Perspective of vaccine-derived poliovirus following a mass vaccination campaign in Cuba: implications for stopping polio vaccination after global eradication

Pedro Más Lago, a Victor M Cáceres, b Miguel A Galindo, c Howard E Gary Jr, b Marlen Valcarcel, d Julio Barrios, a Luis Sarmiento, a Ivonee Avalos, a Jose A Bravo, a Rosa Palomera, a Marite Bello, a Roland W Sutter, b Mark A Pallansch, b and Ciro A de Quadros, e

Background With substantial progress made toward polio eradication, developing the appropriate strategy for discontinuing global oral poliovirus vaccine (OPV) after global eradication becomes increasingly important. At issue is the theoretical risk of independent circulation of potentially virulent OPV-derived strains. Because Cuba uses OPV only in mass campaigns, it represents an ideal site to assess vaccine-derived poliovirus persistence.

Methods Infants born after the 1997 biennial mass campaigns were evaluated for past (neutralizing antibody) or current (virus excretion) evidence of vaccine-derived poliovirus exposure. We obtained sera and/or stool specimens from 861 infants; a second serum from 218 infants.

Results All stool specimens were poliovirus negative. Of 762 infants, 113 (14.8%) had initially detectable poliovirus type 1 antibody, 193 (25.3%) type 2, and 94 (12.3%) type 3. A precipitous antibody decline occurred in initially positive sera.

Conclusions Our results suggest that in a country with high population immunity, vaccine-derived virus is unlikely to establish ongoing circulation.

Keywords Poliomyelitis, polioviruses, eradication, vaccine, excretion, virus, oral polio vaccine, vaccine Sabin, immunization, circulation, transmission, Cuba

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Since the World Health Assembly’s 1988 resolution to eradicate poliomyelitis from the world by the year 2000, the number of estimated polio cases worldwide has declined from more than 350,000 in 1988 to under 35,000 in 2000, with the last remaining global poliovirus reservoirs confined to parts of Southeast Asia and sub-Saharan Africa. The Americas Region (AMRO) of the World Health Organization (WHO) was certified as polio-free in 1994 as was the Western Pacific Region (WPRO) in 2000. It is essential to determine whether vaccination can be safely stopped after polio eradication and, if so, how it should be discontinued. At issue is the theoretical risk of continued circulation of potentially virulent poliovirus vaccine (OPV)-derived strains. Prolonged circulation of vaccine-derived poliovirus increases the likelihood of its reversion to a neurovirulent strain that could eventually assume the transmission characteristics of wild poliovirus. The WHO has identified as the highest priority, research evaluation of persistent vaccine-derived poliovirus circulation in tropical countries. It is believed that tropical climates are more conducive than temperate ones to prolonged vaccine virus circulation. Cuba provides an ideal setting to study vaccine virus persistence because of the absence of wild polioviruses and its unique strategy for administering OPV. Poliomyelitis was eliminated from Cuba in the early 1960s. Since then, OPV has been administered to children through biennial national mass campaigns, or as commonly referred to globally, National Immunization...
Days (NID). Each NID round lasts approximately one week during February and April, targeting all children aged older than one month and younger than 3 years of age.\(^5\) Because OPV is not available in the health system at any other time, it is possible to track the circulation of vaccine-derived viruses from a well-defined point (i.e. the NID).

If vaccine-derived poliovirus were to circulate in Cuba, it would most likely be found in the unvaccinated (therefore susceptible) infants born after the second round of the NID each year in April. In the current study, we used virus isolation and serological tests to assess unvaccinated infants for evidence of exposure to vaccine-derived poliovirus at 7–8 months following the 1997 NID.

**Methods**

**Target sample size**

We determined a target sample size of at least 900 children for this study. This would give a probability of more than 95% for detecting at least one shedding child if the true shedding rate was \(>0.5\%\) and the participation rate was 80%, assuming that the probability of detecting the virus in the stool (specimen sensitivity) was 85%.

