Prolonged Detection of Indigenous Wild Polioviruses in Sewage from Communities in Egypt

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Environmental surveillance for polioviruses has been implemented in Egypt. This paper reports on a study in which 130 sewage samples were collected between January 2001 and December 2001 from eight provinces of Egypt. Samples were analyzed by virus isolation in L20B and RD cell cultures, and wild polioviruses were characterized by sequencing of the VP1 protein coding region. Wild type 1 polioviruses were detected in 57% of the sewage samples and 91% of the study sites, only two of which reported paralytic poliomyelitis cases in 2001. Three genetic lineages of a single indigenous type 1 poliovirus genotype were detectable in sewage, and only one lineage was also detected through surveillance for acute flaccid paralysis. Wild polioviruses persisted in the environment despite implementation of oral poliovirus vaccine immunization campaigns. Continued analysis of sewage samples, critical evaluation of immunization coverage, and performance of surveillance for acute flaccid paralysis are proposed as follow-up activities.

Egypt; environment; environmental monitoring; phylogeny; poliovirus; population surveillance; sequence analysis; sewage

Abbreviations: AFP, acute flaccid paralysis; CDC, Centers for Disease Control and Prevention; KTL, National Public Health Institute (Helsinki, Finland); OPV, oral poliovirus vaccine; PCR, polymerase chain reaction; VACSERA, Egyptian Organization for Biological Products and Vaccine Production.

In 1988, the World Health Assembly resolved to eradicate poliomyelitis from the world by the year 2000 (1). There can be little doubt that considerable progress has been made toward achieving that goal, although the 2000 deadline was not met. Three regions of the world have already been certified as poliomyelitis free: the Americas, Europe, and the western Pacific (2). Under conditions of considerably improved disease surveillance, poliomyelitis cases have declined more than 99 percent in the world from an estimated 350,000 in 1988 at the start of the eradication initiative to only 537 in 2001. Egypt is included among the 10 remaining poliomyelitis-endemic countries of the world.

Egypt’s health authorities have a long tradition of implementing poliomyelitis control activities, and immunization campaigns that include oral poliovirus vaccine (OPV) have been conducted since the mid-1970s (3). In 1989, Egypt joined the global community in implementing strategies recommended by the World Health Organization to eradicate poliomyelitis (4, 5). Such efforts led to a substantial
decline in poliomyelitis cases; 584 were reported in 1992 but only five in 2001 (6, 7).

Poliovirus type 2 was last detected in Egypt in 1979; types 1 and 3 were both isolated from poliomyelitis cases in 2000, and only type 1 was isolated in 2001. All wild poliovirus isolates detected since 1996 have been characterized genetically, and results show that all viruses have been indigenous to the country (Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, unpublished data). Virologically confirmed poliomyelitis cases in 2000 and 2001 were all from a region commonly described as Upper Egypt, which comprises provinces situated south of the Nile delta (refer to figure 1 for a map of Egypt). The four reported poliomyelitis cases in 2000 were from three administrative provinces in Upper Egypt, namely, Asyut (for one case with type 1 virus and one case with type 3 virus, paralysis onset occurred in January and February, respectively), Qena (one case with type 1 virus in May), and Fayoum (one case with type 3 virus in December). In 2001, five confirmed cases with poliovirus type 1 were reported from two provinces, Minya (three cases) and Qena (two cases). Cases in Minya were from the district of Mallawy (for one case, disease onset occurred in January) and Abo Qurqas (two cases reported in January and February). For both cases from Qena in 2001, disease onset occurred in October.

The government of Egypt is highly committed to the poliomyelitis eradication initiative, and persistent transmission of indigenous wild polioviruses has been of great concern. The country’s health authorities have implemented a number of activities to identify and address areas of program weakness (3, 4, 8). Standard indicators used in the poliomyelitis eradication initiative to monitor surveillance and immunization performance were generally satisfactory during 1997–2001 (table 1). Since 1999, there has been an increase in the number, as well as improvement in the supervision, quality, and coverage, of national and subnational immunization campaigns, in which a house-to-house approach has been used to reach children, particularly in rural areas. Surveillance for acute flaccid paralysis (AFP) has also been strengthened considerably, and, as an adjunct to routine surveillance for AFP, environmental surveillance, in which sewage specimens are being evaluated for the presence of wild polioviruses, has been implemented. One hypothesis is that some “reservoir” communities in Egypt sustain poliovirus transmission through “silent”—that is, asymptomatic—infection of susceptible persons and that viruses spread from these reservoirs to other parts of the country. The aim of this study was to identify reservoir communities and target them in OPV immunization campaigns to interrupt transmission of indigenous wild poliovirus.

