Rapid Communication

The Vaccine Origin of the 1968 Epidemic of Type 3 Poliomyelitis in Poland

Javier Martín, Geraldine L. Ferguson, David J. Wood, and Philip D. Minor

Division of Virology, National Institute for Biological Standards and Control, Potters Bar, Hertfordshire EN6 3QG, United Kingdom

Received July 19, 2000; returned to author for revision August 15, 2000; accepted August 29, 2000

A clear association was demonstrated between the use of USOL-D-bac type 3 poliovirus live-attenuated vaccine and the 1968 poliomyelitis epidemic in Poland. The epidemic followed small-scale trials with Sabin and USOL-D-bac type 3 vaccine strains carried out in seven countries including Poland. Factors that might have contributed to the genesis and development of the epidemic were the pattern of virus excretion from vaccinees, mutations found in viruses from the epidemic, and the particular vaccination policies in Poland during the previous years. These findings may provide essential insights into the strategies for stopping polio immunisation once wild poliovirus has been eradicated.

Introduction. The use of live-attenuated and inactivated poliovirus vaccines will most surely complete the eradication of paralytic poliomyelitis and circulation of wild poliovirus from the world in the not too distant future (3). This will eventually lead to the obvious need to stop polio vaccination, particularly with live-attenuated vaccine strains (20). However, some issues regarding the scientific basis on how and when to stop polio immunisation are still not satisfactorily answered (3, 20). One of the most important questions is whether vaccine-derived isolates, which would be the last strains of poliovirus to subsist, would survive for long periods in the human population and/or in the environment and would be able to infect nonimmune children in the postvaccination era, reinitiating poliomyelitis epidemics (6). Live-attenuated vaccine strains replicate in the gut of vaccinees, often giving rise to poliovirus isolates of increased neurovirulence that are excreted to the environment (15). Vaccine-associated paralytic poliomyelitis (VAPP) is very rare (~1 case per 2.5 million doses of Sabin oral poliovirus vaccine) but, in contacts of vaccinees, VAPP affects mostly unvaccinated or inadequately vaccinated individuals (18). Also of great concern is the identification of several cases of long-term excretion of vaccine-derived poliovirus by individuals with antibody deficiencies (3, 6, 11, 13). Very little is known, however, about the molecular and genetic basis of poliovirus transmissibility and whether vaccine-derived strains revert to wild poliovirus transmissibility during person-to-person transmission. Experimental data suggest that vaccine viruses are less transmissible than wild strains but also indicate that vaccine-derived viruses can spread widely under conditions of low population immunity (6). Importantly, no poliomyelitis epidemics of vaccine-derived viruses have been reported since the use of Sabin live-attenuated vaccines became routine in the early 1960s. This is more remarkable in the case of unvaccinated or poorly vaccinated groups of individuals within well-vaccinated regions in which, despite proven exposure to vaccine-derived viruses, only wild poliovirus strains have been shown to spread rapidly and cause epidemics. Examples include religious groups in the United States and Canada, in which few members accept vaccination, who suffered epidemics of wild poliovirus type 1 in 1978/1979 and type 3 in 1992/1993, and certain age groups in Albania with very low vaccination coverage, who suffered a epidemic of wild-type 1 poliovirus in 1996 (reviewed in 6). In this paper we have investigated the possible vaccine origin of an epidemic of type 3 poliomyelitis in Poland in 1968 (12, 13). The epidemic started after small-scale trials with Sabin 3 and USOL-D-bac (USOL) live-attenuated vaccine strains had been completed (1). The vaccine trials, which also included six other countries, were carried out to evaluate the USOL strain as an alternative vaccine to Sabin 3, because of the genetic instability following replication in humans of Sabin 3 and concerns about the duration of the immunity induced (1). The results indicated that the USOL vaccine appeared superior to the Sabin 3 strain with respect of establishment in the gut, immunogenicity, and genetic stability (1, 4, 12, 17).
However, between March and December 1968, an outbreak of paralytic poliomyelitis associated with type 3 poliovirus with a total of 464 cases was reported in Poland. The temporal and geographical distribution of paralytic cases related the outbreak to vaccination of eight children with the USOL-D-bac live-attenuated strain in the city of Poznan 4 months before the epidemic started (1, 12). WHO collaborative studies at the time failed to identify the wild or vaccine origin of selected isolates from the epidemic (13). Here, we show experimental evidence that identifies those isolates with the USOL strain. The properties of the USOL vaccine strain and isolates from healthy vaccinees and cases from the epidemic, together with the circumstances in which the epidemic occurred, were analysed and are discussed in the light of the strategically difficult decision-making process on when and how to stop polio vaccination once wild poliomyelitis has been eradicated.

