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Report on the

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

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1. INTRODUCTION

The eighth intercountry meeting of directors of poliovirus laboratories in the WHO Eastern Mediterranean Region was held in Casablanca, Morocco, from 26 to 28 July 2004. Directors of laboratories in Egypt, Islamic Republic of Iran, Iraq, Jordan, Morocco, Oman, Pakistan, Saudi Arabia, Sudan, Syrian Arab Republic and Tunisia 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; and staff from the World Health Organization (WHO) headquarters and Regional Office for the Eastern Mediterranean.

Dr Raouf Ben Ammar, WHO Representative, Morocco, welcomed the participants and delivered a message on behalf of Dr Husssein A. Gezairy, WHO Regional Director for the Eastern Mediterranean. In his message Dr Gezairy welcomed the participants and thanked the Government of Morocco and His Excellency Dr Mohamed Sheikh Biadillah, Minister of Health, for hosting the meeting. He expressed appreciation for hard work and dedication of the polio laboratory network scientists in providing timely good quality virological investigation results. He urged the laboratories to sound even a "false alarm" if any poliovirus was isolated from a "hot case", or if preliminary tests suggested an isolate was wild or a circulating vaccine-derived poliovirus (cVDPV). He emphasized the value of an early report, even though preliminary, since prompt evidence of WPV transmission was crucial at this stage of the initiative to interrupt decisively the last remaining chains of virus transmission. 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 Mohamed Cheikh Biadillah, Minister of Health, Morocco, in his inaugural address, welcomed all the participants and highlighted the progress achieved in Morocco in the fields of EPI and polio eradication. He expressed appreciation for the role of polio laboratories in polio eradication.

It was agreed to have rotating chairmanship. Elected chairpersons were Ms Hayat Caidi (Morocco), Dr Hinda Triki (Tunisia) and Dr Suleiman Al Busaidy (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 SEVENTH INTERCOUNTRY MEETING OF DIRECTORS OF POLIOVIRUS LABORATORIES IN THE EASTERN MEDITERRANEAN REGION

Dr Humayun Asghar WHO/EMRO

Recommendation	Implementation status
1. Quality assurance should be fully implemented in accordance with the revised 2003 laboratory manual.	Fully implemented in all laboratories. A few weaknesses were identified and were addressed during accreditation visits.
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 through out the Region.	SOPs were developed in the light of those existing in the laboratories and were shared with all the laboratories for necessary amendments to suit their routine work.
3. 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.	NIBSC Sabin standards were distributed to all regional polio laboratories and a cell sensitivity assay was implemented.
4. Laboratory directors/supervisor should critically review worksheets of routine work on a daily basis.	Supervision of routine work improved, and worksheets were modified for better supervision of laboratory work.
5. 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. An intercountry workshop on data management should be organized.	Data entry improved, but analysis was not a regular feature in a few laboratories. A data management workshop was held in Cairo, Egypt, to train laboratory staff handling AFP surveillance data.
6. 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	All laboratories made adjustments in routine work practices to meet accreditation criteria. BSL2/polio was implemented in all laboratories, and inventories of all poliovirus materials were kept current.

	the containment of wild poliovirus (WPV) 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.	
7.	The coordination between EPI/AFP surveillance and the laboratory should be strengthened through regular weekly meeting where data analysed are exchanged to expedite reporting of results.	Coordination improved, especially in endemic countries, and regular meetings and discussions were held, which resulted in improved timeliness of laboratory results.
8.	Governments should review their budget allocations to assure that it 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.	Data obtained from the laboratories indicate that success in obtaining specific allocation of budgets for the polio laboratories has been limited.
9.	Experience of poliovirus network laboratories in virology, quality assurance, laboratory management and data management should be considered as national resource for future development of laboratory support for other priority disease surveillance programmes, while providing continuing strong support to the polio eradication programme. WHO should advocate supporting polio laboratory network for expansion to involve other priority disease surveillance programme.	Some progress has been seen in the form of measles laboratory work assigned to polio laboratories, but it needs to be further addressed with countries to utilize fully the experience of the polio laboratory network for the development and further improvement of the disease surveillance programmes.
10.	Designated regional network polio laboratories should be provided with technical assistance to establish PCR as a molecular technique for intratypic differentiation (ITD) testing and mycoplasma testing of cell culture.	All ITD testing laboratories were trained in PCR techniques through a workshop held in Muscat, Oman, and all laboratories now have capability to do PCR. Mycoplasma testing of cell culture is not established in all laboratories.

3. CURRENT STATUS OF THE POLIOMYELITIS ERADICATION INITIATIVE

3.1 Global status of the poliomyelitis eradication initiative

Dr Esther de Gourville, WHO/HQ

In 1988, the World Health Assembly resolved to eradicate poliomyelitis globally. Since then implementation of the eradication strategies has reduced the number of countries endemic for polio from >125 to 6 in 2003. Three WHO regions are certified polio free: American Region, Western Pacific Region and European Region. However, in 2003, an unprecedented 9 countries reported importations of WPV, including 8 in west and central Africa (Benin, Burkina Faso, Cameroon, Central African Republic, Chad, Cote d'Ivoire, Ghana and Togo) and one in the Middle East (Lebanon). In 2004, imported virus continued to circulate in 5 countries of central and west Africa (Benin, Burkina Faso, Central African Republic, Chad and Cote D'Ivoire), one in southern Africa (Botswana) and one in north-eastern Africa (Sudan). It was noted that the average routine immunization coverage (OPV3) is less than 50% in countries with the most recent importations.

In 2004, delegates from remaining endemic countries participated in a Ministerial Conference held at WHO headquarters in Geneva on 15 January 2004. At the end of the conference, national health officials, together with representatives of main partners in polio eradication, signed the Geneva Declaration for the Eradication of Poliomyelitis, committing them to the necessary action to stop viral transmission by the end 2004. The ministers of health were requested to report progress to the World Health Assembly in May 2004, and heads of the state to the UN General Assembly in September 2004. The salient activities were massive scale-up of supplementary immunization activities during low season in the first six months of 2004, targeting 250 million children, in up to 6 synchronized campaigns in all infected areas of west/central Africa (22 countries) and Pakistan/Afghanistan.

