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Review Article

Oral Poliovirus Vaccine (OPV) Manufacturing in Iran over Five Decades: from tOPV to bOPV and Future Planned Cessation

Behnam Alirezaie[1], Ashraf Mohammadi[1*]

1. *Department of Human Viral Vaccines, Razi Vaccine and Serum Research Institute (RVSRI), Agricultural Research, Education and Extension Organization (AREEO), Karaj, Iran*

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ABSTRACT

By the early 1970s, there were no manufacturers in Iran producing oral poliovirus vaccine (OPV). The only domestic OPV was produced by the Razi Vaccine and Serum Research Institute (RVSRI) in 1974 after receiving the Sabin seeds on 5 January 1973. The quality of six vaccine lots derived from them was deemed satisfactory after re-evaluation by two WHO reference laboratories. Then, the RVSRI OPV produced using human diploid cell (HDC) substrate was manufactured at a scale sufficient to meet nearly all domestic consumption. Vaccine lots that passed control tests were released lot by lot by the national regulatory authority after reviewing the summary protocol and selected independent testing. Following the global switch plan from trivalent OPV (tOPV) to bivalent OPV (bOPV) which protects against type 1 and 3, the RVSRI started manufacturing bOPV in 2014. In 2016, the tOPV was entirely replaced by the bOPV in Iran. For over 50 years, from 1974 to the present, more than 600 million doses of OPV, including both tOPV and bOPV, produced by RVSRI have been approved by the national regulatory authorities and utilized in Iran. Wild poliovirus was eradicated from Iran during this period. Although Iran shares borders with two polio-endemic countries, Pakistan and Afghanistan, there have been no recorded outbreaks of poliomyelitis in Iran for several years. This may be attributed to a sufficient level of herd immunity. Over 50 years of experience in Iran has shown that this vaccine is safe and efficient, and no increased incidence of adverse events following immunization (AEFI) was observed in Iranian OPV recipients. Without a doubt, in the post-eradication era, safe polio vaccines such as Sabin inactivated poliovirus vaccine (sIPV) and/or Virus-Like Particle (VLP) will completely replace OPV in Iran's national immunization program. This change aligns with the global movement to cease the use of OPV to finalize the risks associated with vaccine-derived polioviruses (VDPVs) and vaccine-associated paralytic poliomyelitis (VAPP).

Corresponding Author:
a.mohammadi@rvsri.ac.ir

https://orcid.org/0000-0003-4754-8316
https://orcid.org/0000-0001-5744-767X

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1. Context

Polioviruses (PVs) belong to the family *Picornaviridae* and together with other *Enterovirus* members constitute the largest genus in the family (1). PVs encompass three antigenically distinct serotypes (2), and their particles are small (~ 30 nm) and spherical. PV capsid composed of 60 protomers, each of which comprises four proteins VP1-VP4, VP1 being the most exposed and containing the most neutralization epitopes (2). PV genome is a single-stranded positive-sense RNA with a small protein (VPg) and poly-A tail at the 5' and 3' ends, respectively (2). Its single open reading frame (ORF) encodes a single polyprotein flanked by two untranslated regions (2). PVs are transmitted by the fecal-oral and respiratory routes (2, 3). After entering the body, the virus multiplies in the digestive system mucosa, tonsils, and Peyer's patches and is released into the blood (3). Following viremia, the circulating PV invades the CNS. Subsequently, PV replication in motor neurons and their destruction result in paralytic poliomyelitis (3). It is notable, however, that most PV infections are asymptomatic, and paralysis develops in less than 1% of those infected. Although humans are the only known natural host of the PV, some other primates and transgenic mice that express the human poliovirus receptor (CD155) can be experimentally infected (3).