**MATERIALS AND METHODS**

**Selection of study sites**

Initially, only five sites were selected for collection of sewage samples, representing places in which wild poliovirus was known to circulate in 2000 based on AFP surveillance data. Subsequently, sampling sites were expanded dynamically after field and laboratory staff were trained and logistic arrangements were optimized for specimen handling, transport, and testing. Between January 2001 and

![FIGURE 1. Map of Egypt showing the distribution of environmental samples found positive for wild poliovirus type 1 and of poliomyelitis cases in 2001.](https://academic.oup.com/aje/article-abstract/158/8/807/63972/FIGURE-1)
December 2001, sewage samples were collected from 11 study sites in eight administrative provinces of Egypt. The sites were located in Aswan province at Aswan City; Gharbia province at Tanta City; Minya province at Minya City, Abo Qurqas, and Mallawy; Fayoum province at Fayoum City; Beni Suef province at Beni Suef City; Asyut province at Asyut City and Dairut; Sohag province at Sohag City; and Qena province at Qena City. All sites except Tanta City were in provinces of Upper Egypt. Selection criteria for study sites were confirmed cases of poliomyelitis or partially immunized populations in the previous 3 years, availability of population data, and/or availability of suitable sites for sewage collection.

A converging sewer network connecting individual buildings and/or houses in the community through pipes or water channels to a central sewage treatment plant was found in all study sites except those in Asyut/Dairut and Minya/Mallawy, where sewage from a few buildings or houses was channeled via pipes to a shared pit. When these pits were full, they were pumped out into tanks that were transported away from the premises and emptied at well-known locations in the community. Sampling sites at sewage treatment plants were likely to be representative of the population sizes shown in table 2. However, sites at Asyut/Dairut and Minya/Mallawy were likely to represent only a few hundred persons in a limited number of households with a shared sewage pit that had recently been pumped out and emptied near collection points used in the study.

**Collection of sewage samples**

Sewage samples were collected by trained local authorities. A sampling schedule of once or twice per month per site was recommended. For each of the nine study sites that had sewage treatment plants, 1-liter “grab” samples of raw sewage were collected into clean, leakproof containers from the influent during the peak morning flow. On sample collection days in Minya/Mallawy and Asyut/Dairut, four 250-ml grab samples were collected at 4-hour intervals from selected open canals and were pooled together to make a composite sample. Sewage samples were kept cool and were transported, usually within 1 day of collection, to the Egyptian Organization for Biological Products and Vaccine Production (VACSERA) laboratory in Cairo, Egypt.

**Extraction of virus particles from sewage samples**

Extraction was performed at the VACSERA laboratory, where sewage samples were concentrated approximately 100-fold by using a two-phase separation procedure, as follows. Sewage samples were centrifuged for 10 minutes at 1,000 g and 4°C. The supernatant and pellet were collected into separate containers, and the pellet was held at 4°C. The supernatant was neutralized to pH 7.0–7.5 with a 1.0 normal concentration of sodium hydroxide. The volume of supernatant was measured. To every 1 liter of supernatant were added 79 ml of 22 percent (weight per volume) dextran, 575 ml (weight per volume) of polyethylene glycol 6000, and 70 ml of a 5 normal concentration of sodium chloride solution. The solution was mixed, kept at 4°C for 1 hour, and agitated constantly. The mixture was then poured into a sterile separation funnel and left to stand overnight at 4°C. The following day, the entire lower phase and the interface were collected into a sterile tube (usually 10–20 ml of liquid) to which was then added the pellet that had been held at 4°C following centrifugation of the original sample. The pellet was resuspended, and the mixture was extracted with 20 percent (volume to volume) chloroform by vigorous shaking for 20 minutes at room temperature. The mixture was then centrifuged for 10 minutes at 1,000 g and 4°C. The supernatant was collected and divided into four aliquots, which were stored at –70°C. One aliquot remained frozen for future
reference, one was removed and tested locally at the
VACSER A laboratory, and one each was shipped under cold
conditions to collaborators at CDC laboratories in the United
States and at the National Public Health Institute (KTL) in
Finland. All sewage concentrates were tested in parallel in the
three collaborating laboratories.

