



# **Report of the Joint Containment – European Polio Laboratory Network Meeting**

**St Julian's, Malta, 20–22 February 2007**

**Vaccine-Preventable Diseases and  
Immunization Programme**

## ABSTRACT

Europe is the first WHO region to have completed Phase I of the WHO Global Action Plan for Laboratory Containment of Wild Polioviruses, but globally polio eradication is at a critical point. Virus transmission was not interrupted in the four remaining polio-endemic countries in 2006, heightening the risk of wild poliovirus importation into the European Region. Regional initiatives include new guidelines on outbreak response and strong encouragement of supplementary immunization activities to close immunity gaps at national or subnational level. In February 2007, the WHO Regional Office Vaccine-preventable Diseases and Immunization Programme organized a three-day Meeting of national polio containment coordinators, heads of national polio laboratories and other national, regional and global representatives and experts to maintain the momentum, provide technical support, encourage networking and underline the message that constant vigilance is required.

### Keywords

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## Glossary

AFP	acute flaccid paralysis
AM	aseptic meningitis
BSL	biosafety standard level
EV	enterovirus
CVID	common variable immune deficiency
GAP	WHO Global Action Plan for Laboratory Containment of Wild Polioviruses
IPV	inactivated poliovirus vaccine
ITD	intratypic differentiation
LabNet	Polio Laboratory Network
NCC	national containment coordinator
NT	neutralization reaction
OPV	oral poliovirus vaccine
PCR	polymerase chain reaction
Polio	poliomyelitis
RCC	European Regional Commission for the Certification of Poliomyelitis Eradication
RD	rhabdomyosarcoma
RT-PCR	reverse transcription polymerase chain reaction
VAPP	vaccine-associated paralytic poliomyelitis
VDPV	vaccine-derived poliovirus
aVDVP	ambiguous vaccine-derived poliovirus
cVDVP	circulating vaccine-derived poliovirus
iVDVP	immunodeficient vaccine-derived poliovirus
WPV	wild poliovirus

## Introduction

Europe is the first WHO region to have completed Phase I of the WHO Global Action Plan for Laboratory Containment of Wild Polioviruses, and the WHO Regional Office for Europe is coordinating efforts to sustain the Region's poliomyelitis (polio)-free status. However, concern remains high over global polio eradication, which is at a critical point. Virus transmission was not interrupted in the four remaining polio-endemic countries in 2006, and as a result there is an increasing risk of wild poliovirus (WPV) importation into the Region. In addition, the quality of acute flaccid paralysis (AFP) surveillance in the Region has slowly declined since 2002, while significant high-risk subpopulations and underserved areas remain, for which polio surveillance and immunization indicators are suboptimal. Regional initiatives include new guidelines on outbreak response and strong encouragement of supplementary immunization activities to close immunity gaps at national or subnational level.

To maintain the momentum, provide technical support, encourage networking and underline the message that constant vigilance is required, the Regional Office Vaccine-preventable Diseases and Immunization Programme organized a three-day Meeting of national polio containment coordinators, heads of national polio laboratories and other national, regional and global representatives and experts. Twenty-two European Member States were represented, mainly from western Europe. The goals of the Meeting were as follows:

- to update coordinators on the progress of global polio eradication efforts and containment activities;
- to present the regional results of Phase I of the WHO Global Action Plan for Laboratory Containment of Wild Polioviruses (GAP) on containment and discuss follow-up activities;
- to update coordinators on the third edition of the GAP for Phases II and III;
- to brief participants on the regional containment action plan for Phase II;
- to discuss containment activities in the Region in 2007–2008;
- to update national polio laboratories on the activities and future strategy of the Global Polio Laboratory Network (LabNet);
- to present a new testing algorithm;
- to update national polio laboratories on the performance of the European and global LabNets and discuss the implementation of a new testing algorithm and *Mycoplasma* testing;
- to update participants on the clinical significance of enteroviruses and the persistence of polioviruses in immunodeficient patients.

The Meeting was divided into three parts: introduction, global overview and containment and was followed by the European Polio Laboratory Network Meeting. The programmes of the Meetings are in Annex 1 and the list of participants in Annex 2.

## **Global overview**

Dr Galina Lipskaya, Regional Wild Polio Containment Coordinator, Vaccine-preventable Diseases and Immunization Programme, WHO Regional Office for Europe, opened the meeting and thanked Malta for hosting it. Dr Raymond Busuttil, Director-General of Health, Malta, welcomed participants. He hailed the completion of the first phase of WPV containment in the Region as an important step in a process that started in 1988 when the World Health Assembly resolved to eradicate polio. The Global Polio Eradication Initiative reflected both humanitarian concerns – over 350 000 children in 125 countries were being paralysed every year – and economic ones, since a cheap and effective vaccine had been available from 1961. By 2006, only four countries still harboured indigenous WPV, while the 53 European countries were polio-free. The outlook was, therefore, positive but challenges remained in the next phase. All Member States should adopt a national long-term post-eradication/post-oral poliovirus vaccine (OPV) cessation policy and regulations on polioviruses. Malta would continue to play an active part, working jointly with other countries and WHO.

Dr Eugene Gavrilin, coordinator of the WHO European LabNet, said it was the first time that western European countries in the Network had met after years of contact. The focus had been on countries in the east of the Region but it was necessary to refocus the attention of western countries on polio, lest it became a neglected disease.

Dr Walter Dowdle, Director of Programs, United States Task Force for Child Survival and Development was elected Chairperson. Two introductory presentations gave global overviews of the key issues.

## **Global overview of polio eradication and Phase I containment**

Dr Chris Wolff, Global Containment Coordinator, WHO headquarters, described the main thrust of the global eradication programme and future plans. First he considered the interruption of poliovirus transmission. The remaining four countries where polio was endemic had 500–1500 cases a year in the same geographical areas. Since 2000, 33 polio-free countries had been re-infected 80 times, and the circulation interrupted again in most of them. There were three main challenges: the persistence of polio despite very high immunization coverage, international spread, and too many children still not immunized. Global eradication tools, standards and approaches had been revised by 2006 to address them, and a three-pronged approach launched in 2007 to interrupt WPV globally consisting of:

- new tools to stop transmission, primarily new vaccines and new diagnostics;
- new standards for responding to outbreaks to reduce the risk and consequences of international spread;
- new approaches in endemic areas.

Second, Dr Wolff looked at post-eradication planning and the implementation of a comprehensive work programme to assess, reduce and manage the post-eradication risks. The objectives of Phase II of the GAP had been wholly or partly achieved or were ongoing, and the Region's completion of Phase I was a great achievement. Now it was necessary to determine future vaccination strategy: "The optimum vaccination strategy in a polio-free world depends on

the overall plan for managing the long-term risk of an eradicated pathogen,” he said. The options for long-term polio immunization policy were to:

- stop all polio immunization
- stop all OPV, limited use of inactivated poliovirus vaccine (IPV)
- replace all OPV with IPV
- introduce new polio vaccines.

Only the second and third options were viable, and would depend on national decisions. The risks of IPV would be negligible during cessation in high-income countries; afterwards the risks would come from laboratory accidents, IPV production accidents, immunodeficient vaccine-derived poliovirus (iVDPV) cases (chronic virus excretors) and intentional use. International consensus would be required on destroying/containing all polioviruses, synchronous cessation of all OPV and stock destruction, and international oversight of the reintroduction of live attenuated viruses for outbreak response. Amendment of the International Health Regulations (2005) would be needed; if this was initiated in 2007–2008, it would take over three years for the process of reservations and rejection and entry into force.