A strategic plan for 2004–2008 was launched in October 2003. It contains 4 objectives:

- interrupting wild poliovirus transmission by 2004–2006
- global certification by 2008
- development of post certification policies
- mainstreaming the polio infrastructure for broader health benefits.

The number of polio cases decreased from 1918 in 2002 to 784 in 2003. In 2003, 355 cases were detected in Nigeria, 225 cases in India and 103 cases in Pakistan. By July 2004, 486 cases were reported. However there is evidence of significant increase in Nigeria, where the number of reported cases increased significantly from 202 in 2002 to 355 in 2003, and 383 up to July 2004 compared with 90 for the same period in 2003. The number of polio cases in India decreased from 1600 in 2002 to 225 in 2003, with 25 cases in 2004 compared to 92 for the same period in 2003. In Pakistan, the number of polio cases increased from 90 in 2002 to 103 in 2003, with 19 cases reported up to July 2004 as compared with 45 for the same period in 2003. In Egypt, one polio case was reported in 2003 and one in 2004, compared with 7 in 2002. The proportion of environmental samples positive for WPV declined from 57% in 2001

to 4% in 2003, and less than 1% in 2004. The genetic sequencing suggested reinfection by Nigerian viruses of previously polio-free areas within Nigeria as well as exportation of WPV to previously polio-free countries across west, central, south and north-east Africa.

There is good progress in Asia and North Africa, while there are substantial challenges in west and central Africa. There is funding gap of US$ 100 million for 2004–2005, and emergency response in west and central Africa alone will require additional US$ 100 million.

There are potential risks of OPV in the form of vaccine derived polioviruses, e.g. VAPP, iVDPV and cVDPV. The risk of VAPP appears to be 2–4 cases per million birth cohort. The 19 cases of iVDPV identified during 40 years use of OPV were 7 type 1, 11 type 2, and 1 type 3. Most of them have already died; only two are known to be excreting poliovirus. Circulation of cVDPVs is a major risk associated with the use of OPV in the post-certification era, and the risk is higher than the benefit in the absence of WPV. A policy decision was made for cessation of OPV for routine immunization. The timing for OPV cessation was suggested to be when transmission of WPV is interrupted, all poliovirus strains have been contained and OPV cessation instruments are in place, i.e OPV cessation guidelines, vaccine stockpiles, international response mechanisms, etc.

3.2 Regional status of the poliomyelitis eradication initiative

Dr Faten Kamel, WHO/EMRO

Significant progress has been achieved towards polio eradication in the Eastern Mediterranean Region. At present, 18 countries of the Region have been polio-free for more than 3 years and Somalia reported its last case in October 2002. The three remaining endemic countries reporting cases in 2004 are Pakistan and Afghanistan, which together are considered a common reservoir, and Egypt representing an independent reservoir. Sudan reported a case due to importation from Chad.

The number of poliomyelitis cases in the Region decreased from an annual estimated 35 000 cases in 1988, in other words about 100 cases a day, to only 113 cases during 2003. To date, the number of cases in 2004 is 20, which is significantly less than those of the corresponding period in 2003 (44). As well, genomic sequencing results show diminished genetic diversity of the viruses isolated from Pakistan and Afghanistan as well as Egypt, reflecting progress towards eradication from these countries.

One of the basic strategies of polio eradication is the acceleration and intensification of quality supplemental immunization activities. In 2003, both endemic and recently polio-free countries were supported to conduct national and subnational immunization days. Each of the endemic countries in the Region conducted 4 rounds of national campaigns in addition to 4 rounds of subnational campaigns in high-risk areas. Countries recently polio-free conducted less frequent national and subnational campaigns. All campaigns in the Region were implemented on a house-to-house basis and were characterized by detailed micro-planning, multisectoral involvement and intensified supervision. Monitoring by independent national and international observers showed that these intensified campaigns were of high quality and were very effective in closing the immunity gap among children under 5 years of age.

The second major strategy for polio eradication, surveillance for acute flaccid paralysis (AFP), continued to improve throughout the Region. The required level of sensitivity (non-polio AFP rate exceeding one case per 100 000 children under 15 years of age) has been reached in 2003 not only regionally but also in all individual countries of the Region except three, namely Bahrain and Djibouti, for which only few cases are expected per year, and Palestine, with a difficult security situation. The other global indicator for high quality surveillance, adequate stool specimen collection from more than 80% of AFP cases, was reached and exceeded at regional level and in all individual countries except four, including Bahrain and Djibouti with only two cases reported from each.

The establishment and maintenance of the surveillance system in countries affected by war and in areas with rudimentary or virtually non-existent health care services, such as in Afghanistan, Somalia and south Sudan, is a great achievement. The system has been instrumental in the reporting and investigation of other diseases. The polio surveillance system is supported by a network of 12 poliovirus network laboratories which function at a very high standard and are all accredited by WHO, except the Iraq laboratory which was destroyed in 2003.

Technical Advisory Groups were established for countries that are still endemic to review the epidemiological situation and provide advice to the national eradication programmes. A Regional Technical Advisory Group (RTAG) has been established to guide the eradication activities during the final phase, or "end-game", particularly with regard to the issues of laboratory containment of wild polioviruses, the certification of polio eradication, preparedness for wild poliovirus importation and development of post eradication immunization policies.

A regional plan for laboratory containment of wild poliovirus was developed and endorsed by the Regional Committee in 2000. At present, 18 countries of the Region have developed national containment plans, and 9 of them have completed implementation of phase I (survey and inventory phase) of containment requirements, which is needed before global certification. The other 9 countries are progressing in completing phase I.

All countries of the Region that have been free of poliomyelitis for three or more years, except Palestine, have submitted documentation for certification to the Regional Certification Commission, and have had their documentation accepted. Annual updates from these countries are also submitted to the RCC. The remaining five countries that have not yet submitted certification documentation are facing a difficult security situation (Palestine), have only recently become polio free (Somalia) or are still endemic (Afghanistan, Egypt and Pakistan).