Isolation of viruses in cell cultures

Sewage concentrates were inoculated into L20B and RD
cells for virus isolation. L20B is a recombinant murine cell
line that permanently expresses the human poliovirus
receptor and is susceptible to poliovirus but nonpermissive
to most other human enteric viruses. RD cells are derived
from human rhabdomyosarcoma tumor tissue and are
susceptible to most human enteroviruses except coxsackie B
viruses. Cell-line stocks were derived from cultures obtained
from the National Institute for Biological Standards and
Controls, United Kingdom (L20B), and from CDC (RD).
Cell lines were grown according to procedures outlined in
the World Health Organization’s Polio Laboratory Manual
(9). Cell monolayers were grown in either 50-ml cell culture
flasks (at VACSER A and KTL) or 24-well plates (CDC).
The volume of inoculum of sewage concentrate was 500 µl
per flask for VACSER A and KTL and 50 µl per well for
CDC. The total numbers of cell cultures inoculated were one
RD and two L20B for VACSER A, one RD and four to six
L20B for KTL, and two RD and two L20B for CDC.
Cultures showing cytopathic effects were evaluated for the
presence of polio and nonpolio enteroviruses; any poliovirus
isolates were differentiated as wild or Sabin-like by enzyme-
linked immunosorbert assay, probe hybridization, and/or
polymerase chain reaction (PCR) according to procedures
outlined in the Polio Laboratory Manual. A sample was
considered positive for wild polioviruses if any of the three
collaborating laboratories obtained a positive result for the
presence of non-Sabin-like polioviruses.

Sequence analysis of poliovirus isolates

Sequencing of the entire VP1 protein coding region (906
nucleotides) of the wild type 1 poliovirus isolates was
performed from two overlapping reverse transcription–PCR
amplons. The following primers were used for both reverse
transcription–PCR and sequencing: Y7 5′-GGG TTT GTG
TCA GCC TGT AAT GA-3′ (position 2395–2418) and
panpoliovirus PCR-1A 5′-TTI AIII GC(A/G) TGI CC(A/G)
TT(A/G) TT-3′ (position 2935–2954) (10) for the 5′ part of
the VP1 protein coding region, and panpoliovirus PCR-2S
5′-CIT AIT CII(A/C) GIT T(C/T)G A(C/T)A TG-3′ (position
2875–2894) (10) and Q8 5′-AAG AGG TCT CT(A/G) TTC
CAC AT-3′ (position 3476–3495) for the 3′ part of the VP1
protein coding region.

RNA was extracted from 100 µl of poliovirus-infected cell
cultures by using an RNeasy Kit (QIAGEN GmbH, Hilden,
Germany). A total of 2 µl of RNA was added to reverse tran-
scription-PCR mixtures (total volume, 50 µl) containing 67
mmol of tris(hydroxymethyl)aminomethane hydrochloric
acid (Tris-HCl) (pH 8.8); 17 mmol of ammonium sulfate
(NH4)2SO4); 6 µmol of ethylenediaminetetraacetic acid
(EDTA) (pH 8.0); 200 µmol each of 2′-deoxy-adenosine-5′-
triphosphate (dATP), 2′-deoxy-cytidine-5′-triphosphate
(dCTP), 2′-deoxy-guanosine-5′-triphosphate (dGTP), and
2′-deoxy-thymidine-5′-triphosphate (dTTP) (Roche Diag-
nostics, GmbH, Mannheim, Germany); 1.5 mmol of magne-
sium chloride (MgCl2); 10 units of RNasin Ribonuclease
Inhibitor (Promega, Madison, Wisconsin); 3.6 units of avian
myeloblastosis virus reverse transcriptase (Finnzymes,
Espoo, Finland); 2.7 units of Thermoperfect Taq polymerase
(Integro b.v., Leuvenhorne, Holland); and either 20 pmol of
primer Y7 and 160 pmol of primer panpoliovirus PCR-1A or
160 pmol of panpoliovirus PCR-2S and 40 pmol of Q8. The
mixtures were incubated at 50°C for 30 minutes and at 94°C
for 3 minutes. Thirty-five cycles of amplification (94°C for
30 seconds, 42°C for 30 seconds, and 60°C for 30 seconds)
were followed by an extension step at 60°C for 5 minutes.
The PCR amplicons were purified with the QiAquick Gel
Extraction Kit (QIAGEN) and were used in cycle sequencing
(ABI Prism BigDye Terminator Cycle Sequencing Ready
Reaction Kit; Applied Biosystems, Espoo, Finland) in an
automated sequencer (model 310, Applied Biosystems). Elec-
ropherograms were analyzed by using Sequencing Analysis,
version 3.1, and Sequence Navigator, version 1 (Applied
Biosystems). A total of 906 nucleotide sequences of the VP1
protein coding region were constructed from overlapping
amplicon sequences and were aligned with ClustalX software
(1.64b) (Internet Web site: http://ftp-igbmc.u-strasbg.fr/
BioInfo/ClustalX/Top.html). Distance matrices were cal-
culated by using DNAdist from version 3.572c of the
PHYLIP program package (Internet Web site: http://
evolution.genetics.washington.edu/phylip.html). The dendro-
gram was drawn by using the Neighbor (PHYLIP) program
with the neighbor-joining option and was displayed by using
the NJPlot program (Internet Web site: http://pbil.univ-lyon1.fr/
software/njplot.html).