**Selection of children**

Study participants were enrolled in November–December 1997 through Havana’s system of family physicians using a cluster-sampling scheme. The health care system of Havana comprises 81 health areas, each served by a clinic and staffed by a number of family physicians. Each family living in Havana is assigned a family physician, and each physician cares for an average of 3.4 children within the target age group. To select the children for this study, we randomly grouped the family physicians within each health area into clusters of 15 physicians each. Twenty-five clusters were then selected at random. To be eligible for the study, participants had to have been under the care of a family physician selected by the sampling procedure and either have been born after the April 1997 NID or be younger than 30 days of age (the lower age limit for vaccination) at the time of the NID. Infants whose age could not be determined were excluded. The selected family physicians were contacted and the three month-to-fourth month antibodies of the three poliovirus serotypes were re-tested with a second serum at the appropriate interval prior to the 1998 NID. Written informed consent was obtained from parents or guardians of study participants prior to administering the questionnaire and collecting the samples. In all, 8–10 g of stool were collected in an appropriate vessel and a lancet was used to obtain 0.5 ml of blood through a heel stick procedure. All stool and serum samples were tested at the Pedro Kouri Institute for Tropical Medicine in Havana.

**Isolation and identification of poliovirus in stools**

Isolation of virus was done according to the WHO recommended methods with the following modifications.\(^6\) A 20% suspension was made of each stool sample using PBS (phosphate buffered saline) with antibiotics. The samples were clarified by centrifugation at 10,000 revolutions per minute in an Eppendorf centrifuge. The supernatant was stored in a freezer at \(-20°C\) until inoculation onto rhabdomyosarcoma (RD) and L20b cell lines using 0.2 ml of the supernatant. The tubes were incubated at 37°C and examined 24 hours later. Those that showed cytopathic effect (CPE) were frozen and thawed for passage to new cell tubes. The total observation time was 12 days before a sample was determined to be negative. In those tubes where CPE appeared in the L20b cells, identification was made by neutralization using the Lim-Benyesh-Melnick serum pools for the identification of poliovirus. Because of the lower sensitivity of the L20b cells, those samples that showed CPE only in RD cells were passed to the L20b cells. If the CPE persisted, identification proceeded in the manner described previously. Isolates that appeared to be a non-polio enterovirus were not further typed.

**Determination of neutralizing antibodies**

Neutralizing antibodies were determined by the method recommended by WHO.\(^7\) Microneutralization was done on microtitre plates using twofold serial dilutions of sera beginning with 1:8. At each dilution, 25 µl of diluted serum was mixed with 25 µl of medium (Eagle MEM) containing 100 TCID50 (32–320) of Sabin poliovirus type 1, type 2, or type 3. The virus-serum mixture was incubated for 4 hours at 37°C in an atmosphere of 5% CO₂. Then 100 µl of Hep-2 Cincinnati cell suspension (200,000 cells/ml) were added and the mixture incubated as before for 5 days. Each serum was tested in triplicate. Each test batch was accompanied by the following controls: cell control, serum toxicity control, virus dose and titration controls using an in-house reference serum validated against the international standard. To further characterize the antibodies, study participants having neutralizing antibodies at a titre of at least 1:8 to any of the three poliovirus serotypes were re-tested with a second serum drawn approximately 6–8 weeks after the initial sample.

**Results**

**Study participation**

Of 864 infants enrolled, 3 were later excluded for failure to meet the age criteria for inclusion (Figure 1). Serum and stool were obtained from 739 and serum only from 23 infants between 30 November and 10 December 1997 \((n = 762)\). Reasons for not obtaining a stool sample in these 23 included failure of the parent or guardian to bring a stool sample and loss to follow-up. Between 11 December and 31 December 1997, stool samples were obtained from an additional 99 infants \((n = 838)\).

**Stool survey**

The analysis of the stool survey is based on the 838 infants from whom stools were obtained. Study participants ranged in age from newborn to 8 months (median: 3 months). No poliovirus was isolated from any study participant, giving an upper 95% confidence limit for the percentage shedding of 0.42%, assuming...
a specimen sensitivity of 85%. Non-polio enteroviruses were isolated from 170 (20.3%) of the stool samples.