Before the administration of vaccines, poliomyelitis had long been endemic around the world from 1300 BCE to the 21th century. Therefore, a vaccination program against polio was considered an urgent priority, and potent vaccines were needed. In the late 1940s, propagation of PVs in cell cultures (4) enabled the development of vaccines. Research on an inactivated polio vaccine (IPV) began in the USA in the 1950s. This vaccine consists of a trivalent serotypes which had been prepared in monkey kidney cells and treated with formalin for inactivation. An extensive clinical trial of the IPV vaccine was conducted in 1954 (5) and licensed in 1955. IPV application induces the formation of virus-specific antibodies in the circulation system. Although the virus can replicate in the gut of an immune individual, it does not reach the central nervous system (CNS) due to the presence of specific antibodies in circulation.

OPV was initially licensed in August 1960 by the U.S. Surgeon General. Sabin attenuated PVs developed by serial passaging of the wild strains in vivo and in vitro (5). Vaccination with OPV, similar to natural infection, results in the appearance of not only virus-specific antibodies (IgM and IgG) in the circulation but also of IgA antibodies in the oropharynx and gastrointestinal mucosa. It should be noted that local immunity (IgA antibodies in the gastrointestinal mucosa) is the first line of defense against PV. Therefore, OPV can prevent viral replication in the intestine and reduce transmission when used in mass campaigns. OPV can also indirectly immunize individuals who are exposed to the vaccine by contacting recently vaccinated persons due to shedding of the virus. Additionally, some studies showed that OPV can also induce protection against unrelated pathogens, especially respiratory viruses such as influenza and SARS-CoV-2, by stimulation of innate immunity in vaccine recipients and their unvaccinated contacts (6, 7).

After the introduction of immunization with these two traditional vaccine platforms, poliomyelitis incidence decreased dramatically in many countries. Today, two out of three serotypes of the wild polioviruses (WPV2 and WPV3) have been eradicated throughout the world, and WPV1 remains endemic only in two countries (Afghanistan and Pakistan) (8).

In this review, we examined the history of oral poliovirus vaccine (OPV) production for routine immunization in Iran. We also summarized some features of vaccine production, vaccination, and its consequences in Iran.

2. Data acquisition

This study collected information from several published sources across four databases: Web of Science, PubMed, Scopus, and Google Scholar. Databases were searched for studies indexed without a time limit, using the terms: Polio, Poliomyelitis, OPV, Iran, and AEFI. In addition, data collection in this study was conducted using library research. The collected documents were screened for their titles and abstracts. No automation tools were used for screening and selection of the literature.

3. Results

3.1 History of OPV production in Iran

In the late 1960s, the vaccination against poliomyelitis disease by imported IPV and OPV was initiated in some private clinics in limited areas of Iran. After 1969, oral polio vaccine (OPV) was administered to residents of several cities, including Tehran and Shiraz (9, 10). The only domestic tOPV was produced by the Razi Vaccine

and Serum Research Institute (RVSRI) in 1974 after receiving the first Sabin seeds on 5 January 1973 (11). At that time, the RVSRI needed to start manufacturing stockpiles of monovalent bulks, following the instructions from Dr Albert Bruce Sabin and the WHO technical report series (12).

Following the production and passing control tests, samples were sent to reference laboratories for further analysis. The RVSRI tOPV vaccine quality was found satisfactory by re-examination of two reference laboratories of WHO (11, 13). In 1975, one of the earliest mass vaccination campaigns in densely populated areas in Iran was launched against poliomyelitis by RVSRI tOPV (13). After the successful experience of the mass vaccination campaigns, the tOPV was manufactured by the RVSRI at a scale required to meet nearly the whole amount of domestic consumption. After establishing the Iranian national immunization technical advisory group (NITAG) in 1982, the national poliomyelitis eradication plan was prepared and performed under the supervision of the NITAG (14). Since 1984, mandatory routine immunization against polio with OPV has been established in Iran (15).