## **WHO Global Action Plan for Laboratory Containment of Wild Polioviruses**

Dr Dowdle outlined the third phase of the GAP, which aimed to minimize poliovirus facility-associated risk in the post-eradication/post-OPV era (known as GAP III). This was not yet formally approved, but countries should be thinking about risk management now, because containment would start as soon as implementation was announced. In 2004, WHO had adopted the post-eradication goal of stopping routine OPV use, in order to prevent vaccine-associated polio, eliminate the risk of chronic infection of immunodeficient people and prevent outbreaks of circulating vaccine-derived polioviruses (cVDPV). Once OPV use stopped, some countries would continue high IPV coverage, some would have suboptimal IPV coverage, and some might discontinue immunization. The rationale for GAP III was as follows:

- non-immune populations would increase post-eradication, rapidly so post-OPV cessation;
- a facility-associated reintroduction of WPV would have increasingly serious consequences;
- a facility-associated post-OPV introduction of a Sabin strain could lead to cVDPV and re-establishment of polio;
- facility-associated polio risks must be reduced to the lowest possible level.

GAP III aimed to minimize the facility-associated post-eradication risk of reintroducing WPV or Sabin strains into the community at a time when OPV was no longer being used. It would set precedents by seeking international harmony in national regulations, seeking limits on the number of poliovirus facilities worldwide and proposing international accreditation of facilities. Its strategy, which built on lessons learned from surveying over 200 000 facilities, was to eliminate risk through national destruction and prohibition of poliovirus material, except in a minimum number of essential facilities, and to manage the risks associated with essential facilities through primary safeguard of containment and secondary safeguard of the location of the facilities. The goal after eradication and OPV cessation was, therefore, to reduce the number of poliovirus facilities to fewer than 20 worldwide. Those that remained would serve essential

international functions with regard to vaccines, reference and research, and meet all primary safeguards of containment and secondary safeguards of lowest population risks.

The primary safeguards needed for these essential facilities would include suitable design, construction and operation; the maintenance of biosafety standard level 3 (BSL3); immunization of personnel; reduced use of live WPV, with Sabin strains substituted where possible; contingency plans for containment breaches; and institutional, national and international oversight. Four implementation phases were proposed for GAP III:

- (i) a survey of national biomedical laboratories and WPV inventory
- (ii) establishment of a national long-term poliovirus policy and regulations
- (iii) global destruction and containment of WPV materials
- (iv) global destruction and containment of OPV/Sabin materials.

The next steps were wider circulation and final revision of the Plan before its proposed endorsement by the World Health Assembly in 2008. Countries should not wait but should urgently consider key strategic issues:

- how to achieve reduction/destruction of poliovirus that were not needed
- how to replace needed stocks with Sabin strains
- how to reach facilities at risk of poliovirus-contaminated stocks/materials
- how to ensure effective risk reduction.

## **Containment in the European Region**

### **Completion of containment Phase I**

Dr Lipskaya reported on the implementation of GAP I in the European Region. National surveys and a WPV inventory had been completed by all 53 Member States. Of the 41 220 laboratories included in the analysis, those most likely to possess WPV materials were academic, industrial, research and state-run public health laboratories (11 771). Another 13 369 that might possess WPV were clinical microbiology laboratories with storage capacity. Small diagnostic laboratories lacking storage facilities or cell culture capacity (16 080) were least likely to possess WPV.

Twenty-seven countries reported they had no infectious materials. The remaining 25 countries had 111 laboratories storing WPV infectious materials and 265 storing WPV infectious or potentially infectious materials (of which 206 laboratories were in western Europe, half in the United Kingdom). To help ensure that these data were reliable and complete, all Member States had assessed the quality of their Phase I containment activities and described a range of actions taken, from gaining high-level political support to developing new legislation.

At the 19<sup>th</sup> meeting of the WHO European Regional Commission for the Certification of Poliomyelitis Eradication, the European regional report was officially accepted as documenting completion of Phase I of laboratory containment. The activities had strengthened public health in Member States in various ways:

- strengthening national polio laboratory registration/licensing systems;
- introducing new legislation;
- strengthening collaboration between national public health authorities and the national laboratory system;
- motivating biomedical laboratories to conduct risk assessment of materials in storage and to destroy those of no research or public health value;
- assessing biosafety practice in biomedical laboratories.

## Country reports on containment

Representatives from Member States presented country reports on the following topics proposed by WHO.

### *GAP Phase I*

- National inventory.
- Laboratory inspections/visits to confirm the accuracy and completeness of inventory.
- Laboratory inspections to confirm biosafety requirements.
- Approaches used to identify institutions working with enteroviruses/rhinoviruses or collaborating with polio-endemic countries and storing potentially infectious poliovirus materials.
- National regulations for laboratories applying for licensing/registration.

### *GAP Phase II*

- Strategies to minimize the number of facilities in the inventory.
- Approaches for preparing national authorities to develop long-term post-eradication policies and regulations.
- Initiation of a survey of biomedical laboratories working with Sabin vaccine strains.
- An assessment of the feasibility and new national regulations for laboratories planning to work with polio, including the requirements for licensing/registration

### ***Austria***

Dr Sophia Zasmata reported that the WHO programme for laboratory containment of WPV started in 2000. The national inventory finalized in 2001 included four laboratories in three institutions still retaining WPV. Later two laboratories destroyed WPV, which was officially documented. Since 2003, only two laboratories in one institution, both BSL3 standard, had retained WPV. In one, the Laboratory for Viral Safety, WPV type I is used for polio antibody neutralization tests as a release test for products. In the other, the Laboratory for Viral Vaccines, WPV type I is used for viral inactivation studies as a model virus for chemical inactivation. This was owned by a large transnational pharmaceutical company, which planned to register the vaccine worldwide, and was regulated by the US Food and Drugs Administration (FDA). Changing the FDA registration was complex but the institution was nevertheless planning to replace its WPV once this had been completed. Dr Zasmata suggested that WHO could contact

the FDA and similar bodies to highlight the issue and to promote the replacement of WPV by other viruses in tests for registration of drugs.

### ***Belgium***

Dr Miriam Sneyers summarized the national political endorsement and support for containment activities and the national regulatory process. The laboratory survey in 2002 had revealed eight facilities holding WPV. A 2007 update showed the number had halved to two research laboratories (one university, one industry), one diagnostic (and teaching), and one production. All four were authorized, inspected and reached minimum BSL2 standards. Phase I was now complete and Belgium awaits WHO guidance for the next phase, in particular the need for good coordination on containment in vaccine production.

### ***Denmark***

Dr Blenda Böttiger reported that Denmark's last polio case was in 1977. Immunization coverage was high, with 98% seropositivity for all three poliovirus types. OPV was replaced by IPV in 2001–2003, with the last OPV found in a patient sample in 2001. As of 2007, only two laboratories possessed WPV, both at the State Serum Institute which had built an IPV production facility in 2004. Inspection showed various containment risks that were being tackled to upgrade the facility to BSL3 standard. One challenge is that the country now has few people with a general knowledge of molecular virology and poliovirus specifics, especially among the inspection/control bodies, so international consultancy might be needed and it will be important to keep a knowledge of polio eradication. The role of the coordinator also needs to be clarified: is it to be a partner for discussion and advice, or a controller?

### ***Finland***

Professor Timo Hyypiä reported that Finland had used IPV since 1960. After a poliovirus 3 outbreak in 1984, the whole population was vaccinated with OPV. The last WPV isolation was in 1993 and the last OPV in 2006. The survey of 689 laboratories in 2001–2002, since updated twice, showed WPV is currently held in only two laboratories, one of which is a WHO regional reference laboratory.

### ***France***

IPV was mandatory in France, which had 98% coverage, reported Dr Marie-Claire Paty. Containment began in 2000 with a multisectoral initiative. Of the 7265 laboratories that were surveyed and informed, 10 held WPV (9 in the health sector, 1 in industry) and 44 held potentially infectious material (including 3 laboratories located outside Europe in France's overseas territories). Some planned to destroy their stocks. Another survey was planned for 2007, regulations were being updated, and a stronger inspection regime was being developed through partnership between the Ministry of Health and the agency for the regulation of health products. In discussion it was agreed that France had gone much further than many other countries by surveying laboratories containing not only WPV but vaccine poliovirus as well. In future it could be a model for others.

