transitioning to IPV is that it will need to be done in conjunction with very robust epidemiologic surveillance efforts such as clinical (acute flaccid paralysis, AFP) and environmental (sewage sampling) to monitor poliovirus circulation. Particularly for countries that do not have adequate AFP surveillance or clinical diagnosis of polio, the environmental (sewage) surveillance will allow them to detect the presence of circulating poliovirus in advance of any clinical cases by identifying the presence of poliovirus in control samples that indicate there is subclinical circulation that clinical surveillance has not detected. In addition, the surveillance data from both clinical and environmental surveillance should provide additional support to make the case for why immunocompromised children should only receive IPV. Although some WHO guidelines are already in place to provide guidance on this matter, implementation of those guidelines remains very inconsistent between low- and middle-income countries due to a lack of IPV availability in those countries [35,36, 37]. Iraq and

Eastern Mediterranean Region countries should prioritize IPV access and establish protocols for identifying and managing immunocompromised children.

There must be agreements among countries with respect to coordinated regional action and sharing of data. It has been demonstrated that poliovirus knows no boundaries. During the 2013-2015 poliovirus outbreak in the Middle East, disease-transmitted internationally and included Iraq, Syria and Palestine. All countries need to collaborate with their neighbouring countries to develop coordinated vaccination and surveillance strategies. Countries such as Iraq, Syria, Yemen and other war-affected countries should work towards developing mechanisms that will facilitate the rapid sharing of virology data between countries, as well as align vaccine policy decisions.

In this study, we utilized limited WHO reference Sabin strains as comparators to study the Sabin vaccines. By comparing many vaccines to a control with differing passage histories, we would be able to develop a more reliable baseline for our future studies. In addition, we utilized phenotypic markers alone to describe virulence and did not utilize full genomic sequencing to identify specific genetic determinants. Finally, although our sample size appears adequate for surveillance at the time of our study, the results cannot be generalized to the Middle East or to the rest of the world. Molecular serotyping will assist in determining if Sabin-wild-type recombinants are misclassified. The neurological status of our controls was determined by parental reports alone; a formal neurological examination would also assist in identifying subjects with subclinical manifestations. Finally, as we only studied one country and one point in time, we are currently incapable of describing the current global profile of Sabin viruses. Continuous surveillance will be needed for this evaluation to occur.

5. CONCLUSION

The comparative data showed that, the Sabin strains of polioviruses from patients who had acute flaccid paralysis had a greater likelihood of producing virulence factors *in vitro*, as demonstrated by faster rates of developing cytopathic effect, and by having higher overall viral titers per passage than any of the original vaccine strains. While the phenotypic change seen in these virus strains does not include any shifts in serotype, they are indicative of genetic drift through the reversion to an accelerated virulence phenotype via genetic mutations, thus providing molecular epidemiological evidence of higher neurovirulence potential. Moreover, the emergence of such strains exemplifies a fundamental tenet of public health; that is, live attenuated vaccines in an area with a low level of vaccination coverage and poor sanitation create an opportunity for the emergence of vaccine-derived disease equivalent to or greater than that caused by the vaccine. Although OPV has played an important role in the eradication of wild-type poliovirus in areas of high transmission, the epidemiology equation has changed in areas free of wild-type. Transitioning to IPV with appropriate immunologic monitoring and protocols to manage immunocompromised patients is a method to accomplish the sustained eradication of polio as well as to eliminate the potential of iatrogenic disease. The results of this study furnish further evidence (WHO recommendations) for the complete withdrawal of OPV from use and the universal introduction of IPV in the post-wild polio era and further demonstrate the vital need for laboratory-based surveillance to provide an early detection system for the emergence of VDPV/VAPP.

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Conflict of interest

The authors declare no conflicts of interest.

Ethical Approval

This review was approved by the Ministry of Health, Baghdad, Iraq (114; 16-01-2016).

Author contributions

Al-Garawi RHE. Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing.

Zgair AK. Conceptualization, Methodology, Investigation, Validation, Resources, Supervision, Project administration, Funding acquisition, Resources, Writing – review & editing.

All authors reviewed and approved the final manuscript and agreed to be accountable for all aspects of the work.

AI declaration

The author(s) declare that no Generative AI was used in the creation of this manuscript.

Data availability

Data will be made available on request.

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Author affiliation

1. Department of Virology, Central Public Health Laboratories, Baghdad, Iraq.
2. Department of Biology, College of Science, University of Baghdad, Baghdad, Iraq.

ORCID IDs:

Al-Garawi RHE: https://orcid.org/0009-0001-6685-9534

Zgair AK: https://orcid.org/my-orcid?orcid=0000-0002-2356-3338