**Serological survey**

Serological results were available from 762 study participants. These infants ranged in age from newborn to 8 months, with a median age of 3 months. The age distributions were similar across health areas included in the study. From the screening of the initial serum sample at a dilution of 1:8, 252 (33.1%) of the 762 children were positive for neutralizing antibody to at least one poliovirus serotype (Table 1). The highest rates were for type 2 (25.3%), followed by type 1 (14.8%) and type 3 (12.3%). In general, titres were inversely correlated with age. Also, the percentage positive to at least one serotype declined with age from 73.9% among the 46 infants under one month of age to 0% among the 14 infants aged 7 or 8 months. Because we had expected to find sustained seropositivity in infants born immediately after the NID, we further analysed this group (n = 14). Compared with younger infants, these children were less likely to have other siblings in the household (3/14 [21.4%] versus 345/714 [48.3%; \(P = 0.05\) by two-tailed Fisher’s exact test).

Of the 252 infants whose first sample was positive for neutralizing antibodies to poliovirus, we were able to collect a second sample from 218 (86.5%). The interval between the first and second samples ranged from 38 to 63 days (median

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**Figure 1** Schematic representation of study enrolment and specimen collection

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**Table 1** Seropositivity for the first serum sample by age in months

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>No. tested</th>
<th>Type 1</th>
<th></th>
<th>Type 2</th>
<th></th>
<th>Type 3</th>
<th></th>
<th>Any polio type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. positive</td>
<td>%</td>
<td>No. positive</td>
<td>%</td>
<td>No. positive</td>
<td>%</td>
<td>No. positive</td>
<td>%</td>
</tr>
<tr>
<td>0</td>
<td>46</td>
<td>19</td>
<td>41.3</td>
<td>30</td>
<td>65.2</td>
<td>15</td>
<td>32.6</td>
<td>34</td>
</tr>
<tr>
<td>1</td>
<td>149</td>
<td>61</td>
<td>40.9</td>
<td>78</td>
<td>52.3</td>
<td>44</td>
<td>29.5</td>
<td>103</td>
</tr>
<tr>
<td>2</td>
<td>153</td>
<td>18</td>
<td>11.8</td>
<td>46</td>
<td>30.1</td>
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<tr>
<td>3</td>
<td>112</td>
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</tr>
<tr>
<td>4</td>
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<td>2</td>
<td>1.9</td>
<td>10</td>
<td>9.6</td>
<td>5</td>
<td>4.8</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>108</td>
<td>0</td>
<td>0.0</td>
<td>2</td>
<td>1.9</td>
<td>4</td>
<td>3.7</td>
<td>5</td>
</tr>
<tr>
<td>6</td>
<td>76</td>
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<td>0.0</td>
<td>3</td>
<td>3.9</td>
<td>1</td>
<td>1.3</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>9</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>0</td>
<td>0.0</td>
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<td>0.0</td>
<td>0</td>
<td>0.0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>762</strong></td>
<td><strong>113</strong></td>
<td><strong>14.8</strong></td>
<td><strong>193</strong></td>
<td><strong>25.3</strong></td>
<td><strong>94</strong></td>
<td><strong>12.3</strong></td>
<td><strong>252</strong></td>
</tr>
</tbody>
</table>

a Twofold serial dilutions of sera began at a titre of 1:8.

b Age refers to completed months.
51 days). During this interval, the neutralizing antibody titres of all 218 infants fell (Figure 2). Among those whose initial titre was 1:32 or higher, all but one fell by at least 50%. The one exception was a one-month-old infant whose initial neutralization titres against both types 1 and 2 were 1:54; these subsequently declined modestly to 1:45. The interval between the first and second serum for this infant was 38 days, the shortest in the range of intervals and probably the reason for the modest decline.