### ***Greece***

Professor Antonios Antoniadis reported that Greece had submitted its report on completing Phase 1 in 2006. Its survey showed two laboratories working with enteroviruses (EVs); both had destroyed all WPV stocks and all other infectious and potentially infectious poliovirus materials,

and destruction was officially documented. Both facilities had now been brought up to BSL3 standard. The inventory would be expanded to include all facilities working with EVs. Proposals were being submitted to the Ministry of Health for Phase II activities and funding. Professor Antoniadis thanked colleagues from Italy for their assistance.

### ***Hungary***

Hungary's last imported case of paralytic polio was diagnosed in 1972, said Dr Gyorgy Berencsi. By 2002 the national inventory listed 636 laboratories, of which only 7 had the deep-freeze capacity for long-term storage of poliovirus and samples potentially containing poliovirus. All laboratories except the national polio laboratory destroyed all samples with materials potentially infected with WPV, and all reference strains were replaced by authentic Sabin strains in 2003. The vaccination schedule was changed in 2006 and now only IPV is used. The incineration of more than 4000 ampoules (10 doses per ampoule) of OPV was completed early in 2007; 3216 ampoules would be stored at the National Centre for Epidemiology for unforeseen events. The construction of a BSL4 facility was completed in 2006 to store WPV and to conduct research on Crimean-Congo haemorrhagic fever. All vaccine-associated paralytic poliovirus (VAPP) isolates and wild strains tested since 1950 were stored there at –20 °C. Staff had been trained overseas to BSL4 standard using innocuous samples, and BSL3 and 4 work would be initiated after some minor technical repairs. Sequencing capacity and 'real time' polymerase chain reaction techniques had been introduced to achieve rapid detection and typing of polio and non-polio EVs.

### ***Israel***

Israel completed Phase I in 2006, reported Dr Ella Mendelson. Of the 370 laboratories on its computerized database, only 1, the Central Viral Laboratory, now retained poliovirus materials, and was aiming to upgrade from BSL2 to 3. In 2006 the country switched from IPV/OPV schedules to IPV in a pentavalent vaccine. There was no legislation covering infectious materials of any sort, and compliance with containment was voluntary, but new legislation was planned for Phase II.

### ***Italy***

Professor Paola Verani Borgucci reported on extensive work done in the absence of a national regulatory framework, with a continuing decline in the number of laboratories holding WPV from 17 to 7. Of these, 5 have only WPV, 1 has potentially infectious WPV, and 1 has both. The process had been problematic because there was no specific relevant legislation; in the decentralized system a variety of bodies were in charge; resources were short and workload too heavy; and there had been significant personnel changes in the Ministry. Compliance with BSL2 could not be monitored owing to lack of clarity about the responsible authority. Plans were being developed for Phase II. WHO's help was requested in explaining to national authorities the need to establish or update national regulations covering licensing/registration of laboratories planning to work with polioviruses.

### ***Malta***

Dr Busuttill reported that AFP became a notifiable disease in Malta in 1997, and active surveillance had been maintained since 1998 in both public and private hospitals. All stool samples were sent to the WHO-accredited laboratory in Rome, Italy. High immunization coverage was achieved using both oral and inactivated vaccines; since 1994 coverage with three doses of polio vaccine had ranged from 94% to 96%. Malta was also paying attention to

vaccinating refugees, asylum-seekers, foreign students and travellers to endemic regions. A national survey in 2000–2002 showed that no laboratories or institutions possessed WPV or potentially infectious material; that was still the case in 2007 according to the national containment update.

### ***Netherlands***

Dr Westerhof reported that the inventory completed in 2002 surveyed 988 laboratories: 6 held infectious or potentially infectious material, of which 2 held WPV. Laboratories were being encouraged to destroy their stocks. The Netherlands had a minority group of about 75 000 people who refused vaccination, making them vulnerable to polio importation. It had therefore resumed large-scale environmental surveillance, mainly within high-risk communities, and municipal health organizations were actively encouraging high-risk groups to be vaccinated.

### ***Norway***

The last indigenous case of polio occurred in 1969, said Dr Gabriel Ånestad. In 1969–1980 there were six vaccine-associated cases, and the last imported case was in 1992. Since 1979 Norway had used only IPV. Of the 217 laboratories surveyed, only 1, at the Norwegian Institute of Public health, held infectious and potentially infectious materials.

### ***Portugal***

Dr Maria Teresa d’Avillez Paixão described the comprehensive, collaborative and multisectoral initiatives taken since 2000. A national laboratory containment plan was signed in 2003 and a national post-elimination programme signed in 2004, with extensive guidance issued. National legislation was revised to ensure compliance with WHO objectives. Of the 607 laboratories in the inventory, only 2 had retained WPV: 1 had destroyed its stocks and the only remaining materials were at the National Polio Laboratory. She also highlighted the recent trend for the establishment of private laboratories and/or shift of ownership to transnational companies, which raised accountability and regulatory issues.

### ***Spain***

Spain’s containment activities had started in 1999 with the active involvement of its autonomous regions, reported Dr Maria Cabrerizo. Over 3200 laboratories had responded to the initial survey in 2002: 78 held materials, 9% of which were WPV. Only 2 laboratories now retained WPV in BSL2 or 3 facilities, and 2 retain potentially infectious materials, so good progress had been made with containment. Dissemination work had included regional conferences and publication of articles and leaflets. The strategy was to have WPV in only one national polio laboratory. New legislation would be needed for Phase II. New biosafety rules should be proposed by the European Union.

### ***Sweden***

Containment had been a challenge in Sweden, Dr Helene Norder reported, as it was not seen as a funding priority and the resources available to work on it were very limited. Nevertheless progress continued; of the 11 laboratories identified in 2004 as retaining WPV, vaccine poliovirus or complementary DNA, 9 now held WPV and 2 were planning to destroy their stocks. There was resistance to the idea of regulated laboratory inspections. In discussion, Dr Norder was praised for her persistent and successful work as containment is ongoing process.

### ***Switzerland***

Dr Samuel Roulin described the inventory and updates, the collaboration involved, strict Swiss biosafety regulations, and cantonal inspections. Switzerland, like France, had surveyed for both WPV and vaccine poliovirus. Of 940 laboratories, 13 now possessed infectious or potentially infectious WPV and 22 possessed infectious or potentially infectious vaccine poliovirus. Swiss biosafety legislation would be revised in 2007–2008 in preparation for Phase II. Dr Dowdle commented that it was much easier to work in Switzerland within an existing regulatory framework, and suggested that WHO could help other countries lacking such a framework to put one in place in good time for the next phase.

### ***Turkey***

Dr Neziha Yilmaz reported that the last case of polio was in 1998. In 2000 a working group including provincial health directorates drew up a national containment action plan, with support from the Ministry of Health. No new legislation was introduced. Of the 3407 laboratories surveyed, only 3 laboratories in 2 institutions were retaining WPV infectious or potentially infectious materials by 2006. Two of these laboratories (the virology laboratory at Refik Saydam Hygiene Institute in Ankara and the virology laboratory in the Ministry of Health regional laboratory in Izmir) are accredited by WHO as national and subnational polio laboratories. Refik Saydam Hygiene Institute wished to retain its materials and is hoping to upgrade to BSL3 standard with EU funds. Plans were now under way for Phase II.