Iran achieved nearly 100% immunization coverage against polio in 2002, and this level has remained stable since then (15-17). Following the global switch plan from tOPV to bOPV, the RVSRI had to start manufacturing bOPV in 2014. In 2016, tOPV was entirely replaced by bOPV in Iran. Since 1974, OPV (tOPV and bOPV) produced by RVSRI has been used in Iran for over 50 years (Figure 1). By 2024, approximately 600 million doses of OPV (tOPV and bOPV) were released by the national regulatory authority. This indicates that domestically manufactured OPV by RVSRI met the needs of the national immunization program.

3.2 OPV manufacturing process

Production procedure of OPV has been described in detail elsewhere (18-20) (12). Here, we briefly highlight significant features of the procedure. The overview of the manufacturing process and laboratory tests of OPV is shown in Figure 2.

For the first time, after authorization and approval of Dr Albert Bruce Sabin, the Japan Poliomyelitis Research Institute generously supplied the Sabin seeds for RVSRI (11). Subsequently, the WHO periodically donated the viral seeds. The working seed viruses used for the types 1, 2, and 3 poliovirus components in the RVSRI OPV were the Sabin original Behringwerke, Sabin original Behringwerke, and RNA-derived Sabin original Pfizer strains, respectively (19). Since then, these seeds have been utilized for the production of OPV in Iran.

Cell substrate plays a vital role in viral vaccine production. It has been known that Sabin PVs can be cultivated in three types of cell substrate, namely primary monkey kidney cells (PMKC), human diploid cells (HDCs) such as MRC-5 and WI-38 cells, and Vero (a continuous cell line derived from African Green Monkey kidney cells). RVSRI had established an OPV manufacturing system based on HDC cell cultures. Such cells, which show some features including normal karyology, lack tumorigenicity and are free of exogenous agents (21), meet the requirements of the WHO. Therefore, HDC cells have been chosen for OPV production in Iran.

While some technical optimizations were implemented over the years, the principles remained unchanged. HDC cells that multiply and form confluent monolayers are used for the propagation of the virus (22). On the day of inoculation, the cell maintenance medium containing the seed virus is added to the cell cultures to replace the old cell supernatant. After the appearance of a remarkable cytopathogenic effect (CPE) of PV including rounding up, nuclear pyknosis, cell detachment from the glass, etc. (23), the cell fluid containing infected cells is harvested. The pooled virus suspensions are then passed through a sterile filter (clarification) and stored at below -40°C until used for formulation. There are, however, some critical production parameters in the production of OPV (20).

One of the most critical factors in virus cultivation is incubation temperature. After virus inoculation, cell cultures should be incubated at a constant temperature in the range of 33-35 °C for the maintenance of temperature sensitivity of the Sabin strains. Additionally, cultivated virus must be harvested no longer than four days post-infection (12). Laboratory tests are applied to the different steps of production. Some phenotypic and molecular features of the monovalent bulks of Sabin PV3 propagated in HDC were described elsewhere (24). To avoid redundancy, here, we briefly compared the titer of different harvests of PV1 and PV2 that propagated in HDC during this period (Figure 3).

The coefficient of variation (CV) of the harvest titers was calculated as 5.3% and 4.5% for types 1 and 2, respectively.