Results. Sequence of USOL-D-bac poliovirus vaccine strain. The complete nucleotide sequence of the USOL-D-bac strain (lot 2/66.01.12) used during the 1968 vaccine trials (1) was determined and analysed to establish the predicted amino acid sequence. The genome comprises a 5′ noncoding region (NCR) of 747 nucleotides, a single open reading frame of 6618 nucleotides (2206 codons), a short 3′ NCR region of 71 nucleotides, and a poly(A) tract. Table 1 shows the percentage of sequence homology between the USOL strain and previously sequenced polioviruses.

<table>
<thead>
<tr>
<th>Strain</th>
<th>5′NCR (%)</th>
<th>Capsid (%)</th>
<th>NS (%)</th>
<th>3′NCR (%)</th>
<th>Capsid (%)</th>
<th>NS (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sabin 1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>83.9</td>
<td>70.5</td>
<td>81.6</td>
<td>97.0</td>
<td>82.1</td>
<td>96.3</td>
</tr>
<tr>
<td>Sabin 2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>81.8</td>
<td>71.8</td>
<td>81.5</td>
<td>95.4</td>
<td>82.8</td>
<td>96.2</td>
</tr>
<tr>
<td>Sabin 3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>80.1</td>
<td>79.5</td>
<td>81.6</td>
<td>98.5</td>
<td>95.2</td>
<td>96.2</td>
</tr>
<tr>
<td>23127FIN84&lt;sup&gt;a&lt;/sup&gt;</td>
<td>82.8</td>
<td>84.6</td>
<td>82.7</td>
<td>98.5</td>
<td>97.6</td>
<td>95.9</td>
</tr>
</tbody>
</table>

<sup>a</sup> Nonstructural.
<sup>b</sup> EMBL Accession No. V01150.
<sup>c</sup> EMBL Accession No. X00595.
<sup>d</sup> EMBL Accession No. X00925.
<sup>e</sup> EMBL Accession No. X04488.
were completed in full. Sequence analysis confirmed the similarity of all isolates with the USOL strain, revealed several noncoding and coding changes, and eliminated the occurrence of recombination events with other poliovirus strains. Figure 1 shows the sequence analysis through the VP1-2A junction region of epidemic isolates compared to the USOL strain and previously sequenced type 3 polioviruses. Mutations found in the 5′ NCR and coding regions of the USOL-derived strains are described in the following sections.

**Nucleotide Changes in the 5′ NCR of Virus Isolates.** Few mutations were found in the 5′ NCR of the isolates from both healthy individuals and paralytic cases with respect to the USOL strain. Nucleotide transitions were found at positions 264 (in all isolates from paralytic cases); 118, 234, and 385 (in isolate D10/16); 599 (in isolate 5/91); 620 (in isolates 8/01, 7/61, and 1/6); 625 (in isolate 8/01); 629 (in isolates 8/01 and 7/61); 643 (in isolate 20/92); 659 (in isolate 4/22); 744 (in isolate 5/91). Only two of the nucleotide changes were located in stem structures/regions but none resulted in the disruption of the predicted secondary structure in the 5′ NCR. The mutation at position 264 (U to C), present in all isolates from paralytic cases, results in the strengthening of a basepair (U-G to C-G) in the predicted secondary structure.