### ***United Kingdom***

Dr Karen Noakes described Phase 1 activities: an inventory was being carried out to establish a complete database of laboratories in the United Kingdom and obtain a list of organizations holding infectious or potentially infectious WPV materials; audits, including on-site laboratory inspections, were being carried out; and advice was being given to prepare for changes in the biosafety containment of WPV or to destroy material. The 3374 laboratories identified in the first round were asked whether they had stored samples of WPV reference strains, isolates, vaccine virus, and faecal, respiratory and other samples from polio cases or samples/sewage collected when WPV was circulating (before 1985). Of these, 106 held infectious or potentially infectious WPV (17 held WPV). The Health and Safety Executive, which has primary inspection and enforcement responsibility for all United Kingdom premises carrying out work with hazardous biological agents and genetically modified organisms, carried out thorough on-site polio containment reviews from 2006 in addition to its usual inspection process. Laboratories were asked to destroy poliovirus materials they did not need, and to prepare for changes to the biosafety containment of polioviruses CL2 to CL3 (BSL3) if they planned to keep samples. Measures to maintain awareness included conference presentations, with future plans for information materials and stickers for freezers saying 'poliovirus free' and 'no to polio'.

### ***Conclusion***

In general discussion, it was agreed that progress on containment had been good. Lessons learned included the need to begin preparation for Phase II as soon as possible. It was important to maintain contacts and databases, and safeguard institutional memory and knowledge of polio, especially as most younger health experts had no memory of or contact with the disease and might therefore underestimate the importance of containment. In response to a question about eastern Europe, Dr Lipskaya reported that its problems were different: a majority of the Member States in eastern Europe reported no WPV in laboratories and future containment activities were under discussion.

## **Containment Phase II**

Dr Wolff invited comments on proposed strategies for

- encouraging the reduction/destruction of current poliovirus inventories
- replacing needed stocks with authenticated Sabin strains
- addressing the issue of contaminated non-PV stocks.

### ***Encouraging the reduction/destruction of current poliovirus inventories***

Countries should:

- ensure all facilities are aware of their responsibilities and the future implications of poliovirus retention, including annual confirmation of their current status;
- ask facilities retaining materials to submit to the national coordinator the rationale and expected duration of retention.

This approach was generally supported, but concerns were raised about private sector laboratories run by transnational biotechnology companies. It was suggested that WHO had a role to play in encouraging their compliance, by disseminating information to them about the European and global plan. Given the range of legislation in different countries, including some where compliance was purely voluntary, it was also suggested that WHO inform Member States in Europe about what coordinators were asked to do. There was no intention of putting extra burdens on laboratories that retained materials, or discouraging them from keeping stocks, but they had to understand what was expected of them. Laboratories that met the requirements were free to keep strain stocks and should not be forced to act prematurely; there was no legal constraint at present. However, the view was expressed that this could be a retrograde step, as laboratories were already being persuaded to destroy stocks.

The importance of disseminating information was underlined, and illustrated by experience from the Russian Federation. The Ministry of Health in 1996 ordered the destruction of all WPV in Russian laboratories; all officially confirmed this had been done, but contamination, perhaps through mislabelling, occurred in a laboratory not included in the national polio network – the person working with the viruses did not know about the containment programme. One suggestion was that poliovirus should be included on countries' lists of dangerous organisms.

### ***Replacing needed stocks with authenticated Sabin strains***

Countries should:

- write to all facilities offering to replace WPV strains with authenticated Sabin strains;
- review the national list of laboratories to identify facilities likely to have Sabin strains, and offer to replace them with authenticated strains.

### ***Addressing the issue of contaminated non-PV stocks***

Many countries had already acted on the second point above (review laboratories likely to have Sabin strains). The issue of commercial production of disinfectants was raised – could it be assumed that these laboratories were now using only authenticated strains? Industry should be informed about the legal basis for using WPV strains. There was discussion of the complexities

of mislabelling and cross-contamination, especially in laboratories where staff were poorly informed about polio. Work on poliovirus should be separated from other EVs; staff should be properly trained and immunized, act with maximum caution and not move from working with one type of virus to another. More guidance and consensus on best practice was needed: the European Region could ask the global LabNet to devise an algorithm to address this. The move to molecular testing also needed to be taken into account when advice was given to diagnostic laboratories.

## **Biorisk management for essential poliovirus facilities in the post-eradication era**

Dr Wolff outlined the current status of the new BSL3 standard (GAP Annex 4) for information and comment, as it was still work in progress. He said the unique nature of an eradicated pathogen made a polio biorisk management standard necessary: there was very low tolerance for risk post-eradication, and there would be a radical change in perception of risk from poliovirus. The greatest risk of reintroduction would come from infected/contaminated facility personnel. Risk management was achievable, and WHO needed to clarify how the change should be addressed and assure the world that the risks of facility-based polioviruses were being managed effectively. Facilities and manufacturers wanted guidance. The standard had several purposes:

- to establish performance expectations for poliovirus essential facilities
- to provide a basis for national/international accreditation of facilities
- to provide ‘umbrella’ requirements/guidance for essential facilities.

The process of drafting the document would involve risk assessment, consequence assessment, biosafety and other expert consultations, circulation of draft requirements and review. Consultations to date had concluded that the standard should emphasize identification and management of risk, drawing on past experience with other laboratory-associated infections and underlining that biorisk management required more than equipment and sophisticated facilities. It should have a long time horizon, be adaptable to multiple solutions and new developments in biosafety technologies, and complement but not replace existing guidance. The document should combine an occupational health and safety type of management system with specific biosafety issues, and combine advice on both management and technical issues.

Dr Wolff then outlined details of the draft standard, as follows:

- 1 management and organization
- 2 risk assessment
- 3 poliovirus inventory and information
- 4 transport
- 5 personnel and competence
- 6 health care
- 7 human factors
- 8 general safety
- 9 good microbiological techniques
- 10 clothing and personal protective equipment
- 11 physical requirements of the facility
- 12 equipment, maintenance, calibration and certification
- 13 disinfection, decontamination and sterilization

- 14 security
- 15 accident/incident investigation
- 16 contingency plans and emergency response.

This presentation provoked discussion on a range of points. It was felt that an ongoing testing regime for all facility workers could be burdensome. Outsourcing by facilities of services such as cleaning and caretaking meant contracting companies should also comply with the standard. There was some disagreement on the value of showering out. The question of whether there should be dedicated laboratories was raised: as they would not have to function often, they would be less likely to follow procedures. Vaccine production was a closed system to exclude contamination but it was not possible to locate it all within a BSL4 facility.

### **Approaching GAP 3 in the European Region: tasks and challenges**

Dr Lipskaya proposed an action plan for Phase I follow-up and to initiate preparation for Phase II in 2007–2008. Phase I follow-up would involve:

- verifying the accuracy of national Phase I records;
- verifying the completeness of national data on institutions collaborating with polio-endemic countries with stool sample collections in storage and cell culture capacity;
- ensuring regulatory mechanisms existed for maintenance and update of the national inventory in all Member States; and
- annual progress updates to the European Regional Commission for the Certification of Poliomyelitis Eradication.

The preparation for Phase II would require work on national long-term poliovirus policy and regulations:

- securing administrative and financial resources for implementing and documenting containment activities required at the global certification stage;
- initiating development of a national long-term policy for post-eradication/post-OPV cessation;
- initiating work on establishing national regulations and infrastructures to ensure consistency with international regulations; and
- initiating the destruction of low-value WPV materials.

The Regional Office would work to secure funds for Phase II activity; maintain and regularly update the regional inventory; monitor Member States' preparations for Phase II, with special attention to upgrading laboratories to BSL3 where appropriate; assist Member States in reviewing laboratories remaining in the national inventory; and follow up annual progress updates from countries. It would also pay attention to the wider public health dimensions of containment, including strengthening national laboratory registration/licensing systems; introducing new legislation requirements; strengthening collaboration between national public health authorities and the national laboratory system; and motivating biomedical laboratories to assess materials in storage and destroy those of no research or public health value.