One 4-month old infant had an unusually high initial antibody titre to poliovirus type 3, 1:1211; this specimen was retested with a comparable result. By the second serum collected 53 days later, the titre had fallen to an undetectable level (also undetectable on repeat testing of the second serum). This infant was revisited, found to be healthy and did not have a history of receiving immune globulin.

The serological component of our study reflected 2419 person-months of potential exposure among infants born beyond the immediate 3-month (post-NID) limit suggested by earlier studies. The absence of serological evidence for poliovirus infection corresponds to an exact upper 90% confidence bound of 4.4 infections/100,000/month within this age group based on the Poisson distribution.

**Discussion**

Our investigation provides compelling evidence that vaccine-derived poliovirus does not persist for an extended period following the national mass vaccination campaigns with OPV in Havana. Single serum collection and viral isolation have been used separately by previous investigators and provided some evidence of limited circulation of vaccine-derived poliovirus (i.e. during 3 months following NID). However, our study is unique in concurrently sampling stool specimens and paired sera from a large, susceptible, infant population. Paired sera allowed the determination that positive serology represented maternal antibodies, not exposure to vaccine virus. In addition, we found no serological evidence of virus exposure during 2419 person-months of risk beyond the 3-month period immediately following the NID.

The finding that all positive neutralizing antibodies in the first serum declined in the second serum sample, together with the age prevalence and magnitudes of these titre declines are consistent with their being of maternal origin rather than from poliovirus exposure. As expected, younger infants had the highest titres while older infants, born closer to the time of the NID, had the lowest titres. If the antibodies had resulted from exposure to vaccine virus, their titres would have been higher initially and remained high over the short interval between serum samples. These serological findings combined with the absence of poliovirus in any of the 838 stool samples provide powerful evidence of exceedingly low, if not absent, vaccine virus circulation.

Results of other studies indicate that circulation of vaccine-derived polioviruses in temperate countries may be limited in time. In 1960 in Hungary, stool samples were obtained from 2017 children aged 3–23 months, approximately 4 months after the mass OPV campaigns; no polioviruses were isolated. Data from the Netherlands, a country that changed from using OPV to using inactivated polio vaccine exclusively in their routine programme, demonstrated that almost all vaccine-derived

![Figure 2](image-url)
strains isolated in diagnostic laboratories could be linked to importation, not persistent indigenous circulation. Also, recent molecular studies of vaccine viruses isolated from cases of acute flaccid paralysis in Brazil show little genetic drift, suggesting limited transmission.

In a 1992 study performed by us, we found serological evidence suggestive of rapidly disappearing vaccine strains during the 1–2 months immediately after the NID. In the present study, we failed to detect this expected post-NID vaccine virus circulation. A potential explanation for this finding is that the 1992 mass campaigns targeted an additional age cohort (36–48 month olds) not targeted by the 1997 campaigns, thereby introducing more vaccine virus into the population. Alternatively, this finding may reflect the small sample (n = 14) of study participants potentially exposed to circulating vaccine virus immediately following the NID. We are not certain why so few 7–8 month olds participated in our study. It is possible that this age group was a less captive audience, seen less frequently by their family physicians relative to their younger 0–6-month-old counterparts.

Our study was done in an urban setting. It is likely, however, that the same result would have been found in rural Cuba where the lower population density, high vaccination coverage, and comparable sanitation are at least as inhospitable to vaccine virus circulation. Because Havana has relatively better sanitation and hygiene than may be found in cities in other developing countries, generalization of our findings to other countries may be limited. Furthermore, Cuba’s excellent vaccine coverage has resulted in high levels of immunity throughout the population. In countries with less extensive vaccine coverage over the years, susceptible people in older age groups may contribute substantially to sustaining transmission.