Guidelines were prepared by the Regional Office for the development of national plans for preparedness for wild poliovirus importation. These plans are essentially based on maintaining sensitive surveillance systems capable of early detection of importation. All countries that submitted documentation to the RCC included in their documentation a plan to address wild poliovirus importation. Although importations have been reported in several

countries of the Region, circulation has not been re-established because of the high levels of immunity. The situation in Sudan is being addressed with appropriate response.

Although there has been significant progress in the regional programme of polio eradication, a number of challenges are still facing the programme.

- Stopping circulation of the virus in the remaining endemic countries. According to the National Technical Advisory Group (TAG) for the Pakistan and Afghanistan, the main activities needed are enhancing political commitment, addressing sociocultural factors that limit access to children and ensuring necessary technical support. Significant advocacy efforts were made by the Regional Director in this regard at the highest political level. WHO is providing all necessary technical support, comprising more than 40 international and 180 national staff in Pakistan and 11 international and 120 national staff in Afghanistan. Similarly efforts are being intensified in Egypt to interrupt the last chains of transmission.

- Ensuring access to all children; the security situation in Afghanistan, Palestine, Somalia, in parts of Sudan and Pakistan and, recently, Iraq presents a major challenge which the programme is trying to overcome through a number of initiatives. Special efforts must be made to avoid any gaps in immunity among children less than 5 years.

- Maintaining political support; high levels of national commitment must be sustained in the face of the disappearance of the disease in both polio-endemic and polio-free countries. Yearly progress reports on polio eradication to the Regional Committee help to maintain the visibility of the polio eradication initiative and keep countries alert to the risk of importation.

- Securing financial resources. Despite the significant contributions of national authorities, supplemental funding from external sources is needed to sustain eradication activities. The financial resources required in support of poliomyelitis eradication in the Region for 2004–2005, excluding the price of vaccine, amounts to US$ 68 million (US$ 41.5 million for 2004, which has almost been secured, and US$ 26.5 million for 2005).

The impressive progress towards eradication of poliomyelitis in the Region is the result of the extraordinary efforts of national authorities and the support provided by a consortium of partners spearheaded by WHO, UNICEF, Centers for Disease Control and Prevention in Atlanta, USA, and Rotary International. In addition, significant support was received during 2003 and 2004 from the Department for International Development (United Kingdom), United Nations Foundation, Bill and Melinda Gates Foundation, Arab Gulf Programme for United Nations Development Organizations (AGFUND), the Governments of Russia, Canada, Italy, Netherlands and United Arab Emirates and the United States Agency for International Development.

3.3 Regional status of the polio laboratory network

Dr Humayun Asghar, WHO/EMRO

The poliovirus laboratory network in the WHO Eastern Mediterranean Region is performing at high quality certification standard indicators and this is reflected in full WHO accreditation of all except Iraq national polio laboratory (NPL), which could not be visited due to security problems. All laboratories have passed WHO proficiency panel of unknown viruses for both primary virus culture and intratypic differentiation (ITD) testing. The workload of network laboratories is ever increasing, showing good surveillance in countries of the Region.

All laboratory performance indicators are well above the set targets, except transportation of samples within 3 days, which is below 70%. It remains a concern due to the fact that this has not improved despite repeated reminders to those concerned. The timeliness of results from onset of paralysis to final ITD testing improved; reduced from 43 days in 2003 to 37 days in 2004. Timeliness of ITD results within 14 days after serotyping improved from 86% in 2003 to 91% in 2004. The results of stool samples after receipt in laboratories to final ITD are sent out within 27 days, which is reassuring in that laboratories have capacity to report results of hot cases in a short period of time.

Molecular data are used routinely to identify endemic reservoirs and importation. The genetic sequencing data suggest that WPV transmission is increasingly localized and genotypes are specific to endemic countries of the Region. An importation of WPV type 1 was detected in Habeela, West Darfur, Sudan, which is closely related to Bongor, Chad 2003 virus.

In 2003, 113 WPV cases were reported from the Region: 103 from Pakistan, 8 from Afghanistan, one from Egypt, and one importation into Lebanon, which was closely related to an Indian virus.

As of July 2004, 25 cases due to WPVs were reported from the Region: 20 from Pakistan, 3 from Afghanistan, one from Egypt, and one importation into Sudan.

In Egypt, AFP surveillance is supplemented with environmental surveillance to increase sensitivity for detection of WPV. The percentage of environmental sites positive for polioviruses decreased from 57% in 2001 to 16% in 2002, 4% in 2003, and less than 1% to date. Numerous separate chains of transmission of a single genotype of WPV type 1 were detected. The frequency and number of sewage sample collection sites increased in 2003 and 2004 and now reach 41 samples per month from 33 sites. From Greater Cairo alone, a total of 13 sites are sampled each month. It was noted that there was gradual decline in isolation of polioviruses and non-poliovirus enterovirus (NPEV) starting from September 2003 and became evident in early 2004. The problem was addressed in both the laboratory and field and the problem was resolved.

The regional polio laboratory network is faced with the challenges of sustaining the laboratories' performance, retaining trained human resources, and securing specific budget allocation for polio laboratories. WHO/EMRO is working closely with countries and

advocating for sustaining of certification-standard quality laboratory performance and utilization of expertise of the polio laboratory network for the development and integration with other disease surveillance programmes. The network laboratories were supported with supplies of logistics. Training workshops are conducted as part of refreshing the knowledge and keeping the polio laboratory network scientists interested in polio work. Two training workshops were conducted, one in 2003 in Oman on PCR techniques, and another in 2004 in Cairo on data management.

4. VIRUS SURVEILLANCE AND MOLECULAR EPIDEMIOLOGY IN ENDEMIC COUNTRIES

4.1 Molecular epidemiology of WPVs in poliomyelitis endemic countries of the Region

Dr Mark Pallansch, Centers for Disease Control and Prevention (CDC), Atlanta

Within the Eastern Mediterranean Region, four countries have wild poliovirus transmission in 2004. This includes the three recognized endemic countries of Egypt, Pakistan and Afghanistan as well as Sudan which has suffered an outbreak of type 1 following an importation from the neighbouring country of Chad.