Assessment of general biosafety practice in biomedical laboratories was another important issue. It was incorporated in the regional overview and annual WHO accreditation procedure. Biosafety parameters would be assessed on site visits:

- existing national biosafety regulations/legislation;
- biosafety officer or committee;
- institutional biosafety documentation;
- external/internal biosafety inspection programme;
- alarm system and emergency power back-up;
- security of human pathogen data management;
- restricted access to human pathogen storage areas;
- biosafety of transport (outgoing and incoming materials) in accordance with WHO recommendations;
- reports on leakage or broken sample containers;
- assessment of the effectiveness of disinfectants and decontaminants;
- appropriateness of sterilization practice;
- efficient equipment maintenance;
- staff immunization and health checks;
- appropriate use of personal protective equipment.

The main issue raised in discussion was the need to persuade governments to fund these activities. Key points emerging from the reports and discussions were summarized in a set of recommendations on containment unanimously agreed by the participants.

## **Recommendations on polio containment**

The World Health Organization is recommended to:

- remind Member States of the continuing need for a designated national polio containment coordinator and resources sufficient to meet current and future containment goals;
- inform Member States of current progress on poliovirus containment, the proposed strategy for the post-eradication/post-OPV cessation era, and the preparatory steps recommended for Phase II;
- strengthen collaboration with the European Union and the European Center for Disease Control to harmonize general biosafety standards and practices across all Member States;
- explore mechanisms to ensure poliovirus containment issues are fully considered for research funding, particularly project proposals for review by national and European Union funding bodies involving the collection of human faecal or throat specimens from poliovirus-endemic areas;
- develop an algorithm through the WHO Global LabNet to determine a feasible and effective approach to reducing the risk of poliovirus spread from contaminated non-poliovirus or mislabelled stocks;

- provide an annual update to national containment coordinators on the current status of global polio eradication.

The Regional Certification Commission is recommended to:

- ask national polio containment coordinators to ensure that their annual reports on containment reflect the current state of their national inventory.

National polio containment coordinators should:

- provide an annual update of their national inventory to their national certification commissions and WHO, indicating changes in the number of facilities listed and reports of destruction or transfer of poliovirus materials with accompanying official documentation;
- communicate to all facilities in the national inventory:
  - their responsibility to notify the national containment coordinator annually of the status of wild poliovirus materials and supply written documentation of the destruction or transfer of these materials;
  - the need for a written rationale (or citation of regulatory requirements in some cases) for retaining wild poliovirus materials, specifying the timelines for submission to national authorities;
  - the availability of authenticated Sabin strains.
- review the national list of laboratories to identify facilities likely to have Sabin strains, and contact them with an offer to replace these Sabin strains with authenticated strains;
- identify possible mechanisms for updating the national inventory with information already available through existing channels (e.g. laboratory registration/licensing requirements);
- consider the implications of activities proposed in the draft third edition of the GAP, and discuss them with the national task force for containment, national certification committees and other appropriate national authorities, bearing in mind the opportunity to provide comments to WHO before the plan is submitted to the World Health Assembly in 2008.

## **European Polio Laboratory Network Meeting**

### **Overview of the Global LabNet**

Dr Fem Julia Paladin, Virologist, WHO headquarters, reported on the detection of polioviruses and VDPVs, workload and timelines for reporting, laboratory performance concerns and new initiatives in the Global LabNet. In 2006, WPVs types 1 and 3 were both endemic in Afghanistan, India, Nigeria and Pakistan, and cases or outbreaks occurred in 13 countries following importation, mainly in Africa but also in Asia and the Middle East. In 2005–2006, 30 iVDVP cases were detected, based on retrospective analysis of isolates. Two of these cases occurred in France and Spain, in children of North African origin. Ambiguous VDPVs were found in eight countries in 2006, including the Czech Republic, Israel and Slovakia.

Interlaboratory and interregional collaboration on detection and investigation of importations was working well and new initiatives were under way. Speed of detection of cases with earliest

onset in outbreaks/importations was crucial and generally good, but delays were occurring when samples or isolates were shipped from field to laboratory, or laboratory to reference laboratory. The good news was that 93% of network laboratories were now fully accredited and capacities were increasing. Laboratories of special concern in the European Region had included those in Greece and Turkey, but their testing performance had improved and the laboratory in Greece had been renovated.

In summary, said Dr Paladin, the Network continued to thrive and to display an extraordinary ability to overcome challenges, including problems with workload and infrastructure. Strong collaborative links facilitated rapid sharing of information for use of the programme, and it continued to be responsive to changing needs. Indeed, the Network has been described as the unsung hero of polio eradication.<sup>1</sup>

## New testing algorithm

Dr Fem outlined a new initiative to increase the speed of poliovirus detection and reporting. Some factors such as shipping times lay outside the laboratories' control, but they should consider introducing new technologies and the new algorithm. The main differences from the traditional algorithm were: the shortened cell culture step; changes in how cytopathogenic effect-positive cultures were moved; omission of routine serotyping neutralization reaction (NT) before intratypic differentiation (ITD); passage of all L20B isolates into rhabdomyosarcoma (RD) before ITD; and simultaneous serotype and ITD by polymerase chain reaction (PCR) followed by immediate reporting of poliovirus with non-Sabin-like reaction and referral for sequencing, testing of monotypic Sabin-like viruses by enzyme-linked immunosorbent assay (ELISA), and separation of poliovirus mixtures by NT followed by ELISA on separated viruses.

The algorithm had been evaluated in India, Pakistan and the United States Centers for Disease Control and Prevention in Atlanta (using samples from Maiduguri, Nigeria), testing samples in parallel using old and new algorithms. It enabled faster reporting of WPV. Switching to the new algorithm would bring fresh challenges in the areas of:

- training laboratory personnel in new procedures;
- providing laboratories with the standard protocol;
- establishing new reporting targets and accreditation criteria (14 days for cell culture and seven days for ITD, laboratories to meet new targets by December 2007);
- mobilizing resources: supplies, staff training, equipping more ITD laboratories;
- making changes to laboratory databases for new reporting requirements.

Implementation should be completed in 75% of network laboratories in polio-endemic regions by the end of 2007. In polio-free regions implementation would, however, depend on feasibility and local advantage. In discussion it was acknowledged that laboratories in the European Region needed to explore the value of the algorithm for them, as it was mainly intended for endemic regions.

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<sup>1</sup> Global Polio Laboratory Network: the unsung hero of polio eradication. *Polio News*, 2006, 28:3.

## Overview of the European LabNet

Globalization played an important part in the spread of polio and other viruses, Dr Gavrilin explained. One in thirty-five people in the world was a migrant, and with over 64 million migrants Europe had the highest number in the world. Although a polio-free region, it nevertheless contained at-risk and hard-to-reach groups, which he illustrated with two examples. The first were refugees and internally displaced persons from the conflict zones in south-eastern Europe, where immunization services had been disrupted. VDPVs had been isolated from effluent at two refugee camps in the Czech Republic. No clinical cases or further spread occurred and the general population was well protected owing to historically high immunization coverage, but constant monitoring was needed.

The second example showed the importance of inter-regional collaboration in dealing with a challenge that would not have been thought possible in earlier decades. In 2006, a two-year-old girl from Nigeria went to Singapore for treatment in a private hospital and a WPV was isolated from this child. In hospital she came into contact with a Russian child. As a hot case, her test results were reported by Singapore to the WHO Western Pacific Regional Office, which contacted the Regional Office for Europe, which in turn promptly initiated an investigation. The family of the Russian child was identified and contacted in order to arrange for collection of specimens. Samples were promptly flown to the WHO National Polio Laboratory in Moscow for complete virological investigation. No WPV was isolated from the contact or her family members.

Dr Gavrilin outlined the main ways of learning about polio in Europe:

- AFP monitoring;
- routine EV surveillance;
- stool surveys during EV outbreaks;
- environmental sampling;
- screening bone marrow transplant patients (especially immunodeficient patients from outside the Region) for iVDPV; and
- special projects.

