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Report on the

Seventh intercountry meeting of directors of poliovirus laboratories in the Eastern Mediterranean Region

Amman, Jordan
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1. INTRODUCTION

The seventh intercountry meeting of directors of poliovirus laboratories in the WHO Eastern Mediterranean Region was held in Amman, Jordan, from 24 to 26 August 2002. Directors of laboratories in Egypt, Islamic Republic of Iran, Iraq, Jordan, Morocco, Oman, Pakistan, Saudi Arabia, Sudan, Syrian Arab Republic and Tunisis attended the meeting. Participants also included scientists from the Centers for Disease Control and Prevention (CDC), United States of America; National Institute of Public Health and the Environment (RIVM), Netherlands; National Public Health Institute (KTL), Finland; National Institute for Biological Standards and Control (NIBSC), United Kingdom; Task Force on Child Survival, United States; and staff from the World Health Organization (WHO) headquarters and Regional Office for the Eastern Mediterranean.

Dr Hussein A. Gezairy, WHO Regional Director for the Eastern Mediterranean, welcomed the participants and thanked the Government of Jordan and His Excellency Dr Hakem Al Qadi, Minister of Health, for hosting the meeting. He expressed appreciation for the commitment and dedication of the polio laboratory network scientists in providing quality laboratory work. However, he expressed concern regarding delays in referring specimens from the field to the laboratory for testing, and called on all concerned to devise local mechanisms to improve timely transportation of stool samples. He also advised that every effort should be made to provide certification standard quality results in timely manner and that quality assurance in laboratory procedures should be fully implemented. He thanked CDC and KTL for their support in providing accurate genomic sequencing results of polioviruses in a timely manner, as this greatly facilitated planning and targeting the polio eradication activities.

H.E. Dr Hakem Al Qadi, Minister of Health, Jordan, in his inaugural address, welcomed all the participants and highlighted the progress achieved in Jordan in the fields of EPI and polio eradication. He indicated that Jordan had been polio free since 1992, when the last case was confirmed and underscored the importance of the laboratory network in the eradication efforts.

It was agreed to have rotating chairmanship. Elected chairpersons were Dr Ali Muheidat (Jordan) and Dr Suleiman Al Bussaidy (Oman). The programme of the meeting and list of participants are included as Annexes 1 and 2, respectively.

2. IMPLEMENTATION OF THE RECOMMENDATIONS OF THE SIXTH INTERCOUNTRY MEETING OF DIRECTORS OF POLIOVIRUS LABORATORIES IN THE EASTERN MEDITERRANEAN REGION

Recommendations of the previous meeting were reviewed and main achievements in implementation were as follows.

	Recommendation	**Implementation status**
1.	In line with the recommendation of the Global Technical Consultative Group the time interval between paralysis onset and final ITD report should be decreased to <60 days (target 80%).	In EMR laboratories the period between paralysis onset and final ITD results is within 45 days.
2.	Laboratories should achieve full implementation of quality assurance programme with special emphasis on: documentation of laboratory procedures; sterility testing of media and reagents; temperature monitoring of incubators, refrigerators and freezers; cell sensitivity testing; development of standard operating procedures; and use of standard reference viruses for ITD and cell sensitivity.	Quality assurance (QA) programme was implemented in all EMR polio laboratories. However, there was a need to improve documentation of all laboratory procedures. In cell culture work laboratories showed improvement and introduced most of QA tools. Overall supervision improved and laboratory directors were reviewing the worksheets regularly, but there were occasions when response to findings was not appropriate or not documented.
3.	All laboratories performing intratypic differentiation (ITD) should test each isolate using 2 methods, one antigenic and one molecular, following established quality control procedures and interpretation. The PCR can be used only as supplementary test until training of personnel, provision of supplies and accreditation in the use of the PCR test by the RRL laboratory.	The EMR polio laboratories performing ITD testing used 2 methods (one antigenic and one molecular). Two RRLs (Pakistan and Tunis) also introduced polymerase chain reaction (PCR) as supplementary molecular method.
4.	Continued training of laboratory staff should remain a priority of the programme to keep the trained laboratory workforce intact in the	Training of laboratories staff was continued and six technician and scientist were given extramural training in poliovirus diagnosis

	presence of high turnover of the laboratory staff.	techniques. A specialized training in cell culture techniques was organized in National Institute for Biological Standards and Control, UK, scientist from Egypt, Oman, Pakistan and Syria participated in this training.
5.	Annual laboratory supplies requirement should be calculated based on estimated workload and should be submitted well in advance to avoid delays.	Network laboratories based on estimated workload prepared annual supply lists. In most of occasions estimates were not rationalized.
6.	Governments are encouraged to allocate a specific budget item for polio laboratory functions in their country. The laboratories should develop contingency plans for emergencies and have rapid access to funds to repair or replace critical equipment, if necessary.	Specific budgets were not allocated to National polio laboratories in the countries and this should be followed-up with Ministries of Health of countries of EMR. The polio laboratories staff was not shifted to different disciplines of laboratories, but in some countries were given the task to work in other viral infectious diagnosis.
7.	Specimen collected from AFP cases which are highly suspected as polio, should be flagged as HOT CASES.	The flagging of the highly suspected polio cases improved, however, continued follow-up with EPI was required.
8.	The LABIFA database should be modified to include variables to monitor the movement of samples and isolates between laboratories to track all poliovirus results.	The LABIFA was revised to include all variables required to generate analysis report addressing the tracking of stool samples and isolates. It also included the information on discordant results and VDPVs.
9.	Where facilities exist, maintenance and repair of laboratory equipment should be arranged within country with local companies. WHO should provide assistance to laboratories that do not find such facilities in their countries.	Maintenance and repair of laboratory equipment remained a major concern in countries where very little such facilities exist.

3. OVERVIEW

3.1 Global status of the poliomyelitis eradication initiative

Dr Esther de Gourville, WHO/HQ

The global polio eradication initiative continued to make remarkable progress. Between 1988 and the end of 2002 the number of polio endemic countries decreased from 125 to 7. In 2002, a total of 1918 cases were reported from 7 countries in the world as compared to an estimated 350 000 cases in 1988, in the presence of an improved and sensitive surveillance. Wild type 2 poliovirus has not been detected worldwide since October 1999. Poliovirus transmission continued in 2002 in Afghanistan, Egypt, India, Niger, Nigeria, Pakistan and Somalia. Wild poliovirus importation was detected in Burkino Faso and Zambia in 2002, and in Lebanon and Ghana in 2003. These importations showed an increased risk to polio free countries.

A considerable increase in number of cases occurred in India where 1600 cases were reported in 2002, as compared to 268 cases in 2001. Nigeria reported 202 cases in 2002, which was more than the previous year's cases; this increase was result of outbreak and improved surveillance. All endemic countries except Somalia continued to report wild polio cases in 2003.

AFP surveillance has attained certification standard in most countries of the world. In 2002 the annual non-polio AFP rate per 100 000 population <15 years of age remained well above the required rate of 1.0 in all regions and endemic countries, but concerns remain in a few countries like Algeria. In 2002, WHO regions reached the certification standard requirement of collection of two adequate stool specimens from at least 80% of AFP cases. However, in the Eastern Mediterranean Region Somalia and Djibouti remained below required rate, as did Chad in the African Region.

In 2002, 132 of 145 Global Polio Network Laboratories were fully accredited by WHO, and were providing reliable results. In April 2001, it was recommended to reduce the interval of 90 days to 60 days for virological investigation from onset paralysis to final result. Now it is expected that laboratories should give there results within 49 days and EPI should collect and send samples to laboratories within 11 days. All regions have made good progress, except Western Pacific Region where the polio laboratory infrastructure and staff were utilized for SARS investigation and affected the timeliness of results.

In Nigeria most of the cases were reported from northern states, and in 2003 a few cases have been reported from southern states, which were polio free for more than a year. A decline in number of cases reported was seen as compared to 2002, but in some parts of Nigeria cases occurred recently even after SIAs, putting the quality of campaigns into question.

In India, cases were detected from all along the northern border 2002. In 2003, virus transmission decreased to a great extent and less transmission was detected, but remaining concerns were appearance of wild polioviruses in Andhra Pradesh, West Bengal, Karnataka,

and Rajasthan, which were previously polio free. These were importations from the north, rather than re-establishment of infection. Two indigenous genotypes persisted in India.

The Global Specialized Laboratories provided genetic sequencing results for 88% of polioviruses submitted in 2002. The inability to reach to 100% was due to overwhelming workload in India laboratories. To date in 2003, 86% of genetic sequencing results were provided for polioviruses submitted for sequencing. Remaining poliovirus type 1 genotypes which continued circulation were the South Asia (SOAS) genotype, in Pakistan and Afghanistan; East Africa (EAAF) genotype in Egypt; and West Africa (WEAF) in Nigeria, Niger, Burkino Faso and Ghana. Similarly, poliovirus type 3 genotypes which continued circulation were the South Asia (SOAS) genotype, in Pakistan and Afghanistan; East Africa (EAAF) genotype in Somalia, which has persisted for a number of years; and West Africa (WEAF) in Nigeria and Niger.

Vaccine-derived poliovirus (VDPV) outbreaks had been reported previously in Egypt (32 P2 cases, 1988–1993), Hispaniola (22 P1 cases, 2000–2001) and Philippines (3 P1 cases, 2001) and Madagascar (4 P2 cases, 2002). These were attributed to low immunization coverage and poor surveillance. In October 2002, laboratories started reporting VDPVs from AFP cases, healthy children, sewage sampling and aseptic meningitis. From AFP cases VDPVs were reported from the Syrian Arab Republic, Nigeria, Kazakhstan and China; from healthy children in Thailand and Mongolia; from sewage in Estonia and Slovakia. These VDPVs did not cause any outbreak.

In India wild poliovirus type 2 (MEF-1) was detected in 7 AFP cases, 1 contact and 1 environmental sample between November 2002 and February 2003. The MEF-1 virus is used in IPV production and is a commonly used laboratory reference strain. After long investigation, which is still ongoing, it was found that these were linked with contaminated OPV. The main issue now is to determine source and mechanism of MEF-1 contamination of OPV.

In 2003, a strategy was designed to cope with the funding shortfall and use the available resources in more sensible way. Resources were targeted to the remaining polio endemic countries and 6 other countries at risk of re-establishing transmission (Angola, Bangladesh, Democratic Republic of Congo, Ethiopia, Nepal and Sudan). The main strategy was to increase quantity and quality of SIAs in remaining endemic countries to achieve interruption of wild poliovirus transmission. In addition to these strategies, advocacy efforts were strengthened in the remaining endemic and at risk countries to increase political commitment

3.2 Regional status of the poliomyelitis eradication initiative

Dr Faten Kamel, WHO/EMRO

Significant progress towards the eradication of poliomyelitis is continuing in all countries of the Eastern Mediterranean Region. The number of cases shows relatively regular decrease to a minimum in 2002, in the presence of a well-developed and efficiently performing surveillance system. As of the end of 2002, poliovirus transmission had been interrupted in 18 countries of the Region for more than 3 years. In addition, Sudan has not reported any wild poliomyelitis cases since April 2001. The number of confirmed cases of poliomyelitis reported during 2002 was 110 cases, reported from only 4 countries of the

Region (Pakistan 90, Afghanistan 10, Egypt 7, and Somalia 3). Up to August 2003, 54 cases were reported. Fifty of these cases were from Pakistan, two from Afghanistan, and one from Egypt. One wild poliovirus importation into Lebanon was detected; the virus was genetically linked to virus strains from Uttar Pradesh, India.

In Afghanistan, the cases were detected mainly in the southern region in 2002. During 2003, only two confirmed cases were detected; one in the north-eastern region (Nangarhar province), and the other in the southern region (Kandahar). Both were due to wild poliovirus type 3. These cases were in areas bordering Pakistan where shared transmission between both countries has been observed previously.

In Egypt, in contrast to past years, cases were reported mostly from Lower Egypt and greater Cairo during 2002. All cases were of type 1 with onset in September or later, after intensification of surveillance activities, which started in July 2002. Egypt is supplementing AFP surveillance with sampling from wastewater (environmental surveillance). The proportion of poliovirus positive environmental samples has declined from 16% in 2002 to 5% to date in 2003 (6 of 122 samples). Most of the viruses are from one cluster shared by Cairo, Sharkia, Minya and Beni Suef.

In Pakistan, the impact of efficiently implementing polio eradication strategies resulted in reducing the transmission of poliovirus in Pakistan. The number of cases dropped from 119 in 2001 to 90 in 2002. During 2002, cases were reported from 35 districts, compared to 39 in 2001 and 59 in 2000. During 2003, 54 cases were reported up to August from 33 districts.

In Somalia, during 2002, only three cases were identified, all in the highly populated Mogadishu region and nearby. During 2003, up to the month of August no cases were reported.

All endemic countries of the Region continued to conduct NIDs and SNIDs, with independent monitoring, conducted sufficiently promptly to allow corrections to be made in subsequent rounds. Surveillance has improved, with a non-polio AFP rate in 2002 and 2003 of 2.3 cases per 100 000 children. Adequate specimens are being collected from over 90% of AFP cases.

As the polio eradication initiative moves into its final phase, with only four endemic countries in the Region at the end of 2002, increasing attention is being given to polio “endgame” issues: the laboratory containment of wild poliovirus, the certification of polio eradication and the development of post-certification immunization policy for polio.

Poliomyelitis eradication activities are very closely monitored in the countries of the Region. Surveillance reviews have been conducted in Afghanistan, Egypt, Islamic Republic of Iran, Pakistan, Sudan and Yemen, and in general, these have provided additional confidence in AFP surveillance systems. As well, Technical Advisory Groups (TAG) for the priority countries regularly review the epidemiological situation and national plans and provide technical advice. Their collective conclusions indicate that if high-level commitment to achieve polio eradication is continued with enhanced strategy implementation, it is likely that poliovirus transmission in the Region will be interrupted in the near future.

By the end of 2002, 18 countries had prepared a national plan for laboratory containment of polioviruses. It should be noted that three out of the remaining five countries still have ongoing virus transmission. The first phase of the plan is to conduct a survey of all biomedical laboratories and establish a national inventory of all laboratories that handle or store wild poliovirus material or potential infectious material and to ensure the implementation of enhanced bio-safety level-2 (BSL-2/Polio). This phase has been successfully completed in 7 countries and is currently being implemented in another 11 countries.

According to the recommendations of the Global and Regional Commissions for Certification of Poliomyelitis Eradication, all countries except Somalia have established National Certification Committees (NCC) with appropriate membership. The NCCs of 15 Member States that have high quality AFP surveillance and have not reported cases of poliomyelitis for at least 3 years submitted reports and national documentation to the Regional Commission for Certification of Poliomyelitis Eradication (RCC). The RCC accepted 14 of these reports. It is continuing to review annual updates from countries with satisfactory initial reports until regional certification. The RCC continues to guide all aspects of the certification process in the Region. Some of its members have made country visits to review the status of the certification activities and available documentation

The regional eradication programme, which is now passing through its final and most difficult phase, faces a number of challenges and constraints that must be surmounted in order to achieve polio eradication. It is crucial to maintain the political support and commitment in all countries of the Region. This must be done in endemic countries in order to avoid fatigue, ensure quality and finish the job as soon as possible, and in polio-free countries in order to consolidate the achievement until eradication can be certified. It is also important to ensure access to all children especially those residing in insecure areas and in countries affected by war. Securing the required financial support for implementation of high quality activities is another major challenge facing the programme.

3.3 Regional status of the polio laboratory network

Dr Humayun Asghar, WHO/EMRO

The poliovirus laboratory network in the WHO Eastern Mediterranean Region continued to improve its performance in support of the poliomyelitis eradication programme, and this was reflected in full accreditation of ten laboratories and provisional accreditation of two laboratories as of December 2002. In 2002, 10 860 samples were tested in network laboratories, of which 9402 were from AFP cases, 1128 were from contacts of AFP cases and 330 were from environmental samples, healthy children and other sources. During 2002, 72% of samples were received in laboratories within 3 days of collection from AFP cases, as compared to 62% during 2001. The percentage of specimens received in good condition was 98%; results were reported within 28 days in 96% of cases, and the non-polio enterovirus (NPEV) rate was 14%.

In 2003, as of August 2003, the five network laboratories visited were fully accredited by WHO, and the remaining laboratories will be visited in coming months. All laboratories successfully passed the proficiency panel distributed during 2002. 98% of specimens were received in good condition, 94% of results reported within 28 days, and the NPEV rate was

15%. However, it is of concern that only 69% of samples were received in laboratories within 3 days of collection. The period from paralysis onset to final ITD testing decreased from 45 days in 2002 to 40 days in 2003. Timeliness of ITD results improved remarkably; reporting of ITD within 14 days after serotyping improved from 77% in 2002 to 91% in 2003.

The target of 80% for the transportation of samples within 3 days remains a concern to be resolved. Related to this aspect, low or declining NPEV was discussed and it was emphasized that adequate reverse cold chain and storage conditions and implementation of quality assurance in cell culture laboratories must be ensured before reaching any conclusion on the cause of low NPEV. Molecular data are used routinely to identify endemic reservoirs and importation. No cVDPVs were detected in 2002 and 2003 to date.

The EMR Polio Network Laboratories are participating in the efforts for containment of wild poliovirus and other potential infectious material. These materials are kept under lock and key with restricted access. All polio laboratories are keeping their inventories current.

WHO/EMRO keeps the network laboratories informed of all developments in the polio laboratory network through meetings, sharing of publications, data analysis and advice on improvement of indicators. Regular communications are conducted with laboratories to resolve any technical or logistical problem as and when they arise. All the network laboratories were provided with logistical support.

Funds were provided for establishment of a viral molecular biology laboratory in Pakistan. Re-establishing Iraq NPL was a major challenge, and funds were provided for the purchase of laboratory supplies, i.e. equipment, reagents, furniture and communication equipment.

Training of EMR laboratory staff continued. During the past year 12 scientists from these laboratories were trained in regional reference laboratories (RRLs) and global specialized laboratories (GSLs). A training workshop on antigenic methods, i.e. enzyme-linked immunosorbent assay (ELISA) testing for ITD, was conducted in Oman in November 2002. Another intercountry workshop on molecular methods, i.e. polymerase chain reaction (PCR) and nucleic acid probe hybridization (NAPH) is planned to be conducted in Oman in December 2003, for scientists from four RRLs. It is planned to upgrade the Oman NPL to perform ITD tests.

4. VIRUS SURVEILLANCE AND MOLECULAR EPIDEMIOLOGY IN ENDEMIC COUNTRIES

4.1 Egypt

Dr Iman Al Maamoun, VACSERA, Egypt

The VACSERA RRL was fully accredited by WHO in 2002, with an excellent performance in proficiency panel test with a score of 100% in isolation and identification in primary virus culture and ITD testing. In 2002, the RRL processed 1202 stool samples from 576 AFP cases, the NPEV rate was 19% and results of 92% of stool sample virological investigations were reported within 28 days. In 2003, it processed 655 stool samples from 312

AFP cases, the NPEV rate was 16% and results of 97% of stool sample virological investigations were reported within 28 days.

VACSERA carried out virus culture and ITD on samples referred from Cyprus, Iraq, Lebanon, Syrian Arab Republic and Yemen. In 2002, no wild virus was detected from the 518 stool samples and 45 isolates referred from these countries. Between January and August 2003, a total of 91 stool samples and 13 positive poliovirus isolates were referred from other countries of the Region. Wild poliovirus type 1 was isolated from stool sample of one case and its close contact from Lebanon.

In 2002, seven wild poliovirus type 1 cases were detected in Egypt; six cases were from Lower Egypt and Giza (Giza (2), Sharkia (2), Menoufia (1), Alexandria (1)) and one from Upper Egypt (Assiut). Between January and August 2003, only one wild poliovirus type 1 case was detected from Upper Egypt (Minya).

The molecular sequencing of these viruses showed that all cases belonged to an indigenous single genotype. All cases were closely related to each other and to environmental wild polioviruses. There was strong evidence to suggest that wild poliovirus was circulating in Lower Egypt.

In 2002, 164 environmental samples were collected from 25 sites of 18 provinces in Lower and Upper Egypt. Out of 18 provinces 11 were infected with wild poliovirus type 1. These were Alexandria, Assiut, Behira, Beni Suef, Cairo, Fayoum, Giza, Menoufia, Qena, Sharkia and Sohag. The isolation rate of wild polioviruses from environmental samples decreased from 57% in 2001 to 16% in 2002.

In 2003, the number of collection sites, and frequency of sampling was increased, especially in Greater Cairo (Cairo, Giza and Qalubiya). Between January and August 2003, a total of 164 samples were collected from 27 sites. A total of 32 samples were collected per month from these sites. There was marked reduction in number of positive sites when comparing with last year. Only three sites (Cairo, Sharkia and Minya) were positive for wPVI. The percent positivity of environmental samples tested to date for wild poliovirus type 1 was 5%. In Cairo, detection of wild polioviruses in environmental samples in the absence of clinical cases of poliomyelitis raises doubts about the sensitivity of AFP surveillance.

4.2 Pakistan

Mr Sohail Zahoor Zaidi, National Institute of Health, Pakistan

During 2002, the Pakistan RRL processed 3620 stool samples collected from 1814 AFP cases from Pakistan. 73% of stool samples were transported from field to laboratory within 3 days. The stool adequacy rate was 96%. The NPEV rate was 21% and results of 96% of stool samples were reported within 28 days.

Up to August 2003, a total of 2805 stool samples collected from 1404 AFP cases were tested. Adequate stool samples were collected from 97% of AFP cases, 75% of stool samples were transported from field to laboratory within 3 days, the NPEV rate was 23% and results of 96% stool samples were reported within 28 days.

The RRL Pakistan also provided support to the polio eradication programme in Afghanistan. In 2002, 768 stool samples collected from 384 AFP cases were processed. Only 30% of stool specimens were received in laboratory within 72 hours of collection, but 96% were received in good condition. The NPEV rate was 18%, and the results of 100% of stool samples were reported within 28 days of their receipt in the laboratory.

Between January and August 2003, a total of 784 stool samples collected from 393 AFP cases from Afghanistan were tested. Only 27% of stool specimens were received in laboratory within 72 hours of collection; 99% were received in good condition. The NPEV rate was 23%. The reports of 96% cases were sent within 28 days of receipt of the samples in the laboratory. The laboratory established and standardized a PCR test and passed a PCR proficiency panel distributed in 2002.

4.3 Somalia and south Sudan

Mr Peter King'ori, Kenya Medical Research Institute (KEMRI), Kenya

The polio laboratory at KEMRI performs virology investigation on AFP cases reported from Djibouti, Eritrea, Kenya, St Helena, Seychelles, Somalia and south Sudan. In 2002, KEMRI tested 216 samples from Somalia and 175 from south Sudan. The percentage of samples received in the KEMRI laboratory within 3 days was as low as 12% from Somalia and 9% from south Sudan. The NPEV rate was >10% for both countries, and results were reported within 28 days in 100% of cases. Six wild type 3 polioviruses were isolated from three cases from Somalia.

Between January and August 2003, KEMRI tested 118 samples from Somalia, 93 from south Sudan and 2 from Djibouti. Results of virological investigations of stool samples were sent within 28 days for 100% samples. None of the samples yielded wild viruses.

4.4 Molecular epidemiology of wild polioviruses in poliomyelitis endemic countries of the Region

Dr Olen M. Kew, Centers for Disease Control and Prevention (CDC), USA

Molecular epidemiology is an integral component of wild poliovirus surveillance in the Eastern Mediterranean Region (EMR). Wild poliovirus isolates from AFP cases were identified in the regional reference laboratories (RRLs) by intratypic differentiation (ITD). They are promptly and regularly forwarded to CDC in Atlanta for genetic sequencing. In 2003, only two EMR RRLs (NIH-Pakistan and VACSERA-Cairo) have detected wild polioviruses from clinical specimens, reflecting the retreat of wild poliovirus circulation from most of the Region. ITD results at CDC have been in complete concordance with those of NIH-Pakistan and VACSERA in 2002. Analysis of VP1 nucleotide sequences has 1) permitted detailed reconstruction of the chains of wild poliovirus transmission; 2) identified the remaining endemic reservoirs communities; 3) documented the continued elimination of wild poliovirus lineages (equivalent to the termination of chains of transmission); 4) detected importation of virus from northern India into Akkar, Lebanon in 2002; and 5) identified a type 2 vaccine-derived poliovirus (VDPV) in the Syrian Arab Republic in late 2001. Patterns of wild poliovirus distribution in the endemic areas of Pakistan, Afghanistan and Egypt were represented as spot maps, where different clusters of related lineages, first represented in phylogenetic trees, are colour coded and mapped. Long branches connecting the most closely related pairs of isolates identify gaps in surveillance. Very few such gaps could now be found in the Region, and when found, prompt investigations by the surveillance teams follow. Surveillance gaps in Egypt have been closed by a special project of environmental sampling from multiple communities in both Upper and Lower Egypt, a project coordinated by the Ministry of Health and Population of Egypt and EMRO, and involving the laboratories of VACSERA, KTL-Finland, and CDC.

Poliovirus types 1 and 3 remain endemic to Pakistan and were shared with Afghanistan. Poliovirus type 3 is surviving by a few chains of transmission, and improvement in OPV coverage rates should achieve eradication. Several independent chains of transmission supported poliovirus type 1 circulation, as the virus was still endemic to certain endemic foci where OPV coverage rates remain inadequate. In Egypt, the findings of AFP and environmental surveillance were in good agreement, and despite a reduction in active chains of transmission; these findings support the view that more aggressive immunization activities were urgently needed.

In summary, poliovirus surveillance in the Region was outstanding, and the EMR network laboratories are performing at very high levels of proficiency. The programme has continuous access to highly accurate, detailed and timely virological data. Molecular epidemiology was built upon the solid foundation of excellent work in virus isolation, typing, and ITD, coupled with sensitive surveillance in the field.

5. VIRUS SURVEILLANCE IN NON-ENDEMIC COUNTRIES

5.1 Islamic Republic of Iran

Dr H. Tabatabie, National Polio Laboratory, Islamic Republic of Iran

In 2002, a total of 369 stool specimens were tested from AFP cases. The percentage of virological investigation results within 28 days from the date received in laboratory was 77%, below the target of 80%. This occurred due to a holiday in late March to early April. During this period the university and laboratory were completely closed, while AFP cases samples were kept in a deep freezer by the guard of building. The laboratory work was resumed after the end of the holiday and cell lines were revived from liquid nitrogen, which in total made a delay of 21 days. This happens every year, and it is compensated for efficiently to reach the timeliness target of 80%.

For the last few years a decline in non-polio enterovirus (NPEV) rate was observed. The analysis of stool samples from AFP case, contacts and sewage samples showed that isolation of NPEV decreased during winter and spring. Efforts were made to improve the low NPEV rate. Cell culture media were made according to WHO Laboratory manual using fresh constituents. Cells were obtained and replaced with low passage cells and fresh stocks of cells were stored in liquid nitrogen. Cell sensitivity was performed regularly and both RD and L20B cells were found sensitive. The reverse cold chain was critically reviewed and no problems were observed. Temperature of incubators and other equipments were monitored regularly. The laboratory was working closely with the WHO Regional Polio Laboratory Coordinator to improve the NPEV rate.

5.2 Jordan

Dr A. Muhaidat, National Polio Laboratory, Jordan

The Jordan NPL was provisionally accredited for 2002, due to delay in reporting of stool sample virological investigation results and deficiency in implementing quality assurance (QA) in the laboratory. Due to provisional accreditation status NPL was recommended to refer all stool samples for parallel testing in Tunis RRL to confirm their results.

The NPL implemented the recommendation for accreditation review; revised standard operating procedures (SOPS) and routine worksheets, and implemented internal quality control (QC). In cell culture, laboratory QC procedures were implemented, including regular cell counting at each time of trypsinization of cells, cell sensitivity on both RD and L20B cells, and sterility of media. Two laboratory scientists were trained in VACSERA, Egypt, on cell culture, and isolation and identification techniques, as part of the recommendation.

In 2002, stool samples from 25 AFP cases and 142 contacts were tested in the Jordan NPL, and 100% of results were sent within 28 days. All samples were referred to the Tunis RRL. Two polioviruses type 1 were isolated and were confirmed as Sabin poliovirus type 1 by RRL. Concordance of results between RRL and NPL was 97%; the Jordan NPL could not isolate few NPEV from stool samples. The NPEV isolation rate of Jordan NPL was 7.3%.

Between January and August 2003, stool samples from 15 AFP cases and 69 contacts were tested in the Jordan NPL; 100% of results were sent within 28 days. No poliovirus was isolated. The NPEV isolation rate of Jordan NPL was 10%. The NPL scored 80% in the WHO proficiency panel for isolation and identification of polioviruses. The poliovirus type 3 was missed, and was isolated upon re-inoculation.

The Jordan NPL was fully accredited by WHO for 2003. The NPL was recommended to continue referring all stool samples to RRL for parallel testing, and after six months the WHO Regional Polio Laboratory Network Coordinator may suggest to stop referring after analysing the data.

5.3 Saudi Arabia

Mr Moghram Al-Amri, National Polio Laboratory, Saudi Arabia

The NPL Saudi Arabia was working under strict BSL2/Polio. In 2002, a total of 185 samples were received in the laboratory and 100% were reported to EPI within 28 days. The NPL referred all poliovirus positive samples to Kuwait RRL within 7 days of serotyping results and RRL confirmed 100% of NPL results. The NPEV isolation rate was 2%, which was thoroughly investigated; cell culture sensitivity was performed, and cell lines were replaced with fresh cells from Oman NPL.

Between January and August 2003, results were available for 180 samples out of 186 received in the laboratory. The 100% results of virological investigation of stool samples were reported to EPI within 28 days. The NPL referred 6 poliovirus type 3 positive samples to Kuwait RRL within 7 days of serotyping results and RRL confirmed 100% concordance of NPL results. Results were available for 4 polioviruses and were confirmed as Sabin poliovirus type 3 by RRL. The NPEV isolation rate improved to 5%.

6. GROUP DISCUSSION: CHALLENGES IN IMPLEMENTING QUALITY ASSURANCE

6.1 Overview

For group discussion participants were divided into two groups, one comprising national polio laboratories and other comprising RRLs and NPLs performing intratypic differentiation (ITD). The first group, national polio laboratories, discussed quality assurance (QA) issues related to media and cell culture preparation, cell culture sensitivity, and role of polio laboratories in strengthening the public health laboratories system (PHLS). The second group discussed quality assurance (QA) in intratypic differentiation (ITD) tests and the role of polio laboratories in strengthening the public health laboratories system (PHLS). The outcomes from the group discussions were that laboratories would identify deficiencies and gaps in establishing and implementation of QA, and discuss improvement in implementation of QA. The laboratories were also to give ideas to maximize the benefits from polio laboratory network experience and suggestions on involvement in most appropriate post-certification activities.

6.2 Cell culture preparation

- Implementation of QA system in laboratories helps to prevent risk of errors, improve efficiency and ensure data quality.
- The laboratory director should be responsible for implementation and ensure the compliance with QA.

- All laboratories should send their SOPs to WHO Regional Polio Laboratories Network to revise and develop a uniform format.

- All cell culture reagent preparation and procedures should be performed strictly according to WHO recommended methods, and instructions of the manufacturer should be followed. The HP of double distilled water, temperature of equipment and their performance should be monitored. Fetal bovine serum should be used from authenticated sources and be certified as mycoplasma free.

- Cell sensitivity should be performed using NIBSC standard Sabin reference strains, according to protocol given in the revised 2003 laboratory manual. All laboratories should stock frozen cells from fresh cells and, after testing the viability of cells upon revival from liquid nitrogen, old cells should be destroyed.

- Declining NPEV rates should be addressed thoroughly and trend analysis should be made for previous years to convince that no problem exists in laboratory procedures, otherwise corrections should be made to improve NPEV rate.

6.3 ITD testing

- The network laboratories should follow the procedures that are mentioned in the 2003 revised manual; no attempt should be made to develop their own modified protocols.

- Well-trained technologists must perform the ITD test. All reagents should be prepared strictly according to protocols. Interpretation of ELISA is straightforward, but dilution of different reagents used for this assay may affect the result, therefore strict care should be taken while diluting the reagents. Dilutions mentioned on the label of each reagent must be strictly observed.

- Instruments such as the ELISA plate reader and thermocycler should be calibrated regularly. Appropriate controls should be used and kept at recommended temperature.

- The ELISA kits made by RIVM are sensitive and specific. Therefore, it is recommended that whenever a problem arises in any network laboratory it should immediately communicate with RIVM to resolve this problem.

- Troubleshooting guidelines have been developed, and in case of any problem the 2003 revised laboratory manual should be consulted.

6.4 Utilization of the legacy of the polio laboratory network to strengthen public health laboratories

- The network that has matured and created a well-trained and experienced workforce, can easily be used in other priority diseases surveillance programmes. Remarkable achievements of the network include the following.

- Quality thinking
- Communication between the laboratory and benefiting programme (EPI)
- Data management information for action, which can easily be expanded to other diseases
- Cooperation of various agencies
- Well-equipped laboratory network
- Accreditation system
- Identification of other pathogenic viruses across countries

- Expansion of polio laboratory network to involve into other priority disease surveillance programmes, without jeopardizing polio work, will be most cost-effective investment, because it will not demand intensive effort switching to other programmes.

7. EXPERIENCES IN THE EMR POLIO LABORATORY NETWORK

7.1 Challenges in re-establishing the polio laboratory in Iraq

Dr Al Hamadani, National Polio Laboratory, Iraq

The Iraq NPL was a good performing laboratory before its sudden collapse in April 2003. In 2002 it met all laboratory performance criteria well above the target. The problems started with looting, in the aftermath of the war, which led to complete destruction of the physical structure. Nothing was left behind for the staff to work with and there was no security. The NPL had stored wild poliovirus isolates, original stool specimens and stool suspensions in a deep freezer, which was emptied and looted. This infectious material was sought and collected by NPL staff and most wild poliovirus isolates and original stool specimens were identified and destroyed by incineration. At the same time two institutes that were previously known to retain poliovirus seeds, the National Control of Drug and Research Center (NCDRC) and Al-Bahrain College of Medicine, were contacted and a total of 260 attenuated polio virus seeds were destroyed.

The immediate challenge was re-establishment of polio NPL. The physical structure was re-built and an inventory of NPL requirements for furniture and fixtures, equipment, glassware, reagents and disposable material was submitted to WHO and purchase orders were placed for procurement of laboratory supplies. A continuous power supply, air-conditioning and security to NPL were provided. A series of training workshops was planned for those working in laboratories to reactivate routine surveillance of AFP cases, proper sample transportation and maintenance of reverse cold chain. Two training workshops were held in the NPL for the laboratory workers in paediatric and general hospitals to reactivate AFP case detection and sending of samples to the NPL.

As an emergency measure, one 20 °C deep freeze was purchased and kept in the WHO office for storage of stool specimens before transportation to VACSERA in Egypt. Stool specimen collection kits and stool carriers were distributed to focal points. AFP case investigation forms were also distributed to focal points in the provinces.

Between April and August 2003, stool specimens of 48 AFP cases were sent to VACSERA in Egypt. Results of 41 cases were available and no wild poliovirus was isolated, 7 cases were pending virus culture results. Until the NPL is refurbished samples will continue to be sent to VACSERA.

7.2 Establishment of ELISA and PCR in the national polio laboratory, Oman

Dr S. Al Busaidy, National Polio Laboratory, Oman

The Oman national polio laboratory (NPL), continued to make a good contribution towards the regional polio eradication programme. It has supported laboratory examination of stool samples of AFP cases from neighbouring countries. A training workshop on enzyme linked immunosorbent assay (ELISA), an antigenic ITD method, was held in Oman last year. The laboratory was very successful in establishing ELISA test and passed the ELISA ITD proficiency panel with a score of 100%. No technical difficulty was encountered. According to WHO criteria, ITD tests should be done by two methods, one antigenic and one molecular. A workshop will be held in December 2003 to impart training in the molecular method, polymerase chain reaction (PCR). After successful implementation and passing of the proficiency panel, the NPL will be accredited by WHO to perform ITD testing on poliovirus isolates.

7.3 Implementation of quality control in cell culture, VACSERA

Dr L. El Bassiouni, VACSERA, Egypt

The present cell culture unit was established 3 years ago. The space allocated is sufficient, well organized and completely separate from other infectious laboratories. The laboratory is fully equipped and equipments are regularly calibrated. Ready-made minimum essential medium and other constituents of media are procured from certified sources. The incubator temperature and HP are strictly monitored.

Quality control (QC) is strictly observed, and all staff have been trained in QC methods. Cell culture media are tested for sterility and mycoplasma by PCR test. The cells are obtained from WHO GSLS. To avoid cross-contamination, only one cell line is handled at a time in the bio-safety cabinet. The cell lines are replaced after a maximum of 15 sequential passages. Cell sensitivity testing is performed using authenticated Sabin reference strains according to revised protocol. The cell sensitivity test is performed at the beginning of cell subculture (passage #2), midway (passage #7) and the end of subculture (passage #14) to ensure that cells remain sensitive throughout this period.

7.4 Coordination with the polio unit/EPI in Pakistan

Mr S. Zaidi, Regional Reference Laboratory, Pakistan

The Pakistan RRL has close coordination with EPI/surveillance staff and they are working towards better performance in planning and implementation of national polio

eradication activities. Regular weekly meetings are held between the laboratory and EPI/surveillance staff to review the previous weeks findings. AFP surveillance is regularly informed of data on all cases whose stool samples are received in the laboratory. The analysis of positive cases and culture growing viruses are discussed. Early and rapid data provided by the laboratory help to target immunization activities.

8. LABORATORY PERFORMANCE IN EMR POLIO LABORATORY NETWORK

8.1 Accreditation status of EMR polio laboratories

Dr Humayun Asghar, WHO/EMRO

In 2002, 10 out of 12 EMR network laboratories were fully accredited by WHO. The Jordan NPL and Kuwait NPL showed good performance, but were provisionally accredited by WHO. The main problems were delay in reporting and deficiency in full implementation of Quality Assurance.

Up to August 2003, the national polio laboratories of Jordan, Morocco and Sudan and RRLs in Egypt and Tunisia were fully accredited by WHO. Accreditation of remaining laboratories is ongoing. The compliance of laboratories in implementing the accreditation recommendations has proved beneficial in improving the performance of polio laboratories.

The accreditation data on all laboratories since 1998 showed improvement in performance of all laboratories over the years. There was improvement in timeliness of reporting of the virology results by the network laboratories. The workload in laboratories also increased due to improvement in AFP surveillance. Although internal quality control was implemented in all network polio laboratories, there was still need to improve quality assurance.

During accreditation the reviewers felt that most problems in laboratory performance were related to QA, such as monitoring the temp chart, equipment, worksheets, validation of tests, cell counting and cell culture sensitivity records, and documentation of troubleshooting. Another observation was that few laboratories made modifications in procedures without consultation with WHO.

8.2 New accreditation checklist and its impact

Dr E. de Gourville, WHO/HQ

Review of the accreditation reports has revealed that although the network has now been in place for more than 10 years, there are still laboratories experiencing cell culture problems. The two most common problems in laboratories remain poor management and establishing and maintaining adequate cell culture services.

The accreditation checklists were last revised in February 2000, and in light of current conditions a number of amendments have been proposed. These include the requirements to adopt the laboratory timeliness proposals made in 2001 by the Technical Consultative Group

(TCG), to accommodate the detection of VDPVs, and to take account of processing of samples from sources other than AFP surveillance.

The following is a comparison between the old and proposed new accreditation criteria for the national laboratory checklist.

	Old criterion	New criterion
1	≥ 80% of reports within 28 days	Unchanged
2	Tests on ≥ 150 samples per year	Unchanged
3	≥ 90% poliovirus accuracy of identification of polioviruses	Unchanged
4	≥ 80% poliovirus isolates sent for ITD within 14 days of detection	≥ 80% poliovirus isolates sent for ITD within 7 days of detection
5	Internal quality control implemented	Unchanged
6	≥ 80% score on PT test	Unchanged, except report within 28 days
7	≥ 80% on on-site review	Unchanged

With respect to the supplementary national laboratory checklist, the following is a comparison between the old and the new criteria.

	Old criterion	New criterion
1	ITD results on ≥ 80% of polioviruses reported within 28 days	ITD results on ≥ 80% of polioviruses reported within 14 days
2		≥ 80% wild polioviruses and suspected VDPV referred for sequencing within 7 days of detection
3	≥ 90% score on ITD PT test	≥ 90% score on ITD PT test – score to be recorded for each ITD PT test
4	≥ 90% poliovirus accuracy of identification of polioviruses	To be removed
5	≥ 90% on on-site review	Unchanged

Concerning the accreditation criteria for the regional reference laboratory checklist, the following is a comparison between the old and the new criteria.

	Old criterion	New criterion
1	ITD results on ≥ 80% of polioviruses reported within 28 days	ITD results on ≥ 80% of polioviruses reported within 14 days
2		≥ 80% wild polioviruses and suspected VDPV referred for sequencing within 7 days of detection
3	≥ 90% score on ITD PT test	≥ 90% score on ITD PT test, score to be recorded for each ITD PT test
4	≥ 90% score on isolation and typing PT test	Unchanged, except report within **28** days
5	≥ 90% on on-site review	Unchanged

6	≥ 80% of isolation reports within 28 days	Unchanged
7	Internal quality control implemented	Unchanged

Of the 145 laboratories in the network, 133 (92%) are fully accredited and 11 are provisionally accredited. Two laboratories remain non-accredited and 3 are pending accreditation reviews. The only laboratory serving a critical role in the network that is not currently fully accredited is the regional reference laboratory in Ghana. This laboratory performs the ITD on isolates from Nigeria but is currently only provisionally accredited.

The comparison of 2003 with last year showed that timelines of isolates referred within 7 days to reference laboratory, and ITD result within 14 days improved in all regions except WPR, which could not reach the target due to involvement of network laboratories in SARS work.

8.3 Evaluation of isolation and identification and ELISA ITD proficiency test

Dr Harrie van der Avoort, WHO Temporary Adviser

As in the preceding ten years, the 2003 performance of the laboratories of the EMR polio laboratory network has been excellent. Eleven of the 12 laboratories performing the proficiency test (PT) for isolation and typing scored 100%, while one laboratory missed a poliovirus in a sample containing a mixture of two polioviruses and achieved an 80% score. With a mean score of 98.3%, the EMR network Region contributed to the excellent performance of the global laboratory network; 140 of 141 laboratories participating in the PT for isolation and typing reached the passing criterion with a mean score of 99%. The one laboratory that failed achieved a 100% score on the second try. Seven laboratories are still working on the PT at present.

The 2003 performance of the EMR network in the PT for intratypic differentiation by ELISA was also very good; 5 of the 6 laboratories performing the test reached a 100% score with excellent optical density (OD) values, indicating optimal implementation of the ELISA test in these laboratories. This is also true for the sixth laboratory; however this laboratory performed the test without using the proper controls and was therefore interpreting an invalid test.

In the revised 2003 polio laboratory manual, a chapter was added on troubleshooting for the ELISA test for intratypic differentiation. This may facilitate problem-solving, should problems occur. Laboratories are encouraged to inform the RIVM polio/enterovirus laboratory, which has developed the test and distributes necessary reagents, about any problem encountered and also about successes and failures in solving problems. Full documentation of problems encountered, including OD values obtained and lot numbers of reagents used, is essential for successful troubleshooting and should therefore be provided immediately.

8.4 Proficiency test panels for nucleic acid probe hybridization and diagnostic PCR

Dr Olen M. Kew, CDC, USA

In 2002, laboratories at NIH Pakistan (RRL), VACSERA (RRL), Institute Pasteur-Tunis (RRL), Kuwait (SRRL), and Islamic Republic of Iran (NL) received proficiency test (PT) panels for intratypic differentiation (ITD) by nucleic acid probe hybridization (NAPH) and/or diagnostic PCR. All the laboratories passed. Documentation from the laboratories was generally excellent, and the results were transmitted electronically to WHO, WHO/HQ and CDC. The PT panels serve three primary functions: 1) to test the proficiency of the laboratories in their use of the molecular methods for ITD; 2) to assess the outcome of WHO-sponsored training in the use of molecular methods; and 3) to field test the reliability and durability of the ITD reagents developed at CDC.

The 2002 panels contained 10 unknown samples. The unknowns consisted of various combinations of in vitro RNA transcripts of the sequences specifically targeted by the primers and probes, the same transcripts that were used as positive controls in the molecular diagnostic kits. No additional information (such as serotype) on the unknowns was provided, in order to make the PT as challenging as possible. The PT panels were used in conjunction with the NAPH and PCR kits provided by CDC. The NAPH kits contain four probes: a poliovirus/enterovirus group probe (targeting conserved sequences in the 5'-untranslated region [5'-UTR]), and one probe specific for each of the Sabin OPV strains (targeting variable sequences in the capsid protein, VP1). The diagnostic PCR primer kits contain Standard Diagnostic PCR kits which include: 1) a panEnterovirus primer pair (targeting conserved 5'-UTR sequences, near the conserved sequences targeted by the poliovirus/enterovirus group probe); 2) a panPoliovirus primer pair (targeting VP1 sequences shared among polioviruses but not among other enteroviruses); 3) serotype-specific primer pairs (targeting VP1 sequences shared within poliovirus serotypes; one pair for each serotype); and 4) Sabin-strain-specific primer pairs (targeting variable VP1 sequences characteristic of each Sabin strain; three pairs). The poliovirus serotypes of the unknown samples can be explicitly determined by PCR. However, when the NAPH sample contained only wild poliovirus sequences, the identification cannot be fully defined, as only the group probe will give a positive signal. The unknown could either be a non-polio enterovirus or a wild poliovirus (or some mixture of both), and should be reported accordingly.

Some laboratories in the Global Polio Laboratory Network had difficulty with the 2002 NAPH PT panel because of low concentrations of the unknown RNAs. This problem was aggravated by problems of incomplete resuspension of the lyophilized RNA samples. Nonetheless, nearly all laboratories were able to obtain the correct identifications. Steps have been taken to correct these problems for the 2003 PT panels, which will be similar to the 2002 panels.

9. METHODOLOGIES

9.1 Evaluation of cell sensitivity and of reference standard Sabin strains

Dr Javier Martin, WHO Temporary Adviser

Routine monitoring of the sensitivity of cell lines for virus isolation is an important component of the laboratory’s QA programme. Monitoring the sensitivity of RD and L20B cells on a regular basis gives reassurance of the cell line’s ability to detect poliovirus infection.

2500 ampoules of each of the three Sabin vaccine virus serotypes were prepared at NIBSC and distribution to laboratories of the polio network has already started. They were prepared from monovalent bulk materials of the three Sabin strains that had been released to be used as vaccines in the United Kingdom. The original vaccine preparations were diluted to a target titre of 5.0 log10/0.1ml. The advantage of using such reference materials is that they were authenticated as Sabin strains and that they have an assigned titre that will enable a quantitative evaluation of the cell sensitivity within a laboratory and the comparison of results between different laboratories.

A standard operation procedure (SOP) for virus titration has also been developed based on the WHO standard procedure used for vaccine potency assays. The frequency of testing should be at least once soon after cells are initiated and, if possible, at mid-point and end of the passage history. Records should be kept on laboratory control charts and results reported on standard forms to WHO Regional Polio Laboratory Coordinator/EMRO, WHO/HQ and NIBSC. If the observed titre is below the lower limits of the assigned virus titres for the reference strains, then the cells are of adequate sensitivity. If cells are found to be of low sensitivity, they should be replaced with a new batch of adequate sensitivity either from a laboratory stock or from the corresponding RRL.

9.2 Definitions for VDPV and sensitivity of current strategy for detecting VDPVs

Dr Olen M. Kew, CDC, USA

The recent recognition of VDPVs has important implications for the global polio eradication initiative. Polioviruses can be divided into two broad categories: 1) wild polioviruses, and 2) Sabin vaccine-related polioviruses. The wild polioviruses are divided into serotypes, genotypes (groups of genetically related viruses endemic to wide geographic areas), clusters of closely related lineages and lineages (roughly corresponding to chains of transmission). Sabin vaccine-related polioviruses of each serotype can be further divided into 1) OPV-like (or “Sabin-like”) viruses which show <1% VP1 sequence divergence from the corresponding OPV strain, and 2) VDPVs, which show ≥ 1% VP1 sequence divergence from the corresponding OPV strain. The 1% demarcation is somewhat arbitrary, but will detect nearly all VDPVs of programmatic concern yet exclude typical OPV-like isolates commonly found worldwide. A divergence of 1% corresponds to approximately 1 year of virus replication or circulation, an unusual occurrence for vaccine-related polioviruses.

The large majority of vaccine-related isolates from AFP cases and contacts or the environment are OPV-like. The rare VDPVs are divided into three sub-categories: 1) circulating VDPVs (cVDPVs) that are biologically indistinguishable from wild polioviruses, 2) immunodeficient VDPVs (iVDPVs) isolated from patients with defects in antibody production who are chronic excretors of VDPVs; and 3) ambiguous VDPVs, where the available clinical, epidemiological, and virological data do not permit further classification. Four cVDPV outbreaks have been described: 1) Egypt (type 2), ~1988–1993; 2) Hispaniola (type 1) ~1998–2001; 3) the Philippines (type 1), ~1999–2001; and 4) Madagascar (two independent type 2 lineages) ~1999–2001 and 2001–2002. The key risk factors for cVDPV emergence and spread are gaps in OPV coverage and the prior eradication of the corresponding serotype of wild polioviruses. Additional risk factors are the same as for wild poliovirus circulation: poor hygiene/sanitation, high population densities and tropical conditions. Areas that had previously been reservoirs for wild poliovirus circulation are

potentially at highest risk for cVDPV emergence if the rates of OPV coverage were allowed to decline. iVDPVs occur more sporadically, and are associated with the exposure to OPV of people with rare conditions of immunodeficiency. Most chronic excretors spontaneously stop shedding VDPVs or die from complications of their immunodeficiency (including poliomyelitis). A very small number of people have been found to excrete iVDPVs for 10 years or more. Survival rates of chronic excretors appear to be highest in developed countries and middle-to high-income developing countries.

The occurrence of VDPVs has important implications for current and future polio immunization strategies, for poliovirus surveillance and for containment. Polio Network laboratories currently use two methods for ITD: one antigenic test (either the ELISA or neutralizing monoclonal antibodies, and one molecular test (NAPH, PCR, or PCR-RFLP). Isolates giving discordant ITD results (usually "non-vaccine like" by the antigenic tests and Sabin OPV-related by the molecular tests) are candidate VDPVs, and are promptly referred to a GSL for VP1 sequencing. Most of these isolates turn out not to be VDPVs, but some are, and these are subject to further characterization. A type 2 VDPV in the Syrian Arab Republic in late 2001 was identified by this approach. Complete genomic sequencing of the isolate is nearly complete. Combined clinical, epidemiological, and virological investigation should help determine proper classification of this VDPV case.

VDPVs are important to containment. The available evidence indicates that cVDPVs and iVDPVs are neurovirulent and have potentially high transmissibilities, and therefore should be handled as wild polioviruses for purposes of containment.

9.3 MAb-ITD assay for the detection of wild poliovirus and VDPV
Dr Javier Martin, WHO Temporary Adviser

Intratypic differentiation of polioviruses is a very important step in the characterization of poliovirus isolates from polio surveillance activities. The method based on virus neutralization using Sabin-specific monoclonal antibodies is one of the five ITD methods recommended by WHO. It is routinely used in two GSL with great success. Experimental evidence was presented showing the ability to promptly identify vaccine-derived polioviruses (VDPV) using this method. It is proposed that this method could be used as a rapid "filter" to select out Sabin-homologous strains from the large amounts of virus isolates that are going to be analysed during the next few years. The possible advantages of using this method include the fact that it is already in use in several laboratories so reagents and procedures are available. It is also technically simple and the ITD characterization is carried out at the same time as the serotyping of the virus isolate using the same microtitre plate so it could be performed at the national laboratory level. Furthermore, this method is fast, so wild type and VDPV isolates could be rapidly flagged for further characterization.

9.4 An investigation of the MEF-1 detection in India (1)
Dr Harrie van der Avoort, WHO Temporary Adviser

At the end of 2002 and beginning of 2003, the ERC, in Mumbai, India reported isolation of the wild poliovirus type 2 from 7 cases of AFP, 1 contact and 1 environmental sample. Genetic analysis showed that all these isolates were identical or closely related to MEF-1, the prototype wild P2 strain that is in use for the preparation of IPV and is commonly used as wild type 2 reference strain in research and diagnostic laboratories. Isolation of MEF-1 from diagnostic samples to date in all cases was due to laboratory contamination of these samples. However, this time extensive review of laboratory and epidemiological data has ruled out laboratory contamination as a possible cause for the isolation of MEF-1 from the stools of the 7 AFP cases.

MEF-1 is a field isolate obtained by inoculation of CNS material from more than one case of poliomyelitis in 1942. Extensive characterization of MEF-1 from various sources (laboratories, IPV producers) has shown that there are two species of MEF-1 that differ in three characteristic nucleotides at positions 99, 223 and 300 of the VP1 region. The recent viruses from India are identical to or closely related to either of the two species. On the basis of this genetic analysis, it was concluded that the 7 cases were the result of multiple introductions of MEF-1 from one source, during a limited period of three months.

A very comprehensive field investigation by the Government of India and WHO did not find evidence of a break of containment in any of the laboratories and institutions in the region. During the field investigation, OPV vaccine vials of the 2 batches used in the NIDs held in the regions at the time the AFP cases were found, collected and send to the ERC in Mumbai, RIVM Bilthoven and NIBSC in London.

All three laboratories were able to show by various molecular and biological tests that one batch of OPV from the field indeed did contain both genetically distinct species of MEF-1. This finding has triggered an extensive investigation on the presence of MEF-1 in OPV vials from the same lot from other locations, OPV vials from lots prepared in the

producing institutions involved just before and after the contaminated lot and from recently prepared lots. Samples from the bulk preparations used to prepare these lots of vaccines were also collected for testing for the presence of MEF-1. The investigation is still ongoing. Results will be available within a month.

9.5 An investigation of the MEF-1 detection in India (2)

Dr Javier Martin, WHO Temporary Adviser

Following the identification of 7 cases of paralytic poliomyelitis linked with the MEF-1 wild type 2 poliovirus strain in India in 2002–2003, a thorough investigation of its possible origin was initiated. Both monovalent bulks and finished OPV product of vaccines that were found to be temporally and geographically associated with the poliomyelitis cases were tested for the presence of MEF-1 virus. MEF-1 poliovirus was indeed found in a batch of OPV from a particular manufacturer both by molecular and biological laboratory tests. To date, MEF-1 wild virus has only been found in vials of one particular batch that were retrieved from the field after vaccination campaigns. MEF-1 virus was not found in vials of the same number batch kept in national control laboratories or in the vaccine monovalent bulks that were used to prepare that particular batch. Further investigation is needed to understand the origin of this unfortunate incident.

9.6 Wild poliovirus in cross-absorbed antisera for intratypic differentiation

Dr Harrie van der Avoort, WHO Temporary Adviser

The wildtype-specific and vaccine-like-specific antisera used in the ELISA for intratypic differentiation of polioviruses are produced by cross-adsorption with prototype vaccine-like and wild polioviruses, respectively. Antigen–antibody complexes formed and excess of polioviruses are removed from the antisera by ultracentrifugation at high speed. Removal by centrifugation was considered to be effective to remove all viruses as the antisera obtained could be used in neutralization assays.

Given the unexpected isolation by ERC Mumbai of MEF-1 virus from 7 AFP cases in India, and the experience of the laboratory network that such isolations until now always have been the result of laboratory contamination, the RIVM antisera batches in use at ERC, Mumbai, India, were considered as a possible source for such contamination. Testing these antisera batches at RIVM and at CDC Atlanta indeed showed the presence of wild virus in the SL-specific antisera of all three serotypes (and the presence of Sabin viruses in the NSL-specific antisera).

This unexpected finding led to a series of immediate actions. All 30 laboratories of the global polio laboratory network using the RIVM ELISA test for intratypic differentiation were contacted and asked to consider all ELISA antisera regardless of batch number as contaminated, and therefore to restrict use of these antisera only inside bio-safety cabinets.

RIVM started an investigation on the incident and on an efficient and effective procedure to remove the contaminant from the antisera in use already and to prevent the occurrence of the incident in future. It was shown that inactivation of the antisera by heating during 30 minutes at 56 °C does remove all infectivity.

All 30 laboratories of the global polio laboratory network have confirmed by email that they have inactivated all ELISA reagents in use and in store by heating at 56 °C for 30 minutes. RIVM has included the inactivation step in the SOP of the preparation of the antisera. All antisera distributed in the future will not contain live polioviruses.

Several investigations by WHO and independent consultants at the ERC, Mumbai have demonstrated that a laboratory contamination by the RIVM produced antisera or by other cause can be ruled out as cause for the detection of the MEF-1 virus in the 7 AFP cases in India. The work of the consultants was very much facilitated by the excellent and very detailed documentation of all laboratory activities in the Mumbai laboratory.

10. ENVIRONMENTAL SURVEILLANCE FOR POLIOVIRUSES

10.1 Update on environmental study in Egypt

Dr Tapani Hovi, WHO Temporary Adviser

The collaborative study including parallel cell culture analysis of sewage concentrated in VACSERA, Cairo, Egypt, and KTL, Helsinki, Finland, supplemented with selected studies in CDC, Atlanta, USA, is continuing for the third year. New sampling sites have been enrolled especially in Lower Egypt to cover larger parts of the population, while sampling at some of the older ones has been stopped to avoid work overload in the laboratories. Currently monthly sampling is occurring at 32 different locations. The general approach of the study has remained constant:

- Sampling is targeted to urban populations, which are suspected to maintain wild poliovirus circulation, and where significant proportions of the houses are connected to converging sewage networks.
- The sampling sites are identified in major collector sewers or inlets of sewage treatment plants, each usually representing a population of few hundred thousand.
- One litre of raw sewage is collected at regular intervals, refrigerated and sent to VACSERA.

Sewage concentrated nominally by a factor of 5–100, is inoculated onto one RD and two L20B flasks at both VACSERA and, after freezing and sending to, KTL. Identification and ITD of possible poliovirus isolates are as for clinical samples. Main results so far can be summarized as follows:

- Under the conditions used, environmental surveillance was able to detect wild type poliovirus even during OPV campaigns
- Almost all examined samples contained either polioviruses or non-polio enteroviruses; all serotypes of Sabin-like (SL) polioviruses were found

- Only wild poliovirus type 1 were detected and all sequenced strains belonged to the same genotype as the most recent clinical isolates in Egypt
- After the very intensive circulation of the wild poliovirus type 1 in summer 2001, the proportion of specimens containing wild poliovirus type 1 has dramatically decreased; sewage from three separate cities was still wild poliovirus type 1 positive in June 2003
- Sample sensitivity is proportional to the fraction of sample inoculated into cell cultures; this has to be balanced with concurrently increasing workload
- Parallel vials inoculated with a given sample often yielded strikingly different results because of the virus mixture present; this is causing difficulties in confirming the results in another laboratory
- Genetic diversity of the characterized strains was strikingly wide. In 2001 the strains clustered in three separate lineages; one of them has not been detected since 2001
- Co-circulation of lineages was reflected by frequent occurrence of representative of two separate lineages of wild poliovirus type 1 in a given sample.

10.2 Implications of supplementary environmental surveillance on the polio eradication programme in Egypt

Dr Faten Kamel, WHO/EMRO

An environmental poliovirus surveillance project in Egypt was initiated in 2001 as a collaborative activity between VACSERA, CDC, KTL, WHO and the Ministry of Health and Population with the aim of identifying reservoir communities with "silent" circulation of wild poliovirus in high-risk areas and monitoring the interruption of indigenous wild poliovirus transmission, including targeting reservoir areas in OPV immunization campaigns and evaluating the impact of NIDs.

Initially the project started in Upper Egypt and only one site from Lower Egypt (Tanta) was included. It was found that all sites were positive for wild poliovirus type 1, with more samples positive in Minya, Assiut, Dairut, Sohag, Qena and Beni Suef. Other findings included that no wild types 2 or 3 were found and only one genotype was circulating in all sites. Multiple independent chains of transmission were detected and lineages were much more than those from polio cases.

In 2001 the non-polio AFP rate was close to 1.0 per 100 000 population under 15 years. In fact there was no actual sensitive AFP surveillance, and a culture of fear was prevailing. This implied a need to review the structure and operating procedures for polio eradication to strengthen the surveillance and improve quality supplementary immunization activities.

In the second half of 2002, improvement in AFP surveillance led to reporting of 7 wild poliovirus type 1 cases detected in Egypt: 6 in Lower Egypt and one in Upper Egypt. Molecular sequencing data showed that last year there were six (A–F) arbitrary clusters of

multiple lineages, which were found in 11/18 provinces targeted for environmental sampling. Data showed that 16% of total samples tested were positive for wild poliovirus type 1.

As per the recommendations of technical advisory group (TAG) the numbers of sites were increased in Lower Egypt, and at the same time frequency of sampling in Greater Cairo (Cairo, Giza and Qaloubiya) was also increased. Between January and August 2003, a total of 164 samples were collected from 27 sites. A total of 32 samples were collected per month from these sites. There was marked reduction in the number of positive sites when compared with the last year. Only three sites (Cairo, Sharkia and Minya) were positive for wild poliovirus type 1. The per cent positivity of environmental samples tested to date for wild poliovirus type 1 was 5%. In 2003 one wild poliovirus type 3 case was detected in Minya.

Molecular data showed that only two clusters (B and F) were active during 2003 and wild type poliovirus type 1 isolated from AFP cases in Minya was closely related to Minya environmental wild poliovirus type 1. The last environmental samples positive for wild poliovirus type 1 was collected from Abo Qarkas (Minya) in July 2003.

In conclusion, AFP surveillance remains the gold standard of poliovirus surveillance, but environmental surveillance can provide important supplementary information on circulation of wild poliovirus or VDPV in a population. The concern is that environmental surveillance poses an increase in workload in polio laboratories.

11. DATA MANAGEMENT

11.1 Progress in establishment of modified LABIFA in EMR polio laboratories

Dr Hala Safwat, WHO/EMRO

Remarkable achievements have been made during the past year regarding laboratory Information for Action (LABIFA). The system was modified in 10 laboratories of the Region as well as in EMRO. The Polio Fax laboratory table was reformatted to include additional requested information. Laboratory systems in Iraq and Jordan are to be modified as soon as possible.

In 2002 most laboratories updated EMRO on a weekly basis. Laboratories in Egypt, Oman and Pakistan were the most regular. Other laboratories of the Region are encouraged to improve the frequency of their reporting.

The modified LABIFA is a case-based system, which has enhanced and improved the data quality. There is still room for improvement, especially regarding duplicates and accuracy in recording final results. More efforts are needed to standardize the IDCODES to match cases from the laboratory side with the AFP surveillance. This will permit doing further analysis. Improvement in quality and completeness of newly introduced variables is essential (Hot cases, gender, dates for timing and tracing and tracing of specimens and ITD results).

11.2 Automated data analysis, outputs and reporting: discussion and demonstration of data outputs

Ms A. van Middelkoop, WHO Temporary Adviser

The revised LABIFA system has been installed in 10 of the 12 EMR polio laboratories, with all data converted to a case-based format. Laboratory-specific requests and requirements have led to the situation that each laboratory has its own version of the basic software. The following points were emphasized.

- To ensure correct processing during data entry, the ENTER key must be used rather than the mouse.
- To avoid double counting of cases if there are more than 2 specimens from a case, sequential specimen numbers should be allocated, e.g. 1, 2, 3, and 4. Specimen number 1 should *not* be used more than once for any one case.
- Regional reference laboratories should take special care in entering the correct value of the variable SPECTYP. This variable is used by EMRO to avoid double counting specimens that have been processed by more than one laboratory.
- Laboratories must check that any changes made to the final ITD results are reflected correctly in the results section at the end of the data entry screen.

Laboratories are encouraged to report any problems with LABIFA to the data manager at WHO/EMRO, providing details of the problem.

Full implementation of the LABIFA system requires the following:

- Installation in Jordan and Iraq laboratories.
- Addition of a standard set of lists and reports to all systems.
- Improvement of the error detection process during data entry.
- Addition of an error report that focuses on critical aspects of the data (duplicates, country, inconsistent results).
- Attention to known requests and problems.
- Development of a user's manual.
- Adequate training on the use of LABIFA.

It was suggested that a data management workshop be held in order to update and customize all laboratory systems; provide training on the use of LABIFA; and provide training on the analysis of the data.

12. CONCLUSIONS

The poliovirus laboratory network in the WHO Eastern Mediterranean Region continued to improve its performance in support of the poliomyelitis eradication programme. This was reflected in the full accreditation of ten laboratories and provisional accreditation of two laboratories as of December 2002. The polio network laboratories continued to provide high quality technical support to the polio eradication programme. Remarkable improvements were made in providing accurate and timely virological investigation results. The laboratories continued to maintain certification-standard performance in support of AFP surveillance. Laboratories continued to work closely with the AFP surveillance programme to resolve problems such as timeliness of transportation of samples and maintenance of the reverse cold chain. Remaining challenges to the laboratory network include sustaining high level laboratory performance, provision of laboratory supplies, maintaining enthusiasm of the polio laboratory staff, and ensuring national support for the national polio laboratory.

13. RECOMMENDATIONS

To polio laboratories

Quality assurance

1. Quality assurance should be fully implemented in accordance with the revised 2003 laboratory manual.

2. All laboratories should share standard operating procedures (SOPs) and routine documentation procedures with the WHO regional polio laboratory coordinator by end 2003, to ensure uniformity in laboratory practices throughout the Region.

3. Laboratory directors/supervisors should critically review worksheets of routine work on a daily basis.

4. Media and cell cultures should be prepared strictly in accordance with the revised 2003 laboratory manual and instructions of the manufacturer. Laboratories should discuss possible modifications with the WHO regional polio laboratory coordinator before implementing them.

5. Cell sensitivity assays should be implemented in 2003 using NIBSC standard Sabin reference strains on both RD and L20B cells according to the protocol described in the revised 2003 laboratory manual, scheduled for distribution in 2003.

Data management

6. All variables in LABIFA should be entered, and analysis should be used regularly to monitor performance and track samples. WHO/EMRO should be contacted immediately if problems are encountered.

Annual laboratory accreditation

7. Network laboratories should adjust laboratory practices to ensure capacity to meet WHO accreditation criteria introduced in January 2003. As an important accreditation element, all laboratories should implement bio-safety level-2/polio in accordance with the containment of wild poliovirus and potential infectious materials, Global Action Plan II, Phase-1. Inventories of original stool samples, supernates, isolates or any other potential infectious material should be kept current.

To the Expanded Programme on Immunization

8. The coordination between EPI/AFP surveillance and the laboratory should be strengthened through regular weekly meetings where data analysed are exchanged to expedite reporting of results.

9. In countries where timely transportation of stool samples to laboratories remains a concern, special attention should be given to ensuring that samples are transported to the laboratory immediately after collection.

To Ministries of Health

10. Governments should review their budget allocations to ensure that the budget is sufficient to support polio laboratory functions in their countries. The budget should cover the costs of sustaining the basic laboratory facility, staff, supplies and equipment maintenance.

11. Laboratories of the poliovirus network have matured and gained extensive experience in virology, quality assurance, laboratory management and data management and should be considered national resources for future development of laboratory support for other priority disease surveillance programmes, while providing continuing strong support to the polio eradication programme.

To WHO

12. WHO should advocate supporting the polio laboratory network for expansion to involve other priority disease surveillance programme and offer the expertise of its trained workforce in the establishment of strengthening of public health laboratories.

13. The revised polio laboratory manual should be distributed to all laboratories by the end of 2003, and the Regional Polio Laboratory Coordinator should follow up to ensure that WHO recommended methods for poliovirus diagnosis are implemented.

14. Designated EMR network polio laboratories should be provided with technical assistance to establish PCR as a molecular technique for ITD testing and mycoplasma testing of cell culture.

15. An intercountry training workshop on data management should be organized for data managers of the regional polio laboratories.

Annex 1

PROGRAMME

Sunday, 24 August 2003

08:30–09:00	Registration
09:00–09:30	Opening session Address by H.E. Dr Hakem Al Qadi, Minister of Health, Jordan Address by Dr Hussein A. Gezairy, WHO Regional Director for the Eastern Mediterranean Election of the Chairman and Rapporteur Implementation status of the recommendation of the sixth intercountry meeting of directors of poliovirus laboratories/Dr H. Asghar, WHO/EMRO
Session 1	Overview
09:30–09:45	Global status of polio eradication initiative/Dr E. de Gourville, WHO/HQ
09:45–10:00	Regional status of polio eradication initiative/Dr F. Kamel, WHO/EMRO
10:00–10:15	Regional status of polio laboratories network/Dr H. Asghar, WHO/EMRO
10:15–10:30	Discussion
Session 2	Virus surveillance and molecular epidemiology in endemic countries
11:00–11:20	Egypt, virus surveillance/Dr E. Al Maamoun
11:20–11:40	Afghanistan/Pakistan, virus surveillance/Mr S. Zaidi
11:40–12:00	Somalia and south Sudan virus surveillance/Mr P. King'ori
12:00–12:20	Molecular epidemiology of polioviruses in EMR endemic countries/Dr O. Kew, CDC
12:20–12:45	Discussion
Session 2	Virus surveillance in non-endemic countries
14:00–14:15	Iran/Dr Tabatabai
14:45–15:00	Jordan/Dr A. Muhaidat
14:15–14:30	Kuwait/Dr S. Al–Mufti
14:30–14:45	Saudi Arabia/Mr M. Al-Amri
Session 3	Group discussion: challenges in implementing quality assurance
15:30–17:00	Group 1: Each NPL discuss QC issues related to; media, cell culture preparation, and cell culture sensitivity; and how public health laboratories can be strengthened based on polio laboratory experiences/ Moderators: Dr E. de Gourville, Dr T. Hovi, Dr J. Martin, Dr H. Asghar Group 2: Each RRL including NPL, Iran and Oman, discuss the implementation of QC in ITD testing of polioviruses, and its value in interpreting the results, and how public health laboratories can be strengthened based on polio laboratory experiences/Dr O. Kew, Dr H. van der Avoort

Monday, 25 August 2003

Session 3	Group discussion: challenges in implementing quality assurance (cont'd)
08:00–8:30	Presentations by groups and discussion continuation
Session 4	Experiences in the EMR polio laboratory network
08:30–08:45	Challenges in re-establishing polio laboratory in Iraq/Dr Al Hamadani
08:45–09:00	Establishment of EIA and PCR in NPL, Oman/Dr S. Al Busaidy
09:00–09:15	Implementation of QC in cell culture, VACSERA/Dr L. El Bassiouni
09:15–09:30	Coordination with polio unit/EPI in Pakistan/Mr S. Zaidi
09:30–10:00	Discussion
Session 5	Laboratory performance in EMR polio laboratory network
10:30–10:45	Accreditation status of EMR polio laboratories/Dr H. Asghar, WHO/EMRO
10:45–11:00	New accreditation checklist, responsibilities of laboratories and its impact/Dr E. de Gourville, WHO/HQ
11:00–11:15	Evaluation of isolation and identification proficiency tests and ELISA ITD proficiency test/Dr H. van der Avoort, WHO/EMRO
11:15–12:00	Evaluation of probe hybridization and PCR ITD proficiency test/Dr O. Kew, CDC
12:00–12:30	Discussion
Session 6	Methodologies
13:30–13:50	Evaluation of cell sensitivity and distribution of reference standard Sabin strains/Dr J. Martin, WHO/EMRO
13:50–14:10	Definitions for VDPV and sensitivity of current strategy for detecting VDPVs/Dr O. Kew, CDC
14:10–14:30	MAb-ITD assay for the detection of wild poliovirus and VDPV/Dr J. Martin,WHO/EMRO
14:30–14:50	An investigation of the MEF-1 detection in India/Dr H. van der Avoort, WHO/EMRO, Mr C. Wolf, WHO/HQ
14:50–15:30	Discussion
Session 7	Environmental surveillance for polioviruses
16:00–16:15	Update on environmental study in Egypt/Dr T. Hovi, WHO/EMRO
16:15–16:45	Implications of supplementary environmental surveillance on Egypt polio eradication programme/Dr F. Kamel, WHO/EMRO
16:45–17:00	Discussion

Tuesday, 26 August 2003

Session 8	Data management
09:00–09:20	Progress in establishment of modified LABIFA in EMR polio laboratories/Dr H. Safwat, WHO/EMRO
09:20–09:40	Automated data analysis, outputs and reporting/Ms A. van Middelkoop
09:40–10:30	Discussion and demonstration of data outputs/Ms A. van Middelkoop

11:00–12:00	Open discussion: remaining issues and apprehensions which laboratories may face in the coming phases of polio eradication

Closing session

12:00–13:30	Discussion on conclusions and recommendations

Annex 2

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