Importation and circulation of poliovirus in Bulgaria in 2001

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Objective To characterize the circumstances in which poliomyelitis occurred among three children in Bulgaria during 2001 and to describe the public health response.

Methods Bulgarian authorities investigated the three cases of polio and their contacts, conducted faecal and serological screening of children from high-risk groups, implemented enhanced surveillance for acute flaccid paralysis, and conducted supplemental immunization activities.

Findings The three cases of polio studied had not been vaccinated and lived in socioeconomically deprived areas of two cities. Four Roma children from the Bourgas district had antibody titres to serotype 1 poliovirus only, and wild type 1 virus was isolated from the faeces of two asymptomatic Roma children in the Bourgas and Sofia districts. Poliovirus isolates were related genetically and represented a single evolutionary lineage; genomic sequences were less than 90% identical to poliovirus strains isolated previously in Europe, but 98.3% similar to a strain isolated in India in 2000. No cases or wild virus isolates were found after supplemental immunization activities were launched in May 2001.

Conclusions In Bulgaria, an imported poliovirus was able to circulate for two to five months among minority populations. Surveillance data strongly suggest that wild poliovirus circulation ceased shortly after supplemental immunization activities with oral poliovirus vaccine were conducted.

Keywords Poliomyelitis/prevention and control/transmission; Poliovirus/genetics/isolation and purification; Paralysis/epidemiology; Feces/virology; Vaccination; Immunization programs/methods; Disease outbreaks/prevention and control; Population surveillance; Socioeconomic factors; Gypsies; Minority groups; Child, Preschool; Bulgaria/epidemiology (source: MeSH, NLM).

Introduction

Since 1995, countries in WHO’s European region have been strengthening efforts to interrupt transmission of wild poliovirus. Supplementary immunization activities in individual countries, as well as synchronized supplementary immunization activities in 18 contiguous countries of the Eastern Mediterranean and European Regions of WHO (“Operation MECACAR”) (1, 2), resulted in a dramatic reduction in the incidence of poliomyelitis. At the same time, surveillance of poliomyelitis improved in participating countries. In the European region, the last case of poliomyelitis

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caused by indigenous transmission of wild poliovirus was observed in Turkey in November 1998 (3).

Before 2001, the last instance of wild virus-associated poliomyelitis in Bulgaria was recorded in 1991, when an outbreak that affected 43 cases, mostly Roma infants, was documented (4). In March 2001, a 13-month-old Roma girl in the Eastern harbour city of Bourgas presented with bilateral leg weakness. An epidemiological and virological investigation was initiated after wild type 1 poliovirus was isolated from the patient’s faecal samples. In this report, we describe characteristics of the outbreak, possible mechanisms associated with its occurrence, and characteristics of the poliovirus isolates; findings from serological investigations of healthy children; and interventions implemented to control the circulation of poliovirus.

Methods

Background

Bulgaria is situated in south-eastern Europe. Its population in 2000 was estimated to be 8.15 million, with 1.3 million being children aged 0–14 years; the annual birth rate is approximately 69,000 live births. Vaccination services are provided free of charge through a network of family practitioners. Each family practitioner has a list of the population they serve. District-level health services provide vaccines on the basis of the number of eligible children in each of the 28 health districts. Family practitioners, in turn, report vaccination coverage according to their lists. They also are expected to actively follow up children who fail to attend vaccination appointments or who did not receive age-appropriate vaccination at the correct time.

Surveillance for acute flaccid paralysis

Bulgaria established surveillance for acute flaccid paralysis according to WHO guidelines published in 1993 (5). Key performance indicators of the surveillance include a sensitivity of at least one non-polio-related case of acute flaccid paralysis per 100,000 people aged <15 years annually, with two adequate faecal specimens collected within 14 days of the onset of paralysis for 80% of cases of acute flaccid paralysis (5). A national enterovirus laboratory was accredited by WHO in 1997. Health district personnel investigated and reported cases of acute flaccid paralysis to the Directorate of Epidemiology at the Ministry of Health. After the index poliomyelitis case was diagnosed, national health authorities initiated reviews of records in district hospitals and “zero reporting” (daily reporting that no acute flaccid paralysis cases had been found) by the district epidemiology units.

