Short Communication

Phylogenetic analysis of wild-type 1 polioviruses isolated during the final period of transmission in Turkey

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The last poliomyelitis case associated with a wild poliovirus in Turkey occurred in November 1998. This was the last known case of paralytic poliomyelitis caused by indigenous wild poliovirus in the World Health Organization’s European Region. This study investigated the genetic relationships of wild-type 1 polioviruses at the latest period of transmission. A phylogenetic tree was constructed on the basis of the VP1/2A sequence from 14 wild-type 1 polioviruses isolated from Turkey in 1994–1998, along with those from other areas of the world. The Turkey isolates in the latest period of transmission were closely related to each other, forming a cluster distinct from other strains. The results showed that these viruses had been spreading indigenously in the eastern and south-eastern parts of Turkey, and ceased transmission there during 1998. This finding serves as a reference for future poliovirus surveillance both in Turkey and worldwide.

On 21 June 2002 the Regional Commission for the Certification of Poliomyelitis Eradication certified the European Region of the World Health Organization (WHO) as free of indigenous wild poliovirus transmission (WHO, 2002b). The European Region was the third polio-free WHO region, following the American and Western Pacific Regions. The last known case of paralytic poliomyelitis caused by indigenous wild poliovirus in the European Region was detected in eastern Turkey in November 1998.

The Eradication Programme for Poliomyelitis in Turkey has been implemented since 1989. The routine immunization programme for children in Turkey includes three doses of oral polio vaccine (OPV) by 4 months of age, followed by one dose of booster administration at 16–24 months and another booster dose in the primary school period. In addition to this routine programme, National Immunization Days for OPV have been implemented every year since 1995. On National Immunization Days two doses of OPV were administered to all children under 5 years old, regardless of previous vaccination history. In addition, in the eastern and south-eastern parts of the country a mopping-up programme has been carried out since 1997, with a two-dose immunization schedule implemented by

<table>
<thead>
<tr>
<th>Strain</th>
<th>Specimen collection</th>
<th>Date</th>
<th>Province</th>
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<td>Van</td>
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<td></td>
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<tr>
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<td>Oct 1997</td>
<td>Diyarbakir</td>
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<td>Oct 1997</td>
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<tr>
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<td>Jul 1998</td>
<td>Mus</td>
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Table 1. Wild-type 1 poliovirus isolates from Turkey and the Sabin vaccine strain

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house-to-house visits to all children. As a result of such an intensive implementation of immunization and acute flaccid paralysis (AFP) surveillance, cases of polio had been restricted to a specific geographical area. After November 1998 no case associated with wild polioviruses had been detected.

As a part of the WHO Eradication Program for Poliomyelitis (World Health Assembly, 1988), the virus laboratory of Refik Saydam National Hygiene Center has been engaged in the isolation and identification of polioviruses from AFP. Since 1996 approximately 600–1000 specimens per year from AFP cases and their contacts have been

![Map of Turkey showing provinces](image)

**Fig. 1.** Location of the provinces in Turkey from which the wild polioviruses investigated in this study were isolated. IS, Istanbul; AD, Adiyaman; DI, Diyarbakir; MA, Mardin; SI, Sirnak; MU, Mus; VA, Van.
investigated in the laboratory. In 1997 and 1998, the latest years of wild poliovirus transmission in this country, a total of 32 laboratory-confirmed cases associated with wild polioviruses were reported from eight provinces of the eastern and south-eastern regions of the country. The wild polioviruses were type 1 virus for 30 cases and type 3 virus for two cases. Since that time no case associated with wild viruses has been detected in Turkey.

**Fig. 2.** Phylogenetic analysis of the wild poliovirus type 1 strains based on the VP1/2A nucleotide sequence. (a) VP1/2A nucleotide sequences (nt = 150) of 14 Turkey isolates in 1994–1998 were compared with those of two wild poliovirus type 1 isolates from Turkey in 1990, and 33 isolates from other parts of the world in 1987–1996: 6386/ISR/87 (AF139282), 6467/ISR/88 (AF139280), 9334/SAA/89 (AF139256), 9340/SAA/89 (AF139277), 3727/ABZ/90 (AF139271), 3814/TUR/90 (AF139272), 3816/TUR/90 (AF139289), 919/GEO/90 (AF139290), 919/GEO/90 (AF233119), 3653/HE/CHN/91 (AF111983), 3867/IND/91 (AF139257), 3861/JOR/91 (AF139258), 3865/PAK/91 (AF139261), 7/TAJ/91 (AF233099), 422/RUS/91 (AF233118), 434/MOL/91 (AF233112), 3876/UAE/92 (AF139254), 3862/TUR/92 (AF139265), 5915/VTN/92 (AF139267), 3893/EGY/92 (AF139275), 3876/EGY/92 (AF139288), 5341/UKR/92 (AF233101), 5266/OMA/93 (AF139253), 5137/ETH/93 (AF139260), 5558/NIE/93 (AF139264), 5380/PHL/93 (AF139291), 5794/UZB/94 (AF233102), 6013/TUR/94 (AF233115), 6070/CHN/94 (AF233113), 717/RUS/94 (AF233121), 6405/ING/95 (AF233108), 6433/PAK/95 (AF233117), 6484/CHN/95 (AF233105), 3974/ALB/96 (AJ007963) and 5096/GRE/96 (Fio et al., 1998). The phylogenetic tree was constructed by the neighbour-joining method. Numbers at nodes are percentage of 1000 bootstrap pseudoreplicates containing the cluster distal to the node (Felsenstein, 1985). Turkey isolates were identified by abbreviation of the provinces shown in Fig. 1. Strains whose sequences were obtained from GenBank were identified by laboratory number, three-letter country code and year of isolation: ALB, Albania; AZB, Azerbaijan; CHE, Chechen-Ingush; CHN, China; EGY, Egypt; ETH, Ethiopia; GEO, Georgia; GRE, Greece; IND, India; ING, Ingush Republic; ISR, Israel; JOR, Jordan; MOL, Moldova; NIE, Nigeria; OMA, Oman; PAK, Pakistan; PHL, Philippines; RUS, Russia, SAA, Saudi Arabia; SYR, Syria; TAJ, Tajikistan; TOG, Togo; TUR, Turkey; UAE, United Arab Emirates; UKR, Ukraine; UZB, Uzbekistan; VTN, Vietnam. (b) Time of divergence between strains (shown by year and month in the tree) was calculated from the nucleotide difference between isolates, based on the time of isolation of strains and the evolutionary rate of $1 \times 10^{-2}$ substitutions per nucleotide per year, as described in the text. The isolates were positioned at the time of virus isolation on the horizontal scale. The date (year and month) of isolation of Turkey isolates is shown in Table 1; the date of isolation for other isolates was taken as the middle of the reported year (June).
The purpose of this study was to characterize the molecular epidemiological features of the wild-type 1 poliovirus isolated in 1994–1998, the latest transmission period in Turkey, and to explore any epidemiological relationships both within these isolates and between them and strains from other geographical areas, by constructing a phylogenetic tree based on the nucleotide sequences of the VP1/2A region of poliovirus. Molecular characterization of these strains provides important reference information for future surveillance of poliomyelitis in Turkey in relation to the importation of poliomyelitis from neighbouring countries where wild polioviruses might still exist (WHO, 2003).

Based on epidemiological records and the results of previous studies (Özkaya et al., 2001; Özkaya & Arita, 2002), a total of 14 type 1 wild poliovirus strains, 12 of 30 1997–1998 isolates and two 1994 isolates, were selected as representatives of wild-type 1 polioviruses during the last transmission period in Turkey (Table 1). All Turkey isolates obtained in this period were from the eastern and southeastern regions of the country, except for one strain from Istanbul in 1994 (Fig. 1). The methods used for isolation and identification of the polioviruses were as described by WHO (1992). The isolates had previously been serotyped using an antisemur kit supplied by the WHO European Regional Center, the National Institute of Public Health and Environment (RIVM), The Netherlands. Intratypic differentiation from the Sabin vaccine strain was done by neutralization, in-house ELISA and PCR–RFLP in our laboratory as described elsewhere (Özkaya et al., 2001; Özkaya & Arita, 2002) and was also investigated by RIVM. For reasons of laboratory containment of wild polioviruses (Department of Vaccines & Biologicals, 1999), the virus suspensions were added to an equal volume of guanidine thiocyanate and stored at −80°C for 6 months or more before sequencing.

Viral RNA was extracted and sequenced as described previously (Ishiko et al., 2002). Partial VP1/2A (nt 3296–3445) sequences of poliovirus isolates were determined with a 373A DNA automatic sequencer (PE Applied Biosystems) in cycle-sequencing reactions containing fluorescent dye-labelled dideoxynucleotides (Applied Biosystems). Sequencing templates were 1106 bp PCR products amplified from poliovirus RNAs with the primer pair Q8b [antisense (A) polarity] and Y7b [sense (S) polarity] (Shulman et al., 2000).

To explore the genetic relationships among type 1 polioviruses, the nucleotide sequences of the VP1/2A junction region (nt = 150) were analysed phylogenetically using SINCAsoftware (Fujitsu). The evolutionary distances were estimated using Kimura’s two-parameter method (Kimura, 1980), and unrooted phylogenetic trees were constructed using the neighbour-joining method (Saitou & Nei, 1987).

The VP1/2A nucleotide sequences of two Turkey isolates in 1990 and of 33 isolates from other regions of the world were obtained from GenBank. The other isolates were wild-type 1 viruses isolated after 1988, during the 10 years before the last isolation in Turkey, except for two isolates from Israel in 1987 and 1988. These Israel isolates (Shulman et al., 2000) were obtained from an outbreak in an area geographically close to Turkey, and showed a relatively close relationship to the Turkey isolates in the preliminary study.

The phylogenetic tree of the strains (Fig. 2a) indicated that the 14 wild poliovirus type 1 isolates from Turkey in 1994–1998 formed a monophyletic cluster along with two strains, one from Azerbaijan obtained in 1990, the other from Russia in 1991. Another cluster, formed separately from, but closest to, the recent Turkey isolates, included two 1990 Turkey isolates along with strains from neighbouring countries such as Israel obtained in 1987 and 1988, Saudi Arabia in 1989, Egypt in 1992, Syria in 1992, Russia in 1994, and Georgia in 1990.

Other wild poliovirus type 1 strains isolated worldwide after 1988 were distinctly separate from the Turkey isolates. These were from Africa (Togo, Ethiopia, Nigeria); Europe (Tajikistan, Moldova, Ukraine, Uzbekistan, Ingush Republic, Chechen-Ingush, Albania, Greece); the eastern Mediterranean (Jordan, Saudi Arabia, Syria, the United Arab Emirates, Oman); South-East Asia (Pakistan, India); and the western Pacific (China, Vietnam, the Philippines). The strains from Albania and Greece in 1996 were the most recent isolates. Despite a geographically close location and similar timing of isolation (Fiore et al., 1998), they were clearly differentiated from the Turkey isolates by nucleotide sequence comparison.

The method used to estimate the time of divergence among isolates has been described previously (Tanirumiet al., 1985; Ishiko et al., 1992; Takeda et al., 1994). In brief, the nucleotide difference between isolates (dij) was converted to the value dij′ at fixed time t5, based on the nucleotide substitution rate (b) and isolation time of the strains. The equation was d′ij = b(t5−τi) + b(t5−τj) + dij where τi and τj are the time of isolation of strains i and j, respectively. Then, with d′ij as a distance, a phylogenetic tree was constructed by unweighted pairwise grouping of the arithmetic mean (UPGMA). The branching time (τ5) was calculated by the equation τ5 = t5−(dij/2b). We applied the substitution rate of 1.2 × 10−2 per nucleotide per year, which was estimated for circulating wild polioviruses by Gavrilin et al. (2000).

Based on the phylogenetic analysis shown in Fig. 2(b), the isolates from Turkey in 1994–1998 were grouped into a cluster which separated around the late 1980s from the other 12 strains included in that study. The data indicated that these were progeny of a common hypothetical strain that existed around 1991. Among these, two 1994 isolates from Van and Istanbul diverged from the group of more recent Turkey isolates (Fig. 2a). The isolates from Turkey in 1997–1998, which were the last wild-type 1 polioviruses to prevail in this country, were progeny separated from each
other after around 1996 and maintained in the eastern and south-eastern parts of Turkey, where the lineage of the strains ceased to transmit in 1998 (Fig. 2a). The two 1990 Turkey isolates, whose sequences were drawn from the GenBank database, were shown to have diverged from each other and also from the recent Turkey isolate group during the 1980s. One strain, 3816/TUR90, was closely related to the 1992 isolates from Syria, a country bordering Turkey. The two isolates from Azerbaijan (1990) and Russia (1991) that had been positioned in a common cluster with the recent Turkey isolates (Fig. 2a) were separated from them, although they still showed a closer relationship to the recent strains than two Turkey strains (3814/TUR90 and 3816/TUR90) isolated in 1990.

The results of the VP1/2A phylogenetic study showed that the wild poliovirus type 1 isolates in the latest period of transmission in Turkey belonged genetically to a unique lineage separated from those of neighbouring countries and also from two Turkey isolates previously obtained in 1990. The study indicated that wild-type 1 polioviruses with multiple genetic lineages spread widely in Turkey until the early 1990s, and some showed close linkage with those of neighbouring countries. After the mid-1990s, however, the wild polioviruses were restricted to limited areas of Turkey. Closely related viruses with unique nucleotide sequences were maintained for a while in the eastern and south-eastern provinces, then in 1998 the transmission chain in these areas was cut.

This study clearly demonstrates the success of the national eradication activities and vaccination programmes, along with thorough surveillance, which have been conducted intensively in these areas. Many examples indicate the risks of outbreaks due to imported wild poliovirus, emphasizing the importance of implementing high-quality surveillance in countries free of indigenous wild poliovirus. In addition, nucleotide sequence analysis has become an essential procedure following the emergence and circulation of vaccine-derived polioviruses associated with poliomyelitis cases (Kew et al., 2002). It is proposed that the viruses with <1 % difference from Sabin vaccine virus are classified as Sabin-like; those with 1–15 % difference as vaccine-derived polioviruses; and those with >15 % difference as the wild virus (WHO, 2002a). Among the Turkey type 1 isolates studied here, the differences in nucleotide sequences of VP1/2A region ranged from 17 to 23 % from the type 1 Sabin vaccine strain (data not shown), coinciding with the range of the wild virus defined above.

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References