GenBank accession numbers for the sequences produced in
this study are AF545107–AF545181.

RESULTS

A total of 130 sewage samples were collected from 11
sites in Egypt between January 1, 2001, and December 31,
2001. For only a minority (seven) of all environmental
samples were no viruses isolated in any of the three collabo-
rating laboratories (table 2). Wild type 1 polioviruses were
detected in 74 (57 percent) samples. In addition, most of
these samples and 49 other samples contained nonpolio
enteroviruses and/or Sabin-like polioviruses of one or more
serotypes. It was noteworthy that extensive use of OPV in
Egypt’s immunization program resulted in a high prevalence
of Sabin-like polioviruses in samples but did not seem to
interfere with successful isolation of wild type 1 polioviruses
in cell cultures. Virology results for the two samples
collected from Minya/Mallawy were negative, whereas the
majority of samples from all other sites yielded enterovir-
uses.

Figure 2 and table 3 show the numbers of samples
produced from each site and the numbers of wild-
poliovirus-positive samples. All wild polioviruses from the
environment were of serotype 1, and positive samples were

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collected from 10 of the 11 sites studied in eight provinces (Aswan, Asyut, Beni Suef, Fayoum, Minya, Qena, Sohag, and Tanta). Although all study sites were not sampled for the full 12 months of 2001, there were still 6 or more months of observations from Asyut City, Asyut/Dairut, Beni Suef, Minya City, and Sohag. In these locations, wild poliovirus type 1 was detected in the environment for several consecutive months, for example, 6 months in Asyut City, 3 months in Asyut/Dairut, 8 months in Beni Suef, 4 months in Minya City, and 7 months in Sohag.

Phylogenetic relations were determined among isolates from sewage and those obtained from poliomyelitis cases in Minya and Qena provinces in 2001 and from Minya and other parts of Egypt in previous years. Nucleic acid sequences in the VP1 protein coding region were aligned with each other, and the degree of homology was calculated (figure 3). The difference in nucleotide sequences among the 2001 sewage isolates was about 1–8 percent. Type 1 polioviruses from sewage belonged to the same NEAF genotype as isolates from poliomyelitis cases, and viruses from the environment could be grouped into three clusters, hereafter referred to as genetic lineages. Two lineages had several branches suggestive of the existence of prolonged independent transmission chains. Only one of the three lineages detected in the environment was also detected through surveillance for AFP.

**DISCUSSION**

This study describes the first initiation of environmental surveillance for wild polioviruses in Egypt. Wild polioviruses of serotype 1 were successfully isolated from samples collected in eight provinces of the country. It was hypothesized at the outset of the study that “reservoirs” exist that sustain virus through the low-transmission season and export it to other communities when environmental conditions are more optimal for transmission. However, it was not possible to determine which, if any, of the eight provinces served as reservoirs because all sampling sites were not observed for the full 12 months of 2001. Thus, for those sites sampled for the first time during the peak transmission season in the latter half of the year, any wild virus detected could have always circulated there or may have been imported earlier in the year from other locations.