**FIG. 1.** UPGMA phylogenetic tree based on the 120-nucleotide sequence of the VP1/2A junction region in the genome of 45 type 3 poliovirus strains. Sequencing data from wild strains were obtained from Poyry et al. (16) and Kew et al. (10). The locations of the USOL-D-bac strain and epidemic isolates 11/52 and 20/92, which contained the least (one) and the most (three) nucleotide changes with respect to the USOL strain, respectively, are highlighted.
Amino Acid Mutations in Capsid Proteins of Virus Isolates. Figure 2B shows the location in the Sabin 3 3D-structure model of the mutations found in capsid residues of isolates from the epidemic. All isolates from paralytic cases showed a mutation at VP1-7 from threonine to alanine, at VP1-258 from isoleucine to valine, at VP1-294 from valine to glutamic acid, and at VP2-191 from alanine to valine. Mutations at positions VP1-294 and VP2-191 were also present in all the USOL-derived isolates from healthy volunteers, including the earliest isolate, 5633/67, from Hungary, taken only 17 days after vaccination. Isolates from the epidemic contained a mutation in antigenic site 2a at position VP1-219 from alanine to threonine in isolates 7/61, 8/01, and 1/6 and from alanine to valine in isolate 4/22. Isolate 8/01 also showed a change at antigenic site 1 at position VP1-98 from arginine to lysine. Isolates from healthy individuals contained mutations at antigenic site 2b at VP2-165 from alanine to threonine in strain 5527/67 and at VP2-166 from valine to alanine in strain 10/715 and at antigenic site 4 at residue VP3-80 from aspartic acid to asparagine in isolates 10/715 from Poland, 5527/67 from Hungary, and D10/16 from Romania, and from aspartic acid to valine in strain 5778/67 from Hungary.

Amino Acid Mutations in Nonstructural Proteins of Virus Isolates. The genomes of isolates 11/52 and 1/6 from epidemic cases and strain 10/715 from a healthy vaccinee in Poland were sequenced completely through this region. A mutation was found at amino acid 19 (from isoleucine to threonine) of protease 2A in all three isolates. Both epidemic isolates 11/52 and 1/6 contained changes at amino acid 123 in protease 2A (from isoleucine to valine) and residue 21 in protein 2B (from isoleucine to valine). A mutation at polymerase 3D-133 (from lysine to arginine) was found only in the first epidemic strain 11/52, whereas changes at protease 3C-10 (from methionine to valine) and polymerase 3D-12 (from valine to methionine) were present only in the latter isolate, 1/6.

Changes at Synonymous Codon Positions in the Coding Regions of Virus Isolates. The distribution of synonymous nucleotide mutations with respect to the USOL strain in the region coding for capsid protein VP1 was analysed in order to investigate the genetic relationships between the epidemic strains. As shown in Fig. 2C, all isolates contained a common silent mutation at nucleotide 3127 and different mutations at several other positions that classified them in two groups. Each of the two groups was represented by each of the first two isolates, virus 11/52 from March 25, 1968, and strain 7/61 from April 20, 1968. All of the other five isolates from epidemic cases analysed in this study were from June 1968 when the epidemic was reaching its peak (12). Strains 4/22, 5/91, and 20/92 shared mutations with virus 11/52 at silent positions 2785 and 2821, whereas isolates 8/01 and 1/6 contained changes at nucleotides 2751, 3193, and 3297, identical to those found in virus 7/61 (Fig. 2C).