Supplemental immunization activities

Three types of supplemental immunization activities were conducted in Bulgaria during 2001: an immediate local response after identification of the index case, two rounds of national immunization days, and two rounds of sub-national immunization days. During the four weeks immediately after the virus was isolated in the index case (19 April–16 May), a mass vaccination campaign was conducted among children aged 0–83 months from high-risk groups in Bourgas and in the neighbouring districts of Yambol, Sliven, and Stara Zagora. A first round of national immunization days involved all other children from the same age group in the country during 28 May–1 June. A second round of national immunization days took place for all children from that age group during 25–29 June. The substantial proportion of children among high-risk groups who had not received enough vaccination through routine services meant that two additional rounds of supplemental vaccination were conducted nationwide during 22–26 October and 26–30 November for children at risk aged ≤ 5 years (sub-national immunization days) (Fig. 1).

Epidemiological investigation and specimen collection

Teams of epidemiologists from districts and from the central level investigated each case of acute flaccid paralysis and reviewed clinical records. Vaccination records were reviewed for children who lived in the same household, immediate neighbours, and hospital contacts of people; people in these groups were given oral poliovirus vaccine. Faecal specimens were collected from children exposed in the hospital to case-patients and from children who lived in the same households as these exposed children.

Serological surveys

Between 19 April and 8 May 2001, we sampled 71 children aged 0–83 months who were from minority populations and were hospitalized in any of five district hospitals before the first mass vaccination campaign. We reviewed these children’s vaccination records and collected blood specimens. The Bulgaria National Enterovirus Laboratory measured antibody titres to types 1, 2, and 3 poliovirus in sera by using neutralization assays in microcultures of HEp-2 cells (6). Specimens in which the neutralization titre was <1:8 were considered negative.

Faecal specimen surveys

We conducted three faecal surveys among hospitalized children during April–September 2001. The first survey was made during April and May — immediately after the poliomyelitis index case had been diagnosed — and included 117 Roma and other minority children aged 0–83 months who were hospitalized in nine districts nationwide. A second survey was conducted among 257 children aged 0–83 months who presented with meningitis-like symptoms during June–September 2001. The third survey, implemented during August–September after the second round of national immunization days, involved 155 Roma and other minority children aged 0–35 months from nine district hospitals. These hospitals were selected because the nine districts accounted for approximately 60% of the high-risk children in the country.

Isolation and characterization of poliovirus isolates

Viral isolation was performed on L20B and RD cell cultures at the national laboratory. Viral isolates were identified by micro-neutralization assay, according to WHO’s recommended standard methods (7). Serum pools and type-specific poliovirus antisera were provided by the National Institute of Public Health and the Environment (RIVM, Bilthoven, Netherlands). Intratypic differentiation was performed by polymerase chain reaction with Sabin-specific primers (Centers for Disease Control and Prevention (CDC), Atlanta, USA) (8) and by enzyme-linked immunosorbent assay (ELISA) with cross-adsorbed, type-specific, polyclonal antibodies (RIVM, Bilthoven, Netherlands) (9). The full VP1 genomic region of wild virus isolates was sequenced with the DyeDeoxy Terminator Cycle Sequencing Kit (Applied Bio Systems, Perkin-Elmer, Foster City, CA, USA) after reverse transcription and cDNA amplification, with a panel of synthetic oligonucleotides used as primers. We aligned the sequences obtained with the
corresponding sequences of poliovirus type 1 Sabin reference strains (10) and wild poliovirus type 1 field isolates reported in the European Molecular Biology Laboratory Data Bank or kindly supplied by CDC and by the Indian WHO Regional Reference Laboratory (Mumbai, India). A dendrogram of sequence relatedness was constructed by the method of the VP1 maximum-likelihood bootstrap consensus tree (PUZZLE, version 4.0) (11). The VP1/2A junction and a portion of 5’ non-coding region for all type 1 wild viruses were analysed. Sequence information is deposited at GenBank under accession numbers AY253227, AY255677, and AY255678.