The experience in Egypt confirms the feasibility of using environmental surveillance to detect wild polioviruses under developing-country conditions, as was previously demonstrated by others in Colombia, Nigeria, the Palestinian National Authority, and India (11–16). In Egypt, wild poliovirus was isolated successfully when samples were collected from sewage treatment plants and from an open canal in one of two sites. Success in isolating wild polioviruses from an open canal in Asyut/Dairut but not from a canal in Minya/Mallawy could have been due to a higher level of fecal contamination in waters at the former site. Before sample collection was started, visits were made to both sites, and it was observed that obvious sewage streams led into canals from areas in which septic tanks were emptied in Asyut/Dairut but not in Minya/Mallawy. It is also possible that high ambient temperatures or other factors caused virus inactivation in the canal in Minya/Mallawy, because neither nonpolio nor polio enteroviruses were isolated from two samples collected there in 2001 or from nine additional samples evaluated between July 2000 and December 2000 (data not shown). Negative virology from the limited number of samples led to discontinuation of sampling in Minya/Mallawy.

All wild polioviruses isolated from environmental samples collected in Egypt in 2001 were of serotype 1. Several suggestions can be offered for the failure to isolate wild type 2 or 3 polioviruses from sewage samples. First, it is extremely unlikely that wild serotype 2 would have been detected since it had not been found in Egypt since 1979 or anywhere else in the world since October 1999 (7). It is also possible that type 3 viruses did not circulate in any of the sampled communities in Egypt when sewage samples were collected. Another possibility is that sensitivity of the laboratory procedures for detecting wild polioviruses was limited; analyzed samples represented large groups of people, and excreta derived from uninfected persons would have significantly diluted virus concentrations. However, successful isolation of wild serotype 1 polioviruses in Egypt argues...
TABLE 3. Numbers of environmental samples collected and numbers found positive for wild type 1 poliovirus, by month* and site, Egypt, 2001

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<td>13</td>
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<td>11</td>
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* Jan., January; Feb., February; Mar., March; Apr., April; Jun., June; Jul., July; Aug., August; Sep., September; Oct., October; Nov., November; Dec., December.
† Wild, wild poliovirus type 1 detected; nonwild, no wild polioviruses detected.

against general limitations in sampling and laboratory procedures for wild poliovirus recovery from sewage, unless serotype 3 viruses were present in lower titers than serotype 1 viruses. This scenario would occur if there was a smaller number of type 3 poliovirus excretors in the sampled communities, smaller amounts of virus excreted per person, and/or more rapid inactivation of type 3 virus in sewage. In such situations, there may be selective growth in cell culture of those viruses present in higher titers. To reduce the number of competing viruses in cell culture, selection and separation of wild-type polioviruses from nonpolio enteroviruses and Sabin-like polioviruses could have been investigated by neutralizing interfering viruses or by incubating cell cultures at high temperatures to select for wild viruses, as has been described by Manor et al. (13).

There was widespread transmission of wild type 1 polioviruses in Egypt in 2001. Positive environmental samples were collected from all eight provinces studied, and virus remained detectable for 3 or more consecutive months in sewage collected from five provinces (Asyut, Beni Suef, Fayoum, Minya, and Sohag). Through routine surveillance for AFP, confirmed cases of wild poliovirus type 1 were reported from only two provinces in Egypt in 2001, Minya (specifically from the districts of Abo Qurqas and Mallawy) and Qena. In Minya/Abo Qurqas, environmental samples positive for wild type 1 poliovirus were found at the same time that paralytic cases of the disease occurred, but no positive environmental samples were ever found in Minya/Mallawy, as discussed above. Environmental surveillance started in Qena only after the 2001 paralytic poliomyelitis cases were reported.

The results of environmental surveillance could be interpreted to mean that polioviruses circulated “silently” (i.e., no paralytic poliomyelitis cases were detected when wild type 1 viruses were being transmitted) or that there were deficiencies in surveillance for AFP. Since less than 1 percent of persons infected with wild-type poliovirus present with typical paralytic symptoms, “silent transmission” would imply that only nonparalytic infections occurred in six provinces of the country (17). Silent circulation of wild poliovirus has been described by Manor et al. (13) based on analysis of sewage samples collected in the Palestinian National Authority.
FIGURE 3. Phylogenetic relations among wild type 1 polioviruses from recent poliomyelitis cases (arrows) and from the environment in 2001, Egypt. The scale bar indicates the relative genetic distance (0.01 = 1%) of the strains. In the strain identification code, the number following letter E refers to the consecutive running number of specimens arriving in the laboratory (E3 January through E132 December). The numbers after the sites are the sample collection dates (e.g., 301201 = December 30, 2001).