The European LabNet covered all 53 Member States through its network of 48 laboratories in 37 countries, supervised by 7 global specialist laboratories and the regional reference laboratories. Its achievements were impressive: global networking and high-level laboratory capacity, effective coordination, computerized data management in laboratories, the use of data for immunization planning and monitoring, and better laboratory infrastructure and management in many countries through the provision of financial investment and technical expertise.

The European LabNet's role in the next six to ten years would continue to be vital, not only in OPV cessation and the post-OPV surveillance era, but in applying its expertise and modelling its high standards in dealing with other viruses such as avian flu. There were many examples of its potential for integration or expansion, including EV surveillance, influenza, Crimea-Congo haemorrhagic fever/Dengue/special pathogens, rotavirus, HIV and human papillomavirus. Moreover, the revised International Health Regulations entering into force in 2007 introduced new obligations for notification of WPV and other viruses. The Network's current priorities were to:

- maintain high performance, seek synergies with other programmes and develop biosafety;
- advocate with Member States to maintain long-term support
- participate in global efforts to improve speed of poliovirus detection
- investigate use of the new test algorithm
- increase the number of ITD and sequence laboratories
- collaborate in the development of new diagnostics
- contribute to the research agenda
- develop a concept and plan of action for a VPD LabNet.

## **Significance of enterovirus and environmental surveillance in Europe**

### **Picornaviruses in human respiratory illnesses**

Dr Ville Peltola, specialist in paediatric infectious diseases (Finland), reviewed a number of studies and discussed ongoing work in Finland on the role of rhinoviruses and enteroviruses in respiratory illness. From this he drew the following conclusions:

- the importance of picornaviruses in respiratory illnesses is increasing rapidly;
- the role of EVs in children may be much greater than anticipated;
- rhinovirus-associated wheezing in infancy may be the best predictor of asthma development;
- effective methods are needed to manage and prevent picornavirus infections.

### **Enterovirus infections and type 1 diabetes**

Dr Merja Roivainen, head of the Enterovirus Laboratory in the Finnish National Public Health Institute, reviewed evidence suggesting that EV infection may have a role in the etiology of type 1 diabetes. This could only be ascertained definitively through intervention studies. If the association were found to exist, it might become possible to reduce the risk of type 1 diabetes by immunization with EV vaccine soon after birth. She described current EV surveillance practice in Finland and proposed that the prevalence of EV serotypes should also be analysed in other countries. With better knowledge of which serotypes were most prevalent globally, it might be possible to return to the issue of vaccine development and to the possible role of EV infection in different chronic diseases.

### **Polioviruses in the environment in Israel: implications for eradication**

Dr Lester Shulman, head of the Environmental Virology Laboratory, Ministry of Health, Israel, said environmental surveillance had multiple purposes. It could look for silent circulation (WPV and aVDPVs) and importation before disease became apparent (WPV and all VDPVs); document intervention and eradication; and track possible re-emergence or successors of poliovirus. The method used was plaque isolation of polioviruses. Vaccine viruses evolve along

two pathways: person-to-person transmission (cVDPV) and persistent infections of immunodeficient hosts (iVDPV). Those whose pathways were unknown were classified as ambiguous (aVDPV) (environmental isolates).

Supplementary surveillance for silent poliovirus infections from 1988 to 2006 had detected three types of environmental isolate: OPV vaccine types 1, 2 and 3; aVDPV; and WPV.

In conclusion, surveillance in Israel had detected at least two sources of aVDPV from sewage from documented highly immune populations of over two million. Detection had been intermittent, but excretion into the environment had continued for at least eight years. The isolates were highly diverged and highly neurovirulent. There was waning immunity in the adult cohort, with some individuals losing circulating protective antibody titres against these aVDPVs. The sources had not yet been located, nor was it likely that they would be in the immediate future.

## **iVDPV isolations in the United Kingdom**

Dr Javier Martin described the work of the United Kingdom National Institute for Biological Standards and Control on the detection of iVDPVs. Around 30 iVDPV cases had been reported around the world since widespread use of OPV started in the early 1960s. New additions with onset or collection dates in 2005–2006 were from China (one case coinfecting with types 2 and 3); Syria (one type 2, AFP, classified as iVDPV after follow-up); Iran (one type 2); Spain (child of Moroccan origin and three family contacts, type 2); France (child of Tunisian origin, type 2); and the United States (one type 1 and three community contacts).

Dr Martin reviewed seven long-term poliovirus excretors found in Ireland and the United Kingdom. In one case study, type 1 VDPV was isolated from a healthy child in Ireland as part of a routine health check. The child, originally from Zimbabwe, had been immunized six months before the first poliovirus isolation. Serum immunoglobulin levels were normal at the time of the excretion; 17 poliovirus type 1 isolates were detected during four months, and VP1 gene sequence divergence analysis of isolates was compatible with 10 months of poliovirus excretion.

In a second case study, a healthy man born in 1971 and last known to be immunized with OPV in 1986 was diagnosed with common variable immune deficiency (CVID). In 1995 he was included in a group of CVID patients undergoing virological study. He had not travelled to polio-endemic areas or had close contact with recently immunized children. Poliovirus type 2 was isolated over the seven months of the study and subsequently. The patient lived close to a sewage treatment plant but no poliovirus had been found in sewage samples to date.

In conclusion, Dr Martin stated that VDPVs could replicate for long periods in antibody-deficient individuals. Viruses from long-term excretors invariably reverted to neurovirulent properties typical of WPV. No treatment was available to interrupt poliovirus excretion and this posed a risk for the post-OPV era.

## **Country presentations on polio laboratory activities**

Countries were asked to report on the following points (some countries combined their reports with those on containment, summarized above):

- general update on poliovirus and EV/environmental surveillance;
- workload;
- performance and timeliness data;
- reporting and communication (between laboratories, with national authorities and with the WHO European office);
- interaction between subnational and national laboratories (if relevant);
- virological findings of poliovirus and EV/environmental surveillance.

### ***France***

Dr Bruno Lina reported on EV surveillance by the French laboratory network from 2000 to 2005. These activities were conducted within the framework of a national action plan that required immediate notification of suspected polio cases and/or positive polio isolates to local health authorities, a global immunization programme, enhanced surveillance of human EV transmission and EV environmental surveillance. The voluntary national polio laboratory network established in 2000 comprised 44 laboratories coordinated by the National Reference Centre and Institute for Epidemiological Surveillance. During this period:

- no WPV were identified;
- three Sabin poliovirus (type 1 and 3) were identified;
- large numbers of specimens were tested (30 000–50 000 a year);
- over 12 250 people had at least one positive EV sample;
- major circulating EVs were identified and outbreaks followed up (2000 and 2005);
- geographical coverage was improved; and
- EV circulation peaks were identified in July each year, sometimes followed by a second peak in the autumn.

In 2006, one type 2 iVDPV was identified (in an immunocompromised Tunisian child in Paris), one Sabin-like poliovirus type 2 and no WPV. EV circulation peaked in July and again in the autumn, and the major circulating EVs were identified as serotypes CoxB5, Echo30 and HPEV1. External quality control was conducted for participating laboratories and would be repeated in 2007. An RT-PCR assay was introduced for the direct identification of EV in cerebrospinal fluid.

For environmental surveillance, specimens were collected every four months in the major sewerage plants in Paris and analysed; positive cultures were sent to the reference laboratory and identified by conventional assays. An average of 225 cell culture positive specimens were obtained annually, with frequent identification of EVs and reoviruses often consistent with serotypes identified in clinical cases.

### ***Germany***

Dr Sabine Diedrich described proficiency tests and polio ITD in 2005–2006, AFP surveillance, EV surveillance, investigation of a long-term excreter, a polio seroprevalence study, studies of other members of the *Picornaviridae* family, and projects and ideas for 2007.