Nucleotide mutations identified at other synonymous codon positions in the coding region and at noncoding positions in the hypervariable region of the 5’ end were in good agreement with the suggested genetic relationships between the strains (data not shown). Isolates 10/715 (from a healthy vaccinee), 11/52 (from the first case), and 1/6 (from a later case) contained 0.32, 1.13, and 1.69% nucleotide changes at synonymous third-base codon positions over the entire coding region with re-

### TABLE 2

Comparison of Deduced Amino Acid Sequences at Antigenic Sites between the USOL-D-bac Strain and Previously Sequenced Type 3 Strains

<table>
<thead>
<tr>
<th>Antigenic site</th>
<th>Site 1</th>
<th>Site 2a</th>
<th>Site 2b</th>
<th>Site 3a</th>
<th>Site 3b</th>
<th>Site 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>VP1 89-100</td>
<td>USOL</td>
<td>Sabin</td>
<td>Saukett</td>
<td>21267</td>
<td>009EGY</td>
<td>1838SPA</td>
</tr>
<tr>
<td>VP1 217-223</td>
<td>SDANDN</td>
<td>T-----I</td>
<td>T-----I</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>VP1 286-290</td>
<td>KDGLA</td>
<td>RNN-D</td>
<td>N-----</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>VP3 58-60</td>
<td>ENT</td>
<td>-N-N</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>VP3 77-80</td>
<td>VR</td>
<td>-S-T</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>VP3 70, 71</td>
<td>NLSD</td>
<td>D-D</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>VP3 1/6</td>
<td></td>
<td>E</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>VP3 1/6</td>
<td></td>
<td>K</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>VP3 1/6</td>
<td></td>
<td>E</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>VP3 1/6</td>
<td></td>
<td>E</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
<tr>
<td>VP3 1/6</td>
<td></td>
<td>E</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
<td>-------</td>
</tr>
</tbody>
</table>

*EMBL Accession No. X00925.

*Data from Poyry et al. (16).

*Not available.

*Consensus sequence from the nine isolates from paralytic cases of the 1984 epidemic in Finland. Data from Poyry et al. (16) and Dunn (personal communication).
spect to the USOL vaccine strain, respectively. Taken together these data generated an estimated rate of evolution of 2.70% mutations at synonymous third-base codon positions/year, which is remarkably similar to the sequence evolution rate previously estimated for wild poliovirus during person-to-person transmission (10) or for vaccine-derived poliovirus strains during long-term replication in immunodeficient patients (11, 13). None of the synonymous mutations identified in epidemic isolates were found in any of the USOL-derived strains from healthy individuals including strain 10/715 from a healthy vaccinee in Poland.

Discussion. Substantial evidence was obtained that related the 1968 epidemic of type 3 poliomyelitis in Poland (1, 12, 13) with the use of live-attenuated USOL-D-bac poliovaccine (19). The temporal and geographical evidence of the association of the outbreak with a vaccine trial in the city of Poznan (1, 12) was confirmed by the nucleotide sequence analysis of seven isolates from the epidemic. The nucleotide sequences throughout the genome of the seven epidemic strains were highly similar to that of the USOL strain with the exception of a few mutations that could be assumed to have been incorporated during replication in vaccinees, their contacts, and/or patients. Analysis of nucleotide changes at synonymous codon positions, which has been shown to be an accurate method for measuring poliovirus evolution, was very consistent with the above-mentioned vaccine origin of the epidemic. The number of mutations found in epidemic cases with respect to the