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itionally, environmental studies in Finland during an outbreak in 1984–1985 revealed wild type 3 poliovirus in several provinces in which paralytic cases did not occur (18). Findings in Egypt, Finland, and the Palestinian National Authority may not be exactly comparable because no paralytic poliomyelitis cases had been reported from Finland or the Palestinian National Authority for several years prior to detection of wild viruses in the sewage, whereas wild poliovirus circulation, as documented by surveillance for AFP, was still ongoing in Egypt when environmental surveillance was initiated. Recently, Deshpande (16) reported that wild type 1 polioviruses were sometimes detected in sewage from three areas of Mumbai, India, in the absence of concomitant paralytic polio cases. Egypt and India are both poliomyelitis-endemic countries, and the similarity of the findings in these two countries is intriguing and may warrant further investigation.

While environmental surveillance appears more sensitive than AFP surveillance in these situations, it can be applied in selected instances only. Suitable sampling sites are not always available, and an enormous workload results from representative sampling of large human populations. Mixtures of several different viruses present concomitantly in individual sewage samples also represent a challenge in isolating and identifying the viruses and may have contributed to the only-moderate agreement observed between the results of different laboratories (not shown).

Sewage samples represent a composite of all persons and age groups excreting feces into the sewage network or open canal in a community. Therefore, one important limitation of environmental surveillance is that, while it is possible to determine that poliovirus is circulating, it is not possible, based on testing of sewage samples alone, to identify those persons in the population who are excreting virus. Nonimmune persons in all age groups are susceptible to poliovirus infections (17, 19). All OPV immunization campaigns in Egypt have been targeted at children less than 4 years of age, and performance of surveillance for AFP is monitored among persons less than 15 years of age. One possible, albeit unlikely, explanation for silent transmission of wild polioviruses in some communities in Egypt is that infections occur in older persons not targeted in the immunization campaigns. Community-based stool surveys could be used to identify the persons and age groups sustaining indigenous wild poliovirus transmission in Egypt, but this process would significantly increase the workload for the laboratory and may not be as efficient as other approaches.

The genetic characteristics of viruses from poliomyelitis cases and the environment strongly suggest gaps in performance of surveillance for AFP in Egypt, since two out of three circulating genetic lineages of type 1 poliovirus were detected through environmental surveillance only. Therefore, it is very likely that deficiencies in routine performance of surveillance for AFP caused a failure to detect or report paralytic cases when wild type 1 polioviruses were readily detected for prolonged periods in several provinces through environmental surveillance.

OPV has been used successfully in several countries of the world to interrupt poliovirus transmission. Between January 2001 and December 2001, eight OPV immunization campaigns were conducted in Egypt, inclusive of the provinces from which environmental samples were collected for this study. In each campaign, a dose of OPV was administered to children less than 4 years of age regardless of any prior immunization history. Despite a reported OPV coverage of over 95 percent during the 1999, 2000, and 2001 immunization campaigns, the results from environmental surveillance suggest that the goal of interrupting transmission of indigenous viruses in eight provinces of Egypt has failed. Uninterrupted virus circulation in Egypt during 2001 because of poor OPV quality is thought to be unlikely, but closer scrutiny of OPV coverage may be warranted. OPV has also been used successfully to interrupt outbreaks of poliomyelitis in Finland and the Netherlands, where sewage samples were also analyzed to monitor the outbreaks (18, 20, 21). The latter two countries differ from Egypt in having a much higher standard of hygiene and in using inactivated poliovirus vaccine for routine immunization. However, it is noteworthy that OPV was administered during campaigns of limited duration to interrupt the poliomyelitis outbreaks in Finland and the Netherlands and that both wild polioviruses and Sabin viruses disappeared from sewage after the OPV campaigns were conducted.

In conclusion, the inability of viruses to replicate independently in the environment, the temporal trends of virus detection in sewage, and genetic characteristics of wild type 1 polioviruses from Egypt suggest uninterrupted circulation of indigenous wild polioviruses in several provinces of the country. It is imperative for the success of poliomyelitis eradication in Egypt that further investigations be performed to solve the enigma of prolonged detection of wild polioviruses in sewage in the absence of concomitant paralytic poliomyelitis cases. Critical evaluation of surveillance and immunization performance is planned as a follow-up, as is continuation of environmental surveillance with expansion of sampling sites to include communities in the Nile delta.

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