In Egypt, the number of chains of transmission continues to decline, but the virus is being detected from both AFP cases and the environment in 2004. Only type 1 wild poliovirus has been observed in Egypt for the past three years. A degree of uncertainty as to the extent of virus transmission was introduced as the result of a technical problem with the processing of the environmental specimens. There remains concern about gaps in surveillance as evidenced by the long branch-length of the Assiut–Dairut case WPV type 1.

Pakistan and Afghanistan continue to represent a shared cross-border reservoir for poliovirus. Pakistan has made progress toward polio eradication in the past year. There are fewer active WPV type 1 and 3 clusters this year than for the same time last year. Similar progress is suspected in Afghanistan, but the available information is being interpreted with caution, especially for south-west Afghanistan. The apparent decline in the number of active clusters suggests that the programme continues to break chains of transmission sustaining endemicity in key reservoir areas. Whereas PV1 and PV3 previously circulated in both countries via numerous multiple independent chains of transmission, the chains are greatly diminished, but not yet eliminated. WPV circulation in Pakistan is at the lowest levels ever observed, not only in terms of reduced numbers of cases, but also in the declining genetic diversity of the WPV type 1 and 3 isolates. Nonetheless, several key endemic reservoirs remain: in NWFP, in northern and southern/central Sindh, in southern and central Punjab, and in southern (and possibly central) Afghanistan.

During 2004, an outbreak of type 1 poliovirus was recognized in the Darfur region of Sudan. The virus was imported from Chad as the result of the ongoing outbreak there and subsequently has resulted in multiple cases within Sudan. This is the same region of Sudan with recognized cross-border transmission as seen in 1999. It is likely that civil unrest

contributes to the problem and that control may be difficult; the quality of surveillance is unclear in other parts of the country.

Efforts are continuing to design and develop better methods for showing signs of progress that are evident in the genetic data. Although this effort is far from complete, it is often advantageous to show the reduction in the number of genotypes, the number of clusters, or the reduction in genetic diversity. There is no ideal solution because of the inherent problem of presenting multi-parameter data that include place, time, genetic information, and virus genetic relationships.

In summary, molecular epidemiology data indicate continued programmatic progress within the Region. However, the genetic data continue to highlight focal surveillance gaps in some areas. There is also some concern that the ongoing virus circulation within the Region and adjacent Regions poses a risk of importation to polio-free countries in the Region.

4.2 Egypt: virus surveillance

Dr Iman Al Maamoun, VACSERA, Egypt

The VACSERA regional reference laboratory (RRL) was fully accredited and scored 100% in proficiency testing (PT) panel for both primary culture and ITD. In 2003, the RRL processed 1220 stool samples from 608 AFP cases from Egypt. Its laboratory performance indicators were very good; 97% of stool samples results were reported within 28 days, 100% ITD results within 14 days, NPEV rate 17%. As of July 2004, 881 stool samples were processed from 443 AFP cases. There was a many-fold increase in workload in VACSERA as compared to years before.

In 2003, from 1220 stool samples, WPV type 1 was isolated from a case and its contact in Abu Qurqas, Minya. As of July 2004, WPV type 1 was isolated from one case and its contact from Assiut–Dairut. There was great improvement of timeliness of reporting, especially of hot cases, as the length of time was just 19 days and 10 days for the wild cases reported in 2003 and 2004, respectively. All WPVs isolated were indigenous strains and were closely related to wild viruses isolated from environmental samples.

The RRL carried out virus culture and ITD on samples referred from Iraq, Lebanon, Syrian Arab Republic, Sudan and Yemen. Since May 2003, all AFP cases stool samples from Iraq are tested at VACSERA. In 2003, a total of 443 stool sample and 10 isolates were referred for testing, out of which one WPV type 1 was isolated from Lebanon. In 2004, a total of 317 stool sample and 3 isolates were referred for testing, out of which one WPV type 1 was isolated from Sudan in May.

4.3 Pakistan: virus surveillance

Mr Sohail Zahoor Zaidi, National Institute of Health, Pakistan

In 2003, the Pakistan RRL processed stool samples of 2220 AFP cases from Pakistan. The stool adequacy rate was 97%. The NPEV rate was 25% and the results of 98% cases were reported within 28 days. As of July 2004, stool samples of 1187 AFP cases were tested.

Adequate stool samples were collected from 97% of AFP cases, the NPEV rate was 19% and results of 91% of stool samples were reported within 28 days.

The RRL Pakistan also provided support to the polio eradication programme in Afghanistan. In 2003, stool samples of 599 AFP cases were processed. The NPEV rate was 22% and results of 98% of stool samples were reported within 28 days of their receipt in the laboratory. As of July 2004, a total of 363 AFP cases from Afghanistan were tested. The NPEV rate was 21%. The reports of 91% cases were sent within 28 days of receipt of the samples in the laboratory.

In 2003, poliovirus isolated from 259 AFP cases were submitted for ITD, out of which 72 were WPV type 1 and 31 were WPV type 3. As of July 2004, poliovirus isolated from 103 AFP cases were submitted for ITD, out of which 14 were WPV type 1 and 5 were WPV type 3. In 2004 there was marked decrease in number of WPVs isolated from AFP cases for the same period as compared to 2003. All WPVs were subjected to nucleotide sequencing at CDC, Atlanta, and all WPVs were confirmed. At the same time genetic diversity of WPV type 1 and 3 continued to decline.

The laboratory has successfully made its first sequencing run with the help of CDC, Atlanta and will start it as routine test after training of two scientists from RRL at CDC Atlanta.

4.4 Somalia and south Sudan: virus surveillance

Mr Peter King'ori, Kenya Medical Research Institute, Kenya

The Kenya Medical Research Institute (KEMRI) polio laboratory is designated as the national polio laboratory for Kenya, Djibouti, Eritrea, Somalia and south Sudan. The laboratory has maintained its WHO accredited status since 1999. The laboratory has scored 100% in the PT panel test since 2001.