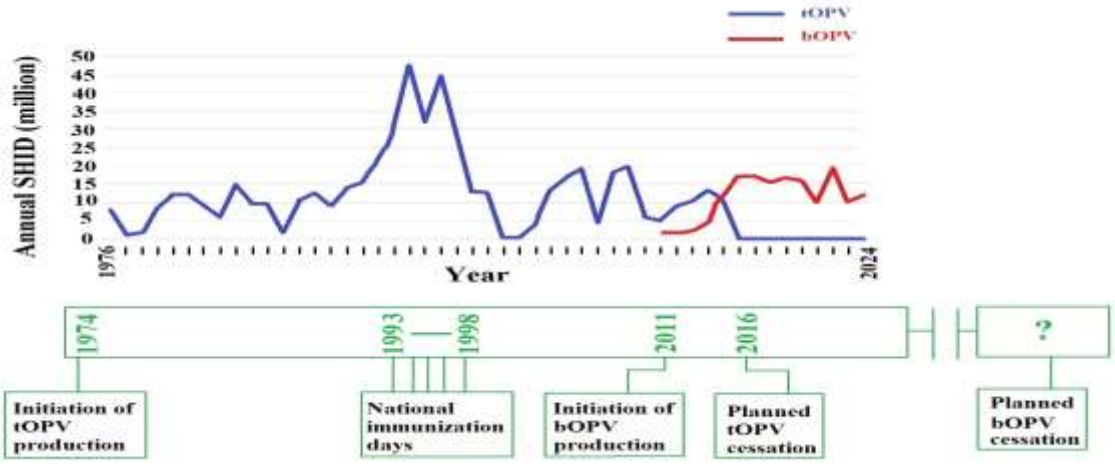

Figure 1. Summary of production and timeline of OPV in Iran.

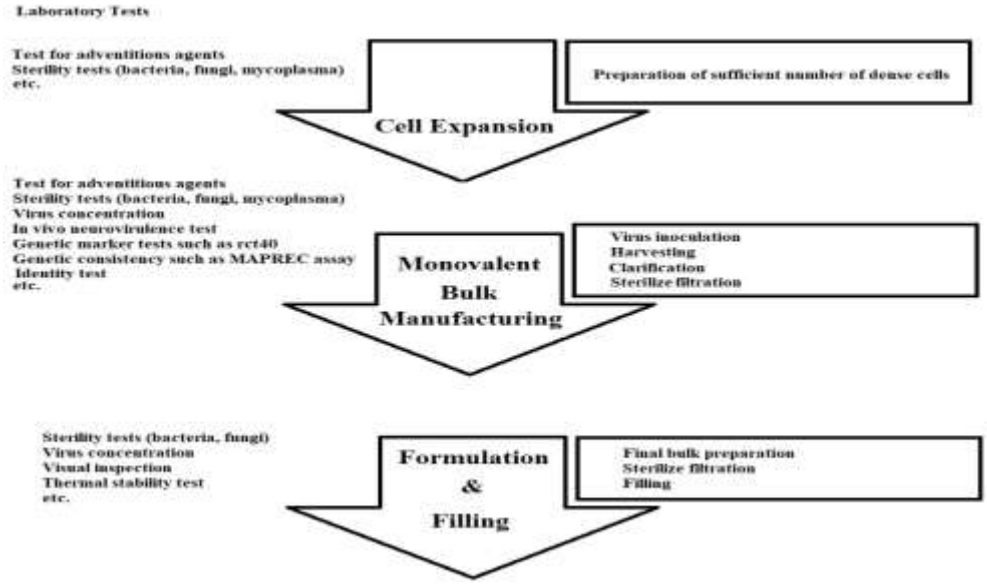

Figure 2. Overview of the OPV manufacturing process.

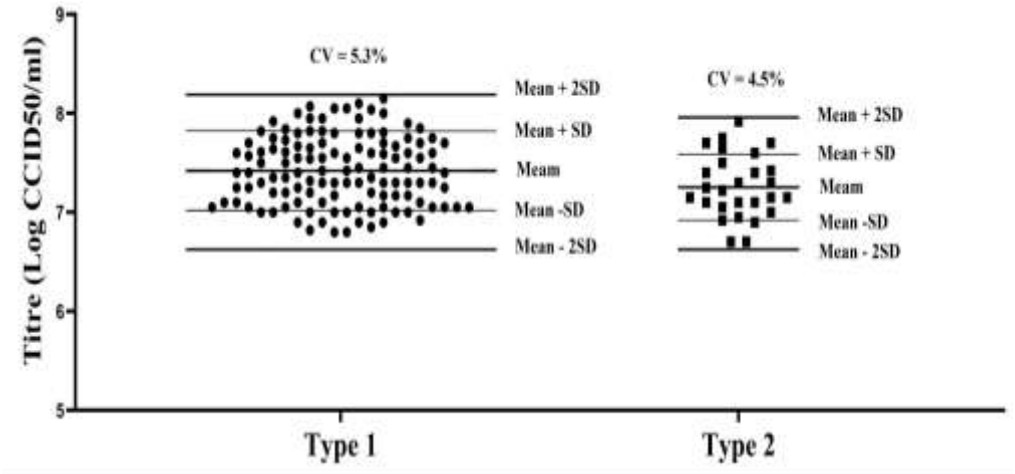

Figure 3. Comparison of different harvest titers of PV1 and PV2 that cultivated in HDC. Titers were determined by $CCID_{50}$ in HeLa cells.