Results

Active surveillance implemented after identification of the index case of poliomyelitis resulted in a substantial increase in the number of cases of acute flaccid paralysis reported compared with previous years in which performance indicators for surveillance of acute flaccid paralysis in Bulgaria were not consistently at optimal level. During 2001, 42 cases of acute flaccid paralysis were reported, including three confirmed cases of polio (Fig. 1). In addition, the detection rate for non-polio related cases of acute flaccid paralysis was 3.0 per 100 000 population aged <15 years, and two adequate faecal specimens were collected within 14 days of the onset of paralysis in 40/42 (95%) reported cases of acute flaccid paralysis.

Cases

Case 1

The index case of poliomyelitis occurred in a 13-month-old Roma girl from Bourgas, who had never received poliovirus vaccine. Although she was hospitalized for high fever, she received several intramuscular antibiotic injections. On 24 March 2001, she lost active movement of both legs and her right arm. On clinical examination, she had weak tendon reflexes in affected limbs. On 17 April, poliovirus type 1 (BUL01239) was isolated from a faecal specimen; this subsequently was characterized as being wild type at the WHO Regional Reference Laboratory, Rome, Italy. Investigation of faeces collected from contacts of the index case allowed the identification, on 20 April 2001, of an additional wild type 1 poliovirus (BUL01306) in a three-year-old girl from Karnobat who had previously received five doses of oral polio vaccine. Interestingly, this girl’s 11-month-old sister had shared a hospital room with case 1 during 21–24 March 2001. The younger sister previously had received two doses of oral polio vaccine, and no polioviruses were isolated from her faecal specimens.

Case 2

An unvaccinated 26-month-old Roma girl from Yambol was hospitalized with tonsillo-pharyngitis and received several intramuscular antibiotic injections. On 24 April 2001, she presented with sudden onset of bilateral leg paralysis. Weak tendon reflexes of her legs and left arm, and weak superficial abdominal reflexes were documented. Wild type 1 poliovirus (BUL01378) was isolated and characterized from faecal specimens collected three days after the onset of paralysis. No wild polioviruses were isolated from faecal specimens of four children exposed at home to case 2.

Case 3

A three-month-old Roma boy from Bourgas developed paralysis of the right leg on 7 May 2001. The patient had received a first dose of oral polio vaccine on 25 April 2001 during the emergency vaccination campaign. He was referred for medical examination and hospitalized on 21 May with severe hypotonia and absence of tendon reflexes of both legs. Residual paralysis, consistent with clinical poliomyelitis, was documented 60 days after the onset of paralysis. Of note: case 3 was hospitalized during 1–23 April in the same ward in which case 1 had stayed during 21–24 March. A serum specimen collected in hospital on 19 April 2001 had no antibodies to any of the three polioviruses. Serology from a specimen obtained on 22 May revealed an antibody titre of 1:64 against poliovirus type 1, no detectable antibodies to type 2, and a titre of 1:16 to poliovirus type 3. A type 3 Sabin-like strain was isolated from one faecal sample after three passages in cell culture. With all available evidence and guidelines from WHO’s regional office, the National Expert Committee classified this case as clinical poliomyelitis consistent with wild virus infection.

Serological surveys

Serological surveys among minority children aged 6–83 months who were hospitalized in district hospitals showed that nine (56%) of 16 children from Bourgas; three (27%) of 11 from Sofia; and 27 (82%) of 33 from Dobrich, Pazardjik, and Plovdiv had antibodies against all three poliovirus serotypes (Table 1). In Bourgas, antibodies against all three serotypes were detected in one in 10 children aged <6 months. In addition, four children from Bourgas hospital (one aged two months and three aged 6–83 months) possessed detectable antibodies against poliovirus type 1 only. The fact that isolated type 1 seropositivity was found in 4/16 (25%) minority children aged <83 months suggests they were exposed to the type 1 strain responsible for the outbreak. No children with isolated antibodies against type 2 or 3 strains were found.