FIG. 2. Mutations in isolates from the 1968 epidemic with respect to the USOL-D-bac vaccine strain. (A) Predicted secondary structure of the first 620 bases of the 5' NCR of poliovirus type 3. The location of the mutation at nucleotide 264 found in epidemic strains is indicated and the sequence compared to that of poliovirus strain representatives of the three serotypes. (B) Ribbon diagram of the α-carbon trace of the Sabin 3 protomer (5), viewed from the side of the virion (the outside is toward the top left of the image and the inside is toward the bottom right). The virus particle consists of 60 protomers, each containing a single copy of VP1, VP2, VP3, and VP4 arranged in icosahedral symmetry. The five- and threefold axes of symmetry are labelled. The equivalent locations of amino acid substitutions identified in epidemic isolates are highlighted. Mutations present in all epidemic cases are underlined; changes found also in isolates from healthy vaccinees are underlined a single line. The presence of sphingosine (SPH) in the hydrocarbon-binding pocket is also indicated. (C) Mutations at synonymous codon positions in the VP1 coding region. The position of nucleotide changes common to two or more isolates is highlighted.
USOL-D-bac vaccine strain in relation to the time of vaccination in Poznan and the time of virus isolation from the patients was remarkably close to that theoretically expected according to the sequence evolution rate that has been estimated for poliovirus (10, 11, 13). Not a single type 3 poliovirus strain had been isolated in Poland during the 1962–1967 period from poliomyelitis patients or in virological surveys of healthy persons and sewage examinations carried out each year (12). There was no known direct or indirect contact between the cases from the epidemic selected for this study or between them and USOL vaccinees or their contacts (1, 13). This observation highlights the fact that a small vaccine trial was the origin of such an extensive poliomyelitis outbreak during which 464 persons became paralysed and, assuming a minimal infection/disease ratio of 200:1 typical of virulent wild poliovirus strains (20), at least 90,000 people were infected.

Several factors were considered in order to understand how the epidemic started and developed. First, no clinical symptoms were observed in any of the vaccinated children or in their unvaccinated contacts during 12 weeks of careful observations after vaccination in Poland (12). Type 3 viruses were, however, isolated during this period from both groups of individuals and therefore, it is reasonable to assume that mutations acquired during replication in vaccinees or their contacts contributed to the reversion of the USOL vaccine strain to wild-type characteristics that prompted the widespread circulation of the virulent strain (8). As extensively reported before, few mutations seem to be necessary for the reversion to virulence of the live-attenuated Sabin vaccine strains; the mutations in most cases are rapidly acquired upon replication in the human gut (15). Similarly, few mutations were identified in the 5′ NCR and coding regions of isolates from the 1968 epidemic with respect to the USOL vaccine strain. All seven isolates from paralytic cases contained a mutation at nucleotide 264 (266 in Sabin 3) (from U to C) that results in the strengthening of a predicted base-pair (G–U to G–C) in domain IV of the internal ribosomal entry site (Fig. 2A), which is conserved in most poliovirus strains (17). However, none of the USOL-derived isolates from healthy individuals, four vaccinees and one contact, obtained during the vaccine trial from Poland and two other different countries, carried that nucleotide change. This observation suggests that, although mutation at position 264 could confer some biological advantage, it was not necessarily selected during replication in the human gut. On the contrary, mutations at capsid residues VP1-294 and VP2-191 were present in all isolates from epidemic cases and healthy individuals from Poland, Hungary, and Romania, including strain 5633/67 taken only 17 days after vaccination. VP1-294 is located in the carboxy-terminal region of VP1 in a position recently shown to be involved in receptor contact in other poliovirus strains (2, 8). The relevance of the mutation at residue VP2-191, situated in the interior of the capsid monomer inaccessible to external and internal surfaces, is unknown. All seven strains from the outbreak, but not those from healthy individuals, contained a mutation at residue VP1-7, located in the amino-terminal portion of VP1, a region that undergoes dramatic conformational changes after virus attachment to susceptible cells that lead to virus cell entry (7). Amino acid VP1-258, also mutated in all epidemic isolates, localizes in the north rim of the canyon, a surface depression that forms part of the receptor footprint (5). Amino acid changes were also detected at antigenic site 2a in four of the epidemic isolates and at antigenic site 1 only in isolate 8/01. Much less is known of the structural and functional significance of mutations in nonstructural proteins in relation to poliovirus attenuation, but mutations were identified at residues 2A-123, 2B-21, 3C-10, 3C-19, 3D-12, and 3D-133 in isolates from epidemic cases, from which only mutation at 3C-19 was also present in isolate 10/715 from a healthy vaccinee in Poland. The distribution of nucleotide mutations at synonymous codon positions among the epidemic isolates indicated that all seven strains had a common ancestor and identified two different genotypic lineages that were cocirculating when the epidemic was reaching its peak.