In addition to providing data regarding the question of ‘if’ polio vaccination can stop, Cuba may be providing a clue regarding ‘how’ it should be discontinued. A strategy of maximizing population immunity followed by simultaneous OPV cessation (as occurs yearly after NID in Cuba) may create the conditions, during a discrete period of time, that favour rapid termination of vaccine virus circulation. It is possible that a similar scenario could be recreated worldwide, through implementation of a Global Immunization Day (GiD), immediately followed by simultaneous global cessation of routine OPV. Although the logistics and the political challenges of a co-ordinated GiD are formidable, there are reasons for optimism. Highly successful regional immunization days, involving tens of millions of children in dozens of countries in the European and South East Asian Regions of WHO, have already been implemented.

In addition, the enthusiasm created by the successful completion of the polio eradication initiative may have favourable political implications for a co-ordinated strategy for OPV cessation.

Several additional studies should be initiated to answer important questions and further clarify possible global ‘stop vaccination strategies’. The needed studies include (1) replication of the Cuban study in populations living in tropical areas with poorer sanitation and lower vaccination coverage; (2) assessment of the role played by children not typically targeted by the NID (5–15 year olds) in the transmission of vaccine-derived virus; (3) evaluation of vaccine-derived virus circulation in countries that have recently changed from an OPV to an IPV schedule; and (4) continued evaluation of individuals with immunodeficiencies that put them at risk for prolonged excretion of poliovirus both in industrialized and developing countries.

The achievement of global polio eradication is expected by the end of 2002, with certification that polio has been eradicated in 2005. A scientifically sound strategy to discontinue all oral polio vaccination will not only eliminate the rare cases of vaccine-associated polio caused directly by the vaccine, but also lessen the possibility that vaccine strains will persist in the post-eradication era. Our investigation in Cuba strengthens the scientific foundation for developing this strategy.

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KEY MESSAGES

- As the accomplishment of global polio eradication nears, it is essential to determine ‘if’ and ‘how’ vaccination can be safely discontinued.
- There was no evidence of prolonged circulation of vaccine-derived poliovirus in infants born after mass vaccination campaigns in Cuba.
- A strategy of maximizing population immunity followed by simultaneous OPV cessation (as occurs yearly after NID in Cuba) may create the conditions, during a discrete period of time, that favour rapid termination of vaccine virus circulation.
- Further study of vaccine-derived poliovirus circulation is needed in environments with lower levels of immunity and sanitation.

References


In this issue of the International Journal of Epidemiology, Mas Lago et al. report the disappearance of oral polio vaccine (OPV) strains from Cuba following a national vaccination campaign, and infer from their results that OPV could be safely discontinued after polio eradication without fear that revertant viruses would continue to circulate and cause polio.

The public health successes accomplished by Cuba with regard to polio and other vaccine-preventable diseases are admirable and deserving of praise. However, there are a number of reasons to hesitate before extrapolating the polio results to other countries.

First, the search for persistent virus was conducted in an age group not ideal for the purpose: infants less than 8 months. Absence of seroconversion among these infants, despite the probability that some of them were exposed to siblings who excreted attenuated polio virus in the weeks after the national campaign.

With regard to the issue of continued circulation of these strains, tests in 1–3-year-old infants would have been more interesting.

Second, no search for poliovirus in sewage is reported here. Although Mas Lago et al. tested over 800 infants, persistent excretion may be even rarer, and the 800 infants presumably included none with B cell immunodeficiency. Sewage testing screens larger populations.

Third, the Cuban population is already highly immune to polio, which would limit the spread of excreted OPV strains. The recent outbreak reported from nearby Hispaniola, where a type 1 revertant became epidemic in a poorly vaccinated population, and similar episodes in Egypt and China, lead to the conclusion that attenuated strains will re-acquire neurovirulence if given the opportunity for multiple passages in the human intestine.

Thus, although the data from Cuba are encouraging for that nation, and perhaps for other countries with reasonably high hygiene and public health infrastructure, they do not allow us to conclude that abrupt discontinuation of OPV, without other measures, will avoid resurgence of paralytic polio due to vaccine strains. More studies are needed before this possibly dangerous strategy, which will allow rapid accumulation of susceptibles in the possible presence of virulent virus, is adopted.

References