During the first faecal survey, conducted in April and May 2001, 117 minority children were sampled in nine district hospitals. One 17-month-old girl from Sofia, who had received a single dose of oral polio vaccine at birth, was found to carry a wild type 1 poliovirus (BUL01478). In addition, six Sabin-like poliovirus strains were isolated from five children (one type 1, four type 2, and one type 3). Two subsequent surveys conducted in June–September 2001 and August–September...
2001 resulted in the isolation of 90 enteroviruses, including 10 Sabin-like polioviruses (seven type 2 and three type 3). No additional wild polioviruses were detected. Fig. 2 (web version only, available at: http://www.who.int/bulletin) shows the geographical location of the cities in which poliomyelitis cases with and without poliovirus isolates, as well as asymptomatic carriers of wild polioviruses, resided.

Sequence alignment analysis of the full length VP1 of the type 1 wild virus isolated from case 1 shows that the strain was only 76% homologous with the Sabin type 1 reference strain and not more than 90% homologous with any previously isolated strains from the European Region. In contrast, the highest degree of homology (98.3%) was found with a wild type 1 strain isolated in July 2000 from a patient in the State of Uttar Pradesh in northern India (IND00018) (Fig. 3). Complete (100%) homology between the strains isolated from the two patients with poliomyelitis (BUL01239 and BUL01378) was found in the VP1 coding region, consistent with the involvement of a single virus genotype in the outbreak. Strains from non-paralysed children were 99.90–99.97% homologous with strains isolated from patients with poliomyelitis. A single mutation (A to G) at nucleotide 2532 of VP1 was found in the strain 478 BUL01 isolated from the healthy carrier in Sofia, whereas the strain 306 BUL01 isolated in the healthy contact in Karnobat immunized with oral polio vaccine possessed two additional mutations at position 3001 (A to G) and 3140 (C to T) of the VP1 coding region.

The strains isolated from the two healthy children did not differ from the strain isolated from case 1 by more than three mutations in the VP1 region; these mutations probably were due to intra- and inter-human virus passage. All the mutations found in the coding region were silent (did not produce amino acid substitutions). Comparison of the sequences in the 5’ non-coding regions, from nucleotides 246 to 543, showed a single mutation at nucleotide 382 (A to T) of all wild strains with respect to the strain BUL01239 isolated from the index case. Homology in the 2A genomic region between all wild strains isolated was 100%.

During the initial limited mass vaccination campaign, a total of 26 552 doses of oral polio vaccine were administered in four districts, including an estimated population of 25 665 children at high risk aged <84 months. During the first round of national immunization days, 412 560 doses of vaccine were administered to an estimated population of 443 055 children not included in the emergency response. In the second round, 447 456 doses were given countrywide to children aged <8 years age. Coverage of each round (estimated on the basis of doses administered) was 93% and 95%, respectively. During two rounds of sub-national immunization days, 91 785 and 94 462 doses of oral polio vaccine were given to high-risk children aged <5 years, with administrative coverage being 94% and 96%, respectively. A survey conducted among the high-risk population after the second round of sub-national immunization days confirmed the coverage data obtained from administrative records. It also indicated that, before mass vaccination, only 73% of children aged >12 months had received three doses of oral polio vaccine through routine services (data not shown).

**Discussion**

This report documents the first temporary circulation of a wild poliovirus strain in Europe in recent history, which occurred through an accumulation of susceptible children in minority populations with low routine coverage of poliovirus vaccination. Serological and faecal surveys conducted among children who were most likely exposed and vulnerable to the virus showed gaps in population immunity and circulation of the

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**Table 1**. Proportion of hospitalized minority children sampled with antibodies to all three poliovirus serotypes by age and health district in Bulgaria, April–May 2001