It is also important to note that the outbreak was restricted exclusively to Poland. No similar epidemic events were reported in any of the countries that were included in the same vaccine trial, although in two countries, Romania (four cases) and USSR (one case), paralytic cases occurred in contacts of children immunised with the USOL-D-bac strain. Isolation of poliovirus strains of different serotypes and other enteroviruses from those areas at the same time made the determination of the aetiology of the paralytic cases very complex (1). A possible explanation for epidemic cases to have occurred only in Poland lies in the fact that regular vaccination programmes in that country differed from those in most other countries in that only type 1 and type 2 strains were included in the live vaccine from 1961. From that time, the type 3 component was given exclusively as inactivated vaccine (IPV), which resulted in low general immunity against type 3 poliovirus (12). This observation was supported by the atypical age distribution of epidemic cases that included a higher than usual proportion of children between 4 and 8 years of age, most of whom had been vaccinated with type 3 IPV (12). The significant differences between the antigenic structures of the USOL strain and the IPV virus, the Saukett strain (Table 2), suggest that failure to specifically neutralise the epidemic strain as well as low immunity may have played an important part in the emergence and establishment of the outbreak. Interestingly, a similar phenomenon was described during the 1984 outbreak of type 3 poliomyelitis in Finland (9), where polio immuni-
sation has been based exclusively on IPV strains since 1957. The USOL and Finland strains, although not genotypically close (Table 1), showed striking similarities in their antigenic structures (Table 2). Following the same reasoning, isolates from vaccinees given Sabin 3 virus, which is more antigenically similar to the Saukett IPV strain, would have had less potential to cause poliomyelitis among the IPV-vaccinated Polish population at the time of the 1968 epidemic.

Other factors such as the level of viral transmissibility and/or virulence could explain the fact that vaccination with Sabin 3 in Poland during the 1968 trials and vaccination with the Sabin strains in general has not been found to be connected with poliomyelitis outbreaks. Little is known about the molecular basis of poliovirus transmissibility, but duration and titers of virus excretion by infected individuals are assumed to be important aspects that determine the ability of a virus to spread to other hosts (6). Studies during this and previous vaccine trials suggested that, although the USOL vaccine appeared to be similar or better than the Sabin 3 strain in terms of their attenuation properties, immunogenicity, and genetic stability, USOL vaccinees excreted higher titers of virus for longer periods of time after vaccination than children given Sabin 3 vaccine (1, 4, 12). This pattern of virus excretion could have been an essential factor that triggered the widespread circulation of the revertant strain(s) from USOL vaccinees or their contacts that eventually led to the establishment of the outbreak.

Considered together, the above data strongly suggest that USOL-D-bac-like strains derived from the vaccine trial in Poznan were responsible for most, if not all, of the paralytic cases during the 1968 epidemic. However, only a small proportion of cases were analysed (less than 2% of the total) and therefore, the possibility of other type 3 poliovirus strains of different origin being responsible for paralytic cases cannot be completely ruled out. Efforts are under way to locate more isolates from the epidemic and confirm this hypothesis.

The conclusions drawn from this paper raise important concerns with regard to the design of strategies for stopping polio vaccination once wild poliomyelitis has been eradicated (3, 20). Here we describe the first example of a widespread epidemic of paralytic poliomyelitis related to vaccine-derived strains, in circumstances that may occur during the first few years after vaccination is interrupted. In the light of our observations, long-term virus excretion from immunodeficient individuals, who have been shown to excrete polioviruses for periods as long as 10 years after vaccination (3, 11), may constitute a serious risk for the re-introduction of poliomyelitis epidemics in the postvaccination era. Evaluation and control of the prevalence of poliovirus infection among immunodeficient individuals is therefore essential before a major decision such as stopping polio immunisation could be taken.