Consequently, the potency trend during this time period indicated that the titer was consistent across different harvests. This result is also in agreement with other studies that indicate HDC cell substrate is suitable for PV cultivation (11, 24-28).

Before filling the vaccine, the final bulk should be formulated. According to the WHO TRS, each dose of OPV should contain at least 6.0 $\log_{10}$ $TCID_{50}$, 5.0 $\log_{10}$ $TCID_{50}$, and 5.5 $\log_{10}$ $TCID_{50}$ per human dose for types 1, 2, and 3, respectively (19). The stabilizer for the OPV contains I M magnesium chloride (29). After testing, the final bulk suspension is distributed into plastic tubes with fifteen-dose presentations. Vaccine lots that passed control tests are released lot by lot by the national regulatory authority after reviewing the summary protocol and selected independent testing.

3.3 Protective effect of vaccine

In the 1960s (the pre-vaccination period), poliomyelitis was an endemic disease in Iran (13, 30). Since the early 1970s, OPV produced by RVSRI has been used for routine vaccination and campaigns in Iran. Before general distribution, the efficacy of the vaccine in the prevention of poliomyelitis had been demonstrated in Iran in a field trial. In this study, 3,000 children who received three doses of the vaccine at four to six week intervals were analyzed, showing no severe reactions and favorable seroconversion results (31). After the preliminary clinical trials, a mass vaccination campaign against poliomyelitis was launched in Iran using the vaccine. The poliomyelitis incidence (confirmed cases), which had been endemic in Iran, decreased steadily after the mass vaccination from over 700 in 1974 to half that rate in 1980 (30).

The vaccine was shown to be most effective, and 96%, 94%, and 91% of vaccinated children had a protective level of antibodies against types 1, 2, and 3, respectively, among those who were vaccinated following three national immunization days (NIDs) in Iran (32). Another seroprevalence study in Sistan-va-Baluchestan province near the south-east border of Iran, conducted on children who had received at least five doses of tOPV, found seroprevalence of 94.1%, 96.7%, and 78.3% against PV1, 2, and 3, respectively (33).

In addition to these, several studies that have been previously carried out showed that some factors, such as interference with other enteric pathogens, could affect the immunogenicity of OPV in developing countries (9, 34-38). In total, only 73% and 70% of vaccinated children with tOPV in developing countries have detectable specific neutralizing antibodies to PV1 and PV3, respectively (39). On the other hand, it seems that IgG antibody concentration is insufficient to evaluate the immunity level, and the level of serum and secretory IgA needs to be measured. Therefore, detecting IgA antibodies has been proposed to assess the immunity level (40). For example, it has been discovered that poliomyelitis (acute flaccid paralysis [AFP]) cases had no significantly different IgG levels compared to others, but their IgA levels were significantly lower (41). A study by Buisman et al. revealed that preexisting PV-specific circulating and secretory salivary IgA antibodies caused protection against PV infection after challenging with PV (42). Furthermore, several other studies have shown the crucial role of mucosal immunity in defending against PV (43).

Administration of OPV has resulted in the complete eradication of the disease caused by WPVs. Iran has been free of indigenous WPV-associated disease since 1997 and exogenous WPV-associated disease since 2001. It should be noted that WPVs are still circulated in Afghanistan and Pakistan, the southeastern neighboring countries with which Iran has a frequent large interchange of people. WP1 has recently been detected in East-West Iran's border cities by environmental surveillance of wastewater (without causing an outbreak) (44).

Accordingly, it seemed that high immunity in Iran prevented the spread and circulation of the WPV1 (44). The surveillance system of poliomyelitis has played a pivotal role in assessing a polio-free status in Iran. In addition, to prevent the circulation of possible exogenous WPVs and exogenous and endogenous VDPV in Iran, routine and mass vaccination needs to be continued for the maintenance of a sufficiently high level of herd immunity.

3.4 Adverse events following immunization (AEFI)

Although OPV is classified as a safe vaccine, similar to other biological and drugs products, it can cause various side effects. OPV-related AEFI can be divided into two groups: minor (non-serious) adverse effects and rare neurologic complications, including vaccine-associated paralytic poliomyelitis (VAPP).

Some studies showed OPV can cause some minor side effects, including fever, gastrointestinal disorders, especially diarrhea and abdominal pain, and headache (45). Minor side effects usually resolve after a short period without permanent consequences. In addition to the minor adverse effects, there is a rare neurologic complication,

including VAPP, due to OPV. This side effect occurs with different frequencies from one region to another. The risk of VAPP among vaccine recipients or their close contacts has been reported as 1.4 to 0.1 per million doses in various parts of the world (45, 46). This risk per million doses ranged from 0.05 to 0.99, 0 to 0.65, and 1.18 to 8.91 for serotypes 1, 2, and 3, respectively. The risk of VAPP is highest (up to ~6.6-fold) in the first dose compared to the subsequent doses (47). Moreover, it has been shown that VAPP occurs more frequently (up to ~3000-fold) in primary immunodeficiency (PID) patients (48).

Therefore, OPV should not be administered to persons who suffer from PID or to children under treatment with corticosteroids and other immunosuppressant drugs. Individuals with compromised immune responses may experience severe illness, paralysis, or even death. They may also excrete immunodeficiency-associated VDPV (iVDPV) for an extended period. However, PID can be difficult to detect and diagnose at the time of birth. No increased levels of AEFI were observed in Iranian OPV recipients, and only a few VAPP cases (primarily in PID patients) have been reported through acute flaccid paralysis (AFP) surveillance (49-58).

4. Conclusion

MRC-5 cell substrate has been used for the production of OPV in Iran. This cell line is suitable for the manufacturing of vaccines because of some features, including normal karyology and lack of tumorigenicity.

Moreover, the use of such cell banks, which are free of all known contaminations (adventitious agents) instead of primary cells, decreases the risk of contamination of the vaccine.

Over 50 years of experience in Iran has shown that this vaccine is safe and efficient. WPs have been eradicated from Iran. Although PVs have been eradicated from most ports of the world, such as Iran, outbreaks of WPs have been reported in limited areas of the world, including two neighboring countries of Iran, Afghanistan, and Pakistan.

Therefore, it could be a potential source of emergent outbreaks of PVs in Iran. To prevent the circulation of possible exogenous PVs in Iran, routine and mass vaccination needs to be continued for the maintenance of a sufficiently high level of herd immunity. However, in the post-eradication era, risk-free polio vaccines such as Sabin IPV (sIPV) and/or virus like particle (VLP) will completely replace OPV in Iran under the global OPV cessation for the finalization of the risk of exposure to vaccine-derived polioviruses (VDPVs) and vaccine-associated paralytic poliomyelitis (VAPP).

Lastly, we cannot conclude this article without mentioning the name of the late Prof. H. Mirchamsy, Prof. A. Shafyi, Prof. P. Ahourai, Prof. S. Bahrami, and late Prof. J Razavi. Their critical roles were instrumental in the establishment of OPV production in Iran.

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Authors' Contribution

The manuscript was written by BA. AM edited and approved the content.

Ethics

Not applicable.

Conflict of Interest

The Authors are employed by the Razi Vaccine and Serum Research institute, the manufacturer of OPV in Iran.

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Not applicable.

Data Availability

The data that support the finding of this study are available on request from the corresponding author.

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