EV surveillance was based on the registration and investigation of patients with aseptic meningitis/encephalitis by regional laboratories. Its aims were to contribute to etiological clarification, improve patient management, recognize disease clusters as a precondition for intervention and estimate age-specific incidence. It was conducted by questionnaire and a reporting form, with the results reported weekly by e-mail and displayed on the internet. Stool/cerebrospinal fluid investigation was funded by the Ministry of Health and untyped viruses were sent to the national laboratory. The results of the first 15 months were very promising, and at the end of the pilot phase it would be decided whether this would replace AFP surveillance.

Polio seroprevalence was studied in 2006 for the first time since the switch from OPV to IPV. It revealed a high level of immunity in young people aged under 18 years that should be maintained through vaccination programmes.

The work programme for 2007 had three main activities: continuation of reference tasks at national and international level within the WHO polio eradication initiative, establishment of new diagnostic procedures and preparation of antisera.

### ***Greece***

Dr Maria Logotheti described the activities of the Greek national polio laboratory. It was making good progress following renovation and she thanked the regional reference laboratory in Rome, Italy, for its support. She described the results of AFP surveillance in 2006: four of the 34 samples contained adenovirus, but no poliovirus or NPEV were detected. There were several areas for improvement: the cell bank had not been fully tested for *Mycoplasma spp.*, specimens were not being collected from several AFP cases, environmental poliovirus surveillance was not yet being implemented and EV detection should be optimized.

### ***Italy***

Dr Lucia Fiore outlined the main activities of the Higher Institute for Health, Rome: active AFP surveillance, environmental surveillance, monitoring of immunodeficient patients, polio vaccine control, and supporting nine eastern European countries with virological AFP investigations. Italy had a network of 20 regional reference centres, 6 subnational laboratories and 20 local hospital networks. In 2002, its mixed IPV/OPV vaccination schedule was replaced by IPV only. Italy supported six countries with national laboratories (Albania, Bulgaria, Greece, Montenegro, Serbia and The former Yugoslav Republic of Macedonia) and three without national polio laboratories (Bosnia and Herzegovina, Malta and Monaco). Laboratory visits and official reports had been conducted in Albania, Bulgaria, Greece, Montenegro and Serbia, and training activities carried out with Bulgaria and Greece.

She concluded that:

- AFP surveillance had constantly improved since 1997 and met quality indicators in 2003–2005, but performance in 2006 was suboptimal owing to problems of cooperation with doctors in the field;
- no circulation of WPV or VDPV was found despite high immigration from endemic countries;
- environmental surveillance in seven major cities provided additional evidence of the absence of circulating WPV or VDPV in the post-OPV period;

- collaboration between linked countries, regional reference laboratories and WHO had been satisfactory;
- communication with all countries had improved, although there were still some problems with Bosnia and Herzegovina;
- viral isolates needed to be sent faster, particularly from Montenegro and Serbia;
- samples should be transported door-to-door to avoid double shipping payments, particularly from Bulgaria.

Future plans were to improve quality indicators for AFP surveillance, continue environmental surveillance, find financial resources for surveillance activities and continue support for linked countries.

### ***Norway***

Dr Gabriel Ånestad said that the national enterovirus laboratory network comprised nine laboratories, which reported their findings monthly to the national polio laboratory. All stool specimens from AFP cases were investigated at the national laboratory, which employed the equivalent of two full-time staff on polio-related and EV work. Reporting and communication between the national polio laboratory and the Regional Office were conducted by direct web entry and e-mail. In 2006, 403 EV isolates, mainly from stools or cerebrospinal fluid, were reported to the national polio laboratory and 70 were typed. EV activity was most prominent in late summer and autumn. No poliovirus was isolated.

### ***Poland***

Dr Zdzislaw Jarzabek reported that the last outbreak of polio in 1968 involved 468 people. Vaccination coverage remained high – 99% in 2005. IPV was introduced in 2000 for the first dose and in 2003 it replaced the second OPV dose. IPV was now used routinely for the first three doses; a single supplementary OPV dose was given to children aged six years.

Samples from hot cases were investigated through the system of AFP surveillance since 2003. The notified rate dropped significantly in 2003–2005 (0.69, 0.77 and 0.49, respectively) but substantial improvement was observed in 2006, when 68 AFP cases were reported (1.13 per 100 000 children under 15 years). No polioviruses were isolated from AFP contacts or diagnostic specimens in 2005–2006, probably related to the replacement of the first three doses of OPV by IPV in children aged under two years in 2004. Every AFP case was routinely screened by local public health workers receiving notification of the hot case criteria. There were five hot cases in 2005 and one in 2006.

VAPP cases were reported through AFP surveillance and vaccine-associated adverse effects surveillance. A national expert committee reviewed cases reported through both systems. There were five VAPP cases in 1998–2003 but none in the last three years. The EV surveillance system was based on 16 provincial laboratories; these and the national polio laboratory were the only laboratories carrying out EV diagnostic work using cell lines. They needed newer equipment, staff training and better quality control measures.

WHO support was very important for maintaining national polio laboratory activity. Dr Jarzabek highlighted staffing difficulties at the national polio laboratory: in 2002 he worked with two

technicians and one assistant, but from 2003 he had worked with only one technician, and there were no trained staff to take over.

### ***Portugal***

Dr d'Avillez Paixão gave an overview of laboratory activity. She also described advocacy measures to promote the containment programme from 2004 to 2006. The national post-eradication polio programme had been approved and guidelines issued, mechanisms developed to update the inventory and immunization guidelines published. Portugal's open borders encouraged visitors from polio-endemic countries and migrants from eastern Europe, so the scientific community needed constant reminders of the need for caution when bringing samples into the country and AFP surveillance should be intensified. New private hospital and laboratory partnerships, often involving transnational organizations, required new thinking about regulation.

### ***Russian Federation***

Dr Olga Ivanova described laboratory activities and quality control. Proficiency testing had improved year by year and reached 100% in 2001–2006. Other improvements included quarterly *Mycoplasma spp.* tests. Better surveillance supported by the hot case approach had identified 74 AFP cases in 2006.

EV surveillance was carried out as part of polio surveillance, as a public health requirement and a matter of scientific significance. Based on registration of aseptic meningitis patients, over 10 300 EV infections were registered in 2006, of which 3194 were AM cases.

### ***Spain***

Dr Gloria Trallero reported that OPV was used from 1963, switching to IPV in 2004. Coverage was high and rising – over 90% since 1996. The last WPV case was in 1988. The national polio laboratory in Madrid coordinated a network of nine laboratories in the autonomous regions. From 1998 to 2002 the national polio laboratory used classical virological techniques and molecular detection by RT-PCR. From 2002 onwards it used intratypic identification of poliovirus isolates by RT-PCR molecular typing followed by complete VP1 gene sequence analysis.

Patients aged under 15 years had undergone AFP surveillance since 1998, and patients hospitalized with respiratory or neurological syndromes (mainly AM) since 1999. Environmental surveillance was also conducted. Data were sent monthly to the national polio laboratory, which also conducted annual quality control. In 2005 the network studied over 7330 samples and detected 507 EV and 16 poliovirus, all Sabin-like, mainly in infants.

EV surveillance had detected an average of 8.5% of EVs from all samples studied. EV surveillance was complementary to AFP surveillance (86% and 14% of poliovirus recovery, respectively). The EV yield from the stools studied was 16%, of which 4% were poliovirus.

One iVDPV type 2 isolation was an importation from Morocco, isolated from a Moroccan child born in 2004. He was diagnosed with a primary immunodeficiency, went to Madrid for a bone marrow transplant in 2005 and died in 2006. Nine family members and one hospital worker were tested for poliovirus excretion; in three family members a transient excretion of related virus was detected lasting the longest (seven months) in one of them. Currently, none of them excrete VDPV. In the last sample from the index child, the virus had a sequence divergence of 4% from

the Sabin 2 reference strain. The VDPV had two recombination sites with Sabin 1, both located in the non-structural region.

Dr Trallero concluded that Spain had high vaccination coverage levels, with special policies targeting risk groups, good sanitary standards and a high quality surveillance system with high participation. EV surveillance was complementary to AFP surveillance and both systems were able to detect the imported iVDPV: the risk of polio transmission was minimal owing to high vaccine coverage. Efforts should be continued to improve rapid notification and sample collection, by making health professionals aware of the risk of importation and improving surveillance of immigrant groups.

### ***Sweden***

Dr Helene Norder reported that environmental screening for poliovirus by PCR was performed during 2003–2004 on sewage samples from eight plants. Detection of hepatitis A virus by PCR was used as control. The sensitivity of surveillance for polio was hampered by PCR on cerebrospinal fluid becoming the routine diagnostic procedure. From 2006 all suspected AM cases should be confirmed virologically.

A national seroimmunity study of around 5000 people would start in 2007 and cover polio, diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b, measles, mumps and rubella.

Most laboratories used mainly PCR for diagnosing EV infections, leading to less isolation and typing of EV. The Swedish Institute for Infectious Disease Control routinely investigates approximately 100 samples a year using four cell lines (RD, L20B, GMK and MRC5), and performs typing on isolated viruses and on about 100 isolates from other laboratories by neutralization in cell culture and confirmatory molecular typing. The workload was heavy and resources low.

### ***Turkey***

Dr Alper Akçali and Dr Zehra Unal reported on the work of the national polio laboratory (Ankara) and subnational laboratory (Izmir). The last polio cases caused by WPV were observed in Turkey in 1998. National AFP surveillance was conducted in collaboration with the Primary Health Care Directorate of the Ministry of Health and provincial health directorates according to the 2002 national action plan. Every AFP case and every case of suspected polio was studied and notified. If contacts were available, specimens were collected from five contacts of every case in a child aged under five years. Some attempts were made to implement EV surveillance in hospitalized children with diagnoses such as meningoencephalitis, but were limited by cost and lack of medical interest.

In 2006, 217 samples were examined at the national polio laboratory for EV identification: 73 samples of 35 cases, 63 samples of contacts, 6 stool samples of patients with an illness other than AFP and 36 samples of sewage. The subnational laboratory received samples from eight provinces in western Turkey covering nine million people, i.e. 13% of the total population.

### ***United Kingdom***

Dr Miren Iturriza-Gomara reported that most primary isolation was done by the Health Protection Agency and National Health Service laboratories. All diagnostic laboratories were accredited by the national clinical pathology accreditation scheme. Diagnostic laboratories

characterized EV/PV isolates by neutralization assay or immunofluorescence. Poliovirus isolates were referred to the national polio laboratory. There had been a shift in diagnostic laboratories from virus isolation to RT-PCR for diagnosis of EV infections, and to respond to this, molecular detection and characterization of EV had been offered at the national polio laboratory from 2004. Virus isolation and neutralization carried out in parallel with molecular testing in 2004–2005 showed 100% correlation.

Molecular methods had a number of advantages for EV characterization:

- increased sensitivity
- characterization directly from clinical sample, not subject to selection in cell culture
- detection and characterization of Ab-escape mutants
- detection and characterization of novel genotypes or variants
- reduced turnaround times (3–4 days) in comparison to classical methods (10–14 days).

In discussion this new diagnostic paradigm was hailed as the shape of things to come.

## Recommendations

The European Region remains at risk of importation of wild type poliovirus from countries where one is currently in circulation. Technical and financial support from governments to national polio laboratories is crucial in ensuring rapid detection of any importation and adequate response to prevent further spread.

WHO is recommended to:

- explore the possible implementation of the new WHO test algorithm for poliovirus isolation and confirmation in selected countries of the Region in collaboration with WHO headquarters.

The WHO Regional Certification Commission is recommended to:

- remind all national governments of the importance of their full commitment to polio eradication, including full support of national polio laboratories;
- explore the possible implementation of national or regional supplementary polio surveillance (enterovirus and environmental) in countries/regions where AFP surveillance is poor or non-existent.

National polio laboratories are recommended to:

- encourage all health care professionals involved in enterovirus diagnostics to collect and submit faecal samples to national polio laboratories for poliovirus detection and characterization;
- monitor the speed of specimen collection and delivery and, in cases of delay, alert the responsible public health authorities;
- expedite the implementation of *Mycoplasma spp.* detection in cell cultures using any method recommended in the WHO Polio Laboratory Manual or, if this is not possible, to

request remote testing in global specialized or regional reference laboratories by submitting nucleic acid archival cards (protocol available from the Regional Office).

## **Close of Meetings**

Dr Dowdle closed the Meetings by thanking the hosts from Malta, speakers, participants and WHO staff for an informative and productive conference. Dr Lipskaya thanked him for his expert chairmanship.

*Annex 1*

**PROGRAMME OF THE JOINT CONTAINMENT – EUROPEAN POLIO  
LABORATORY NETWORK MEETING**

**20 February**

- 09.00–09.30 Global overview of polio eradication (Roland Sutter)  
09.30–10.00 Global overview of containment  
10.00–10.30 Completion of the containment Phase I in European Region (Galina Lipskaya)
- 10.30–11.00 Coffee break*
- 11.00–11.30 Presentation from the United Kingdom  
11.30–12.00 Presentation from France  
12.00–12.30 Presentation from Germany  
12.30–13.00 Presentation from Italy
- 13.00–14.00 Lunch*
- 14.00–14.30 Presentation from Switzerland  
14.30–15.00 Presentation from Sweden  
15.00–15.30 Presentation from Portugal
- 15.30–16.00 Coffee break*
- 16.00–16.30 Presentation from Spain  
16.30–17.00 Presentation from Belgium  
17.00–17.30 Presentation from Greece

**21 February**

- 09.00–09.30 Presentation from Turkey  
09.30–10.00 Presentation from Denmark  
10.00–10.30 Introduction to Containment Phases II and III, Global Action Plan 3
- 10.30–11.00 Coffee break*
- 11.00–11.45 Approaching GAP 3 in the European Region: tasks and challenges. Plan of action on containment in 2007–2008  
11.45–13.00 General discussion
- 13.00–14.00 Lunch*

## **European Polio Laboratory Network Meeting**

### *Overview and general aspects*

- 14.00–14.30 Global LabNet overview. Presentation of the new testing algorithm (Fem Paladin)  
14.30–15.00 Polio LabNet in the European Region. Implementation of the new testing algorithm in the Region (Eugene Gavrilin)  
15.00–15.30 Discussion  
*15.30–16.00 Coffee break*

### *Significance of enterovirus and environmental surveillance in the Region*

- 16.00–16.30 Picornaviruses in human respiratory illnesses (Ville Peltola)  
16.30–17.00 Enterovirus infections and type 1 diabetes – an update (Merja Roivainen)  
17.00–17.30 aVDPV isolations in Israel (Lester Shulman)  
17.30–18.00 iVDPV isolations in the United Kingdom (Javier Martin)

## **22 February**

### *Presentations from Member States*

- 09.00–09.30 Presentation from France  
09.30–10.00 Presentation from Germany  
10.00–10.30 Presentation from the United Kingdom  
*10.30–11.00 Coffee break*  
11.00–11.30 Presentation from Greece  
11.30–12.00 Presentation from Italy  
12.00–12.30 Presentation from Portugal  
12.30–13.00 Presentation from Spain  
*13.00–14.00 Lunch*  
14.00–14.30 Presentation from Norway  
14.30–15.00 Presentation from Poland  
15.00–15.30 Presentation from the Russian Federation  
*15.30–16.00 Coffee break*  
16.00–16.30 Presentation from Sweden  
16.30–17.00 Presentation from Turkey  
17.00–18.00 General discussion and recommendations

*Annex 2*

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