In 2003, KEMRI tested 195 stool samples of 98 AFP cases reported from Somalia and 174 of 87 AFP cases from south Sudan. No WPV was isolated. In 2004 to date, 86 stool samples of 43 AFP cases from Somalia and 64 of 32 AFP cases from south Sudan were tested in the laboratory. No WPV was isolated. The NPEV rate decreased both in Somalia and south Sudan as compared to the previous year. This decrease in NPEV rate was more in south Sudan then Somalia. This was a major concern and is being addressed in the laboratory and field. Two stool samples of an AFP case from Djibouti were tested in 2004.

5. LABORATORY EXPERIENCES IN DEALING WITH SPECIFIC PROBLEMS

5.1 Egypt RRL: decline in NPEV/SLPV in environmental sampling

Dr L. El Bassiouni, VACSERA, Egypt

It was observed that there has been a gradual decline in non-polio enteroviruses (NPEV) and Sabin-like (SL) polioviruses since September 2003, reaching to minimum in January

2004. This decrease in NPEVs and SLs was seen in both VACSERA and KTL. During this period 5 WPV type 1 were isolates from environmental samples collected from Giza–AboRawash (KTL), Minya–AboQurqas (VACSERA), Cairo–ElSalam (VACSERA and KTL), and two samples from Minya–AboQurqas (VACSERA and KTL).

It was evident that environmental surveillance had lost its sensitivity and investigations were started both in the field and laboratory to identify the cause behind the problem. Focus was on sample collection, transportation from field to laboratory, and storage, processing and virus isolation system in the laboratory. The sample collection and transportation in the field was found to be according to prescribed instruction.

In the laboratory, all equipment, glassware, disposables, reagents and laboratory procedures were checked for changes or modifications since the beginning of environmental study. Nothing of significance could be noted; however, it was noted that Dextran T40 was 3 year old, but it was kept in good condition. All old reagents were replaced with fresh ones.

Bacterial and coliform counts were performed to see if any chemical had been added to sewage which might kill the bacteria, and as a result virus too. Results were satisfactory and confirmed that samples were collected properly and that no chemical affected the samples

In consultation with KTL, validation of the concentration step was performed in which a known amount of poliovirus type 1 Sabin was mixed into a selected sewage sample, the mixture was concentrated, and the concentrate was analysed for poliovirus to determine the sensitivity of the process. Results showed that there was a problem in the concentration process.

WHO/EMRO recruited a consultant to resolve the problem of decline in sensitivity of the laboratory in isolating NPEV and polioviruses. The consultant visited the field and worked with laboratory staff, checking the procedures and trying different methods:

- effect of different material of sewage sample containers
- effect of pH or chemical on poliovirus infectivity in raw sewage sample
- non specific binding between the wall of the tubes and the virus
- spiking for virus detection before and after concentration step
- spiking for virus detection by KTL method versus the WHO/VACSERA method.

No significant results could be obtained, except that spiked MEM with 2% FBS, showed cytopathic effect in tissue culture up to $10^{-7.3}$.

As per the consultant's recommendation, 6 unconcentrated sewage samples were sent to KTL for processing in parallel with VACSERA. KTL isolated NPEV and SL polioviruses from all samples, including two WPV type 1 from two sewage samples. This showed that there was a definitive problem in processing of sewage samples in VACSERA. Accordingly, VACSERA is implementing recommendations made by the WHO consultant to resolve the problem.

5.2 Islamic Republic of Iran: maintaining sensitive cell culture

Dr H. Tabatabai, Islamic Republic of Iran

In virus isolation systems, a sensitive tissue culture is necessary to obtain correct and timely results. In poliovirus laboratories, the NPEV rate is used as a surrogate marker of the sensitivity of cell lines being used.

Quality of components of growth media, e.g. water, fetal calf serum, pH, should be maintained, because the environment condition in which cells grow is important for their development to support the replication of viruses. Mycoplasma contamination affects the cells and retards their growth and sensitivity.

It is routine in the national polio laboratory of the Islamic Republic of Iran that each cell line is handled separately at different times and that designated media bottles are used. The quality assurance is fully implemented; regular cell counting, incubator temperature monitoring, cell sensitivity testing with WHO Sabin standards, and documentation of all laboratory procedures. Both RD and L20B cell lines are discarded after 12–15 passages and fresh low passage cells are revived from liquid nitrogen.

5.3 Iraq: re-establishment of polio laboratory

Dr Faisal Al-Hamdani, Iraq

The national polio laboratory in Iraq, destroyed in 2003, has been re-established and efforts to restore laboratory activities are ongoing. The NPL was moved to its temporary location in the WHO office in July 2003, as the central public health laboratories were undergoing radical rehabilitation. The NPL was refurbished with equipment, reagents and accessories through WHO/EMRO. All poliovirus materials were destroyed by incineration. Coordination with EPI is continuing to improve AFP surveillance. The director of NPL is serving as a member of national containment committee. The NPL is also working on measles diagnosis.

5.4 Jordan: meeting accreditation standard performance

Ms Najat Najjr, Jordan

The NPL was provisionally accredited in 2002; as a result, recommendations were made to improve implementation of quality assurance in tissue culture and other laboratory procedures. The NPL successfully implemented all recommendations resulting in full accreditation of the NPL in 2003. The NPL is now working under strict bio-safety level 2. All standard operating procedures and routine worksheets were revised, and quality assurance is assured in the tissue culture laboratory. Two scientists were trained at VACSERA, Egypt, for one month each in cell culture, isolation and identification techniques. The NPL scored 100% in the proficiency testing panel, and the NPEV rate improved to 14%. However, concerns remain on NPEV isolation, because the NPL was not able to isolate all NPEV as compared to results obtained from parallel testing of stool samples in the Tunisia RRL. The NPL continues to send stool samples to the Tunisia RRL for parallel testing.

5.5 Morocco: molecular sequencing of old stocks of polioviruses

Ms Hayat Caidi, Morocco

The virology laboratory of the military hospital was storing wild polioviruses and uncharacterized polioviruses which were collected during 1971 to 1982, at the time when wild polioviruses were circulating in Morocco. These were transferred and stored in the national polio laboratory, National Institute of Hygiene, Rabat. As part of the implementation of phase 1 of wild poliovirus containment activities, the national containment coordinator was advised to destroy these materials.