<table>
<thead>
<tr>
<th>Age (months)</th>
<th>Bourgas</th>
<th>Sofia</th>
<th>Dobrich, Pazardjik, and Plovdiv</th>
</tr>
</thead>
<tbody>
<tr>
<td>0–5</td>
<td>1/10</td>
<td>0/1</td>
<td>0/0</td>
</tr>
<tr>
<td>6–35</td>
<td>8/12</td>
<td>3/10</td>
<td>18/23</td>
</tr>
<tr>
<td>36–83</td>
<td>1/4</td>
<td>0/1</td>
<td>9/10</td>
</tr>
<tr>
<td>All</td>
<td>10/26</td>
<td>3/12</td>
<td>27/33</td>
</tr>
</tbody>
</table>

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**Fig. 3. Dendrogram summarizing nucleotide sequence relatedness among wild polioviruses type 1 isolated in Bulgaria (BUL) and recent wild type 1 polioviruses isolated in Tajikistan (TAJ), India (IND), Syrian Arab Republic (SYR), and China (CHN), across the interval of nucleotides 2480–3385 (VP1 capsid region).** The three letters represent the country, the next two digits the year of identification, and the last three digits are specific to the particular isolates presented. Numbers at nodes represent quartet puzzling support values (similar to bootstrap values).
wild type 1 poliovirus. These findings justified a vigorous intervention. Prompt vaccination of children at high risk within one month of the onset of paralysis in the index case and supplementary vaccination countrywide within two months may have prevented further spread of the virus strain within and outside Bulgaria.

No definitive conclusions were reached about the transmission path that allowed a strain very similar to those circulating recently in the Indian subcontinent to be imported into Eastern Europe. Anecdotally, a review of all manifests from four ships that reached Bourgas harbour during February and March 2001 showed that 34/67 (51%) crewmembers were of Indian nationality (data not shown). Considering that a sufficient pool of susceptible children was present in and around Bourgas at the time of onset of paralysis in the index case, it is unlikely that silent circulation of the virus could have occurred before February 2001. Molecular evidence that showed that none of the four isolates differed by more than two base pair mutations in the sequences analysed suggests a limited period of circulation in Bulgaria before the viruses were identified. The presence of isolated antibody titres to type 1 poliovirus among minority children in Bourgas — where the index case was diagnosed — suggests that poliovirus might have entered Bulgaria through that district. The fact that wild viruses were not detected after 8 May 2001, despite enhanced surveillance and repeated faecal surveys among children at high risk, suggests that circulation might have been interrupted by the first mass vaccination campaign. At the very least, discrete transmission among a small number of non-immune individuals was not enough to result in a clinical case of poliomyelitis after May 2001. Active surveillance of acute flaccid paralysis and faecal surveys in neighbouring countries, as recommended by WHO (12), did not result in identification of wild virus outside of Bulgaria. Supplemental immunization activities among high-risk children were undertaken in many of these countries (WHO, unpublished data, 2001).

Increased awareness of the risk of poliomyelitis and implementation of active surveillance resulted in improvements in performance indicators for surveillance of acute flaccid paralysis in Bulgaria. Investigation of the outbreak rapidly identified the existence of greater-than-expected gaps in immunity among minority populations. The rapid implementation of the national vaccination campaign shows excellent collaboration between local health services, governmental services, the community network, the laboratory network, and international partner organizations. Although both rounds of national immunization days seemed to have achieved high coverage, additional supplemental immunization activities among high-risk minority children were conducted to provide multiple doses of oral polio vaccine to many children who had never been vaccinated.

Suboptimal vaccination in the Roma population contributed to the outbreaks in 1991 and 2001. Other outbreaks occurred in Europe among population groups with lower vaccination coverage in Spain during 1982–84 (13), the Netherlands during 1992–93 (14), and Romania during 1990–92 (15). These examples show that, without appropriate control actions, population groups with lower vaccination coverage can sustain the circulation of wild polioviruses within a country for up to three years. In addition, outbreaks of paralytic disease caused by circulating vaccine-derived polioviruses also could occur (16). High-risk communities are present in all European countries. All attempts must be made to reach minority children with immunization and other health services.

Until the transmission of wild poliovirus is interrupted globally, it will remain possible for poliovirus to be reintroduced into a European country, as well as into other regions of the world already free from poliomyelitis (17). Countries in polio-free areas of the world therefore should maintain vigilance, identify and actively vaccinate underserved populations, and develop the mechanisms for rapid detection and appropriate response to such an event.

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Conflicts of interest: none declared.

Résumé

Importation et circulation du poliovirus en Bulgarie en 2001

Objectif Préciser les circonstances dans lesquelles des cas de poliomyélite sont survenus chez trois enfants en Bulgarie au cours de l’année 2001 et décrire la riposte de santé publique.

Méthodes Les autorités bulgares ont étudié les trois cas de poliomyélite et leurs contacts, effectué un dépistage par prélèvement d’échantillons fécaux et sérologiques chez des enfants appartenant à des groupes à haut risque, mis en place une surveillance renforcée de la paralysie flasque aiguë et organisé des activités de vaccination supplémentaires.

Résultats Les trois cas de poliomyélite étudiés n’avaient pas été vaccinés et vivaient dans des zones socialement et économiquement défavorisées de deux villes. Chez quatre enfants Rom du district de Bourgas, on a retrouvé uniquement des anticorps dirigés contre le poliovirus de sérotype 1, et le virus sauvage de type 1 a été isolé dans les prélèvements fécaux de deux enfants Rom asymptomatiques des districts de Bourgas et Sofia. Ces isolats de poliovirus étaient génétiquement apparentés et appartenaient à une seule et même lignée évolutive ; les séquences génomiques montraient une similitude inférieure à 90 % à celle des souches de poliovirus isolées précédemment en Europe, mais étaient similaires à 98,3 % à une souche isolée en Inde en 2000. Aucun cas ou isolement de virus sauvage n’a été retrouvé après les activités de vaccination supplémentaires organisées en mai 2001.

 Conclusion En Bulgarie, un poliovirus importé a pu circuler pendant deux à cinq mois parmi des populations minoritaires. Les données de la surveillance semblent indiquer que la circulation du poliovirus sauvage a cessé peu après l’organisation des activités de vaccination supplémentaires par le vaccin antipoliomyélitique oral.
Resumen
Importación y circulación del poliovirus en Bulgaria en 2001

Objetivo
Caracterizar las circunstancias que rodearon la infección por poliomielitis de tres niños en Bulgaria durante 2001, y describir la respuesta del sistema de salud pública.

Métodos
Las autoridades búlgaras investigaron los tres casos de poliomielitis y sus contactos, sometieron a análisis fecales y serológicos a los niños de los grupos de alto riesgo, introdujeron mejoras en la vigilancia de la parálisis flácida aguda y emprendieron actividades de inmunización suplementaria.

Resultados
Los tres casos de poliomielitis estudiados no habían sido vacunados y vivían en áreas socioeconómicamente desfavorecidas de dos ciudades. Cuatro niños romanos del distrito de Bourgas presentaban títulos de anticuerpos para el serotipo 1 del poliovirus únicamente, y en las heces de dos niños romanos asintomáticos de los distritos de Bourgas y de Sofía se aisló el virus salvaje de tipo 1. Los aislados de poliovirus estaban relacionados genéticamente y representaban una única línea evolutiva; las secuencias genómicas eran idénticas en menos del 90% a las de las cepas de poliovirus aisladas anteriormente en Europa, pero se asemejaban en un 98,3% a las de una cepa aislada en la India en 2000. No se detectó ningún caso, así como tampoco ningún aislado del virus salvaje, después del comienzo de las actividades de inmunización suplementaria en mayo de 2001.

Conclusión
En Bulgaria, un poliovirus importado pudo circular por espacio de dos o cinco meses entre poblaciones minoritarias. Los datos de vigilancia autorizan a pensar que la circulación del poliovirus salvaje se interrumpió poco después de emprender las actividades de inmunización suplementaria con la vacuna contra el poliovirus.

Referencias

Fig. 2. Map of Bulgaria showing the residence of poliomyelitis cases positive at isolation for type 1 wild virus (●), poliomyelitis case negative for wild virus isolation (◇), and asymptomatic carriers of type 1 wild poliovirus (◆).