**Materials and Methods.** USOL-D-bac strain and vaccine trial. The USOL-D-bac strain was developed in 1962 from a type 3 poliovirus isolated in 1960 in Czechoslovakia from a paralytic patient (19). The attenuation properties of the strain were extensively studied before the 1967-1968 vaccine trials including massive vaccine trials in Czechoslovakia and small-scale trials in Russia and Switzerland (1, 4, 19). The vaccine batch (lot 2/66.01.12) used in the 1967-1968 trials was prepared at the Institute Sieroterapico Milanese (Milan, Italy) and was subjected to exhaustive quality tests in the National Institute for Medical Research (London, UK) and the Institute of Poliomyelitis and Viral Encephalitis (Moscow, USSR) (1). Small-scale vaccine trials took place in France, the United Kingdom, Japan, Romania, Russia, Hungary, and Poland between September 1967 and March 1968 (described in detail in Ref. 1). In Poland there were three sets of vaccinations. On November 3, 1967, in Poznan City, eight children were vaccinated with the USOL virus. On November 15, 1967, in Bialystoc (considerably distant from Poznan), 10 children were immunised with the Sabin 3 strain. In addition, on February 16, 1968, nine children in Slupsk were given the USOL vaccine (1, 12).

**Poliomyelitis Outbreak and Virus Isolates Selected for Analysis.** The 1968 epidemic in Poland (described in detail in Ref. 12) endured from March to December 1968. More than half of the 464 paralytic cases were reported in Poznan City and Province and, although the epidemic spread to other parts of the country, it was restricted mostly to the northern and western provinces of Poland surrounding the Poznan Province. The epidemic diminished in September 1968 following mass vaccinations with live-attenuated vaccine in August, with only 5 cases reported in October, 2 in November, and 1 in December (12). Isolates from paralytic cases available from the WHO collaborative studies (14) included viruses from the first two cases, 11/52 (taken on 25 March 1968) and 7/61 (taken on 20 April 1968), and five later isolates from five other unrelated patients: 8/01 (from June 24, 1968), 4/22 (from June 17, 1968), 1/6 (from June 21, 1968), 5/91 (from June 19, 1968), and 20/92 (taken on June 25, 1968). Our analysis also incorporated isolates from USOL healthy vaccinees: one isolate (10/715) from Poznan City taken at day 66 after vaccination; three isolates taken from healthy vaccinees in Hungary, 5633/67 (17 days after vaccination), 5527/67 (49 days after vaccination), and 5778/67 (77 after vaccination); and one isolate (D10/16) from a healthy contact of USOL vaccinees in Romania taken 52 days after vaccination. Isolates were prepared in primary monkey kidney cells from faecal suspensions at the National Institute for Medical Research (Hampstead Laboratories, London, UK). Faecal samples from epidemic cases were collected between 2 and 21 days after the onset of paralysis (14).
Reverse Transcription, PCR, and Nucleotide Sequencing of Poliovirus Genomes. Poliovirus RNA was purified from HEP2-c cell culture supernatants and used for reverse transcription and PCR using standard procedures. DNA fragments containing the 5′ end of the viral cDNAs were obtained by the use of the 5′/3′ RACE kit (Boehringer) according to the manufacturer’s instructions. Sequencing of the purified viral RT-PCR DNA products was carried out using the ABI Prism 310 Genetic Analyser as specified by the manufacturer. Primers were designed by the “primer walking” strategy.

Analysis of Sequence Data. Sequence data were stored as Standard Chromatogram Format (*.scf) files and analysed using the Wisconsin Package Version 10.0-UNIX (GCG) and AlignIRV11 (LI-COR) software. Genetic relationships between poliovirus strains were estimated by sequence comparison in the 120-nucleotide VP1/2A junction region of the genome (positions 3317 to 3436 in Sabin 3 genome) (10).

ACKNOWLEDGMENTS

We thank Geoffrey Schild, Vladimir Vonka, and Andrew Macadam for their useful comments, Alan Heath for his help with the statistical analysis, and Tapani Hovi and Olen Kew for generously providing poliovirus sequences through the VP1-2A region.

REFERENCES