No molecular data were available on these viruses, so isolates were sent to CDC for molecular characterization by genomic sequencing. A scientist from NPL carried out this study at CDC. The 35 isolates were sequenced (22 PV type 1, and 13 PV type 3). All isolates were wild poliovirus positive for a larger sequencing PV1 fragment (~990 nt), and most sequences were an exact match for most isolates. So it is evident that some isolates were suspected laboratory contamination. Further genetic analyses have excluded the presence of any wild reference poliovirus contamination, and the viruses were the indigenous Moroccan strains that circulated in Morocco during 1971 to 1982. Phylogenetic comparison with other viruses isolated in different countries collected during the same decade showed much divergence. All wild polioviruses and other infectious material were destroyed on 23 July 2004 in the presence of NPL staff and the WHO/EMRO regional polio laboratory network coordinator.

5.6 Oman: establishment of ITD testing capabilities

Dr Sulieman Al-Busaidy

The WHO-recommended methods for the intratypic differentiation (ITD) of polioviruses are ELISA, nucleic acid probe hybridization, PCR-RFLP, and diagnostic PCR, using specific approved methods. In the WHO Poliovirus Laboratory Network, reporting of ITD results is the responsibility of the regional reference laboratories. However, ITD may be performed in national poliovirus laboratories that have been specifically accredited to do so. For the NPL to be accredited to perform any of the specific ITD methods, the staff received training in ITD testing methods. Two workshops were held at the Central Public Health Laboratory (CPHL), Muscat, Oman, to train personnel in the WHO/EMRO poliovirus laboratory network in the identification and intratypic differentiation of poliovirus isolates using the poliovirus diagnostic ELISA and PCR method.

Following these two training workshop, the Oman NPL successfully established both ELISA and PCR techniques without any difficulties. The NPL is waiting for WHO accreditation to perform ITD.

6. QUALITY ASSURANCE

6.1 Accreditation status of regional polio laboratories

Dr H. Asghar, WHO/EMRO

In 2003, 11 out of 12 regional network laboratories were fully accredited by WHO. The Iraq NPL could not be visited due to security problems. As of July 2004, the national polio laboratories in Jordan, Morocco and Sudan were fully accredited by WHO. All NPLs implemented recommendations made during accreditation visits.

Accreditation data for all laboratories since 1998 show improvement in performance of all laboratories over the years. The most significant was the improvement in timeliness of reporting of the virology results by the network laboratories. The workload in laboratories increased due to improvement in AFP surveillance. Internal quality control was implemented in all network polio laboratories, but still there was need to improve quality assurance.

During accreditation, reviewer felt that most of the problems in laboratory performance were related to partial implementation of quality assurance in tissue culture laboratories; cell counting, proper recording of routine worksheets, cell culture sensitivity records and documentation of trouble-shooting.

6.2 Evaluation of cell sensitivity for poliovirus infection

Dr Javier Martin, WHO Temporary Adviser

Monitoring the sensitivity of RD and L20B cells on a regular basis is an essential process of laboratory quality assurance and gives certification of the cell line's ability to detect poliovirus infection.

During 2003, the National Institute for Biological Standards and Control (NIBSC) distributed authenticated Sabin standard poliovirus reference strains to all laboratories of the WHO polio network. The reference strains had an assigned virus titre and were designed to be used to prepare and validate laboratory quality control (LQC) standards for routine cell sensitivity testing. A detailed protocol was also devised and included in an updated version of the WHO polio manual.

Based on the initial experiences in some laboratories, it was recommended that the protocol should be updated. Amendments to the protocol include the establishment of an upper limit of virus titre (+0.5 log of expected titre) for the test to be valid, the need to test all three serotypes in both cell lines and the acceptance as valid of those tests were 90% CPE (instead of 100% CPE) or 10% CPE (instead of 0% CPE) are achieved. In those cases, the next lower or higher virus dilutions are to be considered 100% or 0% CPE, respectively, when applying the Karber formula to calculate the virus titre.

It is also important to insist that laboratories perform three independent tests for each reference strain (done on different days) and of course, to use freshly made dilutions each

time. It was also noted that some laboratories did not perform the titration of the laboratory quality control standards in parallel with the NIBSC standard of the corresponding serotype. In order to assign a virus titre to the laboratory quality control standards, it is strictly necessary to perform three independent valid tests done in parallel with the corresponding NIBSC standard. Once the results of the three tests are available and the virus titre of the NIBSC standards is within the expected limits, a titre for the LQC standards can be established. The LQC will then be used for routine testing of cell sensitivity for poliovirus infection.

If the virus titre of a NIBSC standard falls out of the expected limits (± 0.5 log of the assigned virus titre) the assay should be repeated once, to discard human error. If the results are confirmed the whole procedure is invalidated and has to be reviewed in order to identify the source of the problem. A virus titre higher than expected could be due to procedural error/s such as a mistake during the preparation of the virus dilutions or overspill from adjacent wells. A virus titre lower than expected could also reflect a procedural mistake but, particularly if the results are confirmed in a second test, it most likely reveals low sensitivity of the cells to poliovirus infection. All elements and actions related to cell culture and the process of cell sensitivity testing should be reviewed including SOPs, staff, management, reagents/materials, equipment, records, etc. If a defect is identified, it should be immediately corrected. The same rules apply to the LQC standards once they have been validated for use in routine cell sensitivity tests.

If cells are confirmed to be of low sensitivity it should be a case of great concern since efficient and sensitive poliovirus isolation is central to the AFP surveillance process. The cells in use should be discarded and replaced with a batch of cells of documented sensitivity for poliovirus infection. If necessary, new appropriate cells should be requested from a regional reference laboratory.

6.3 Evaluation of proficiency tests for isolation and typing and for intratypic differentiation of polioviruses by RIVM ELISA

Dr H. van der Avoort, WHO Temporary Adviser

The regional polio laboratory network has confirmed its reputation of being an excellent partner in the polio eradication initiative activities in the Region with excellent scores in the yearly proficiency tests performed in 2003–2004. All but one of the laboratories participated in the test for isolation and typing (the laboratory in Iraq was not yet functional at the time the panel was distributed). All 11 regional laboratories passed the test with a mean score of 97.3%. Eight laboratories reached an optimal score of 100%, while 3 laboratories scored 90%, as they were not able to resolve correctly the mixture of polioviruses present in one of the samples. The regional performance fits well to the global performance (146 laboratories participated with a mean score of 98%).

All 6 laboratories performing the RIVM ELISA for intratypic differentiation of polioviruses scored 100% and provided excellent data showing that the ELISA worked very well in all laboratories.

At the moment there is a shortage of P3 reagents for the RIVM ELISA. RIVM is working continuously to overcome this shortage and appeals to all laboratories not to spill ELISA reagents, and use the available reagents only for WHO-related activities. Problems with the ELISA tests should be reported *immediately* to the RIVM, preferably by email, with a copy to the laboratory coordinator. Full documentation by sending copies of worksheets is necessary for quickly resolving the problem.

7. GROUP DISCUSSION: CHALLENGES IN IMPLEMENTING QUALITY ASSURANCE

7.1 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, discussion was focused on full implementation of quality assurance programme in laboratory procedures and in tissue culture laboratory in particular, and their experiences with implementation. The following were talking points.

- Quality assurance as a tool to monitor the laboratory performance on a daily basis and its role in tracking problems and trouble-shooting.

- Cell culture: media and cell culture control (cell sensitivity, cell counting, media sterility).

- Monitoring of equipment and effects of minor temperature changes on the cell culture growth and virus isolation.

- How to avoid cell culture cross-contamination?

The group was expected to identify deficiencies and gaps in establishing and implementation of quality assurance, and suggestion for full implementation.

The second group discussed quality assurance in ITD testing. The discussion was focused on full implementation of a quality assurance programme, and their experiences with implementation of quality assurance. The following were talking points.

- Value of quality assurance in ITD testing, both antigenic and molecular, validation and interpretation of ITD.

- Difficulties in establishment of PCR in laboratories.

- Trouble shooting in (enzyme linked immunosorbent assay (ELISA), nucleic acid probe hybridization (NAPH) and polymerase chain reaction (PCR).

- Why PCR has been preferred to be molecular method of choice for ITD testing.

The group was expected to identify deficiencies and gaps in establishing and implementation of quality assurance, and suggestions for full implementation.

7.2 Group 1: quality assurance in laboratory procedures, particularly in tissue culture

Full implementation of quality assurance system is necessary to avoid errors, improve efficiency and give credibility to results and performance. The laboratory director should establish and supervise the implementation of quality assurance through regular monitoring of data.

In the tissue culture laboratory, media preparation and cell culture procedures should be strictly documented, including sterility testing, cell counting, cell sensitivity, changing of cell lines after every 15 passages, and temperature monitoring of incubators.

Cell sensitivity testing should be performed regularly according to WHO protocol with NIBSC standard Sabin reference strains.

Cross-contamination in cell culture or virus isolation laboratories has serious consequences in the form of loss of time, credibility of laboratory and financial implications due to actions taken in response to incorrect results. It is better to avoid such accidents in first place and the only way is to perform laboratory procedure under strict biosafety by trained staff. The procedures should not be performed in a panic or when time is insufficient to perform test.

The group was of the opinion that full implementation of quality assurance is practicable and it is of great help to monitor day to day activities in the laboratory, and it helps in trouble-shooting where and when required without any delays.

7.2 Group 2: quality assurance in ITD testing

With regard to ELISA, it was emphasized that all isolates giving discordant results should be transferred immediately with all worksheets to the global specialized laboratory (GSL). Double reactive isolates can be regrown in order to resolve the problem, but it is better to refer the isolate rather than waste time.

Reactivity of all controls should be recorded regularly, and internal controls should be constantly monitored. It is better to plot the OD readings values and compare the trend.

With regard to PCR, all measures should be taken to avoid carry over effect. The samples should be arranged in a way to avoid carryover. All controls should be used in order to validate the test. Negative controls should be included. Monitoring of data is crucial for trouble-shooting.

Trouble-shooting should be looked at carefully. All problems should be discussed with the laboratory coordinator and other network laboratories to solve the problems.

8. CHALLENGES TO THE POLIO ERADICATION PROGRAMME

8.1 Risk of decline in AFP surveillance

Dr Faten Kamel, WHO/EMRO

Sustaining the AFP surveillance activities to maintain certification standard quality indicators is a challenge, especially in countries that have been polio free for a long time. Overall activities at regional level are good. However, the level of achievement of AFP surveillance indicators differs in polio endemic and polio free countries. In polio endemic and recently polio free countries, non polio AFP (NPAFP) rate and adequacy of stool collection met the target of 2.0 and is well above 80%, respectively. In polio free countries, the NPAFP rate and adequacy of stool sample is just maintained above 1.0 and 80%, respectively. There will be more risk of decline in AFP surveillance when the Region is polio free, while it will be more important to maintain it until certification of polio eradication, and especially in the post-OPV era.

Countries are kept informed of the progress and of their performance through communication via Polio Fax, and feedback is provided for corrective measures. Other measures like surveillance reviews, regular visits to countries, meetings, workshops and discussion with country staff are undertaken to keep the surveillance up to date. All polio free countries are submitting annual updates on documentation of national certification. The Regional Certification Commission comments to National Certification Committees on any change in performance indicators, and suggests appropriate measures. The countries have developed preparedness for importation. Endemic countries are supported with human resources by WHO to fill up gaps in technical expertise. The Regional Office is working towards mainstreaming the polio staff through integration into other disease surveillance programme.

8.2 Acceleration of OPV cessation: implications for surveillance and laboratory workload

Dr H. van der Avoort, WHO Temporary Adviser

Good quality surveillance must be continued until after global polio eradication and certification. Network laboratories are dealing with heavy workloads due to increasing AFP surveillance in most countries. Now that polio eradication is near, the polio laboratory network will need to tackle new challenges: the absence of wild poliovirus circulation and cessation of OPV use will have consequences for the activities of the laboratories. For example, they will no longer use L20B cells for detection of polioviruses, and there will be no need for ITD as every poliovirus isolated needs to be sequenced directly. With the proven flexibility of the network in previous years, these changes will be implemented easily.

A bigger threat to the network is the insufficient finances to keep the network alive in its present form in the long run. Loss of interest in polio, which will no longer be a big issue in many countries after global eradication is achieved, will lead to loss of expertise to detect importations of poliovirus and loss of potential for optimal intervention should such an event happen.

Knowing the consequences of Sabin virus contamination in diagnostic laboratories, it is essential that its use should be minimized. Although it is a difficult option, reshaping the laboratory network would be considered, such that only good performing NPLs would be maintained. Testing of faecal specimens collected or stored for other purposes or environmental surveillance in risk areas, or serology, may be used as possible solutions to fill gaps with declining AFP surveillance. The most crucial are decision time for formulation of essential tasks for the network, selection of key laboratories in each region/risk area, assurances that samples will continue reaching the laboratories, and clearly defining the role of laboratories in all circumstance, especially in emergencies.

8.3 Long term poliovirus excretion by immuno-deficient individuals: the United Kingdom experience

Dr Javier Martin, WHO Temporary Adviser

Immune competent individuals excrete poliovirus for short periods after vaccination with live-attenuated oral poliovaccine (OPV). This excretion period rarely exceeds 3 months. However, immunodeficient patients can excrete vaccine-derived poliovirus (iVDPV) for several months and even several years. To date, 21 such cases have been confirmed around the world. B-cell deficiency disorders associated with long-term polio excretion include X-linked or sporadic agammaglobulinaemia (XLA) and common variable immunodeficiency (CVID), which can remain undiagnosed for more than 5 years.

Only 3 of the 21 reported long-term excreters are known to be currently excreting poliovirus. The length of polio excretion ranges from 1 to 20 years. An individual in the United Kingdom is known to have been excreting Sabin 2-derived poliovirus for an estimated 20 years and is still excreting at present. Most iVDPV strains showed increased neurovirulence in laboratory experiments and have been associated with paralytic poliomyelitis although some iVDPV carriers remain asymptomatic. Treatment with therapeutic immunoglobulin provides protection against the paralytic disease but does not seem to be very efficient at interrupting polio excretion. Several attempts at trying to clear the polio infection from the United Kingdom patient, which included oral immunoglobulin, milk IgA and the RNA mutagen ribavirin, have so far been unsuccessful.

The current poliovirus strain ITD strategy used in network laboratories is believed to be adequate to detect iVDPV strains, although more sensitive molecular tests based on nucleotide sequence differences are being developed at present.

Although there is not sufficient experimental evidence, long-term poliovirus excretion is believed to be a rare event that involves between 0.01% and 1% of B cell disorders. There is a low prevalence of these diseases particularly in children over 2 years of age in the least

developed countries. Persistent poliovirus carriage is uncommon among persons who are receiving immunoglobulin therapy and among persons with HIV infection. However minimal the risk posed by iVDPV strains might be, their mere existence emphasizes the need to interrupt the use of OPV after global eradication is achieved and to devise optimal immunization strategies to minimize the risk of long-term poliovirus excretion by vaccinees.

8.4 Environmental surveillance in the search for VDPV strains in Europe

Dr Tapani Hovi, WHO Temporary Adviser

Environmental surveillance is being used as a supplementary approach in poliovirus surveillance in some European countries. Poliovirus (PV) strains isolated from the environmental specimens are referred to the corresponding regional reference laboratory (RRL) for intratypic differentiation (ITD) just like those isolated from clinical specimens. KTL is serving as the RRL for 12 European countries (4 Scandinavian, 3 Baltic and 5 central European). For ITD of PV strains, KTL is using the EIA and RFLP of RT-PCR amplicons in the VP3-VP1-region. All strains not giving typical SL result in either test are subjected to sequencing of the VP1 coding region of the genome to reveal possible wild type nature or genetic drifting of the vaccine virus.

Because OPV is used in routine immunization in only part of the above countries, PV isolates sent for ITD do not represent all the sub-region evenly and recently, most of them have been isolated in Czech Republic, Slovakia, Latvia or Estonia. During last about 24 months the "routine" analysis of about 70 strains has revealed apparent VDPV, with more than 1% genetic drifting of the VP1 coding region, in 4 strains. None of these was associated with a clinical case of poliomyelitis.

One strain serotyped as PV3 had been isolated from a healthy child during a stool survey, and turned out to an apparent PV3/PV2 recombinant with the junction site within the VP1. Each portion of the VP1 gene was less than 0.3% different from the corresponding Sabin sequence and therefore, this strain was not finally classified as a VDPV.

A PV3 isolated from sewage in Tallinn, Estonia, in late 2002, was characterized in detail and turned out to be a vastly drifted (VP1 more than 13%) apparent Sabin 3/Sabin 1 recombinant. This type of genome has been previously found in immunodeficient (IMD) individuals. However, no IMD persons excreting PV were known in the country and could not be found. Likewise, no subsequent sewage sample was found to contain this type of drifted virus. Although the likelihood of detecting products of one (IMD) individual in a city sewage representing 0.5 million people is very small, no evidence for any circulation of this virus was obtained, and this remains the option number one.

The other two "routine" VDPV isolates are PV type 2 strains found in sewage in two different localities in Slovakia in 2003. Again, the strains are vastly drifted, about 14% at the VP1 gene, and no cases were associated with them. In subsequent intensive search in the sewage of one of the two localities (Skalica), a large number of additional VPDV strains have been found, the last one in June 2004. Phylogenetic analysis of 51 VP1 sequences indicates that all strains are related but may differ more than 5% from each other and cluster in 3 major

groups. Extending the sewage sampling upstream in the sewage network has revealed a group of houses with about 600 people which include one of the major sources of this VDPV. Preparations are being made to carry out a stool survey among these people to reveal what kind of persons are excreting the virus. From the data available, one can speculate that most of the divergence may have been generated in an IMD person and subsequently, the virus has "escaped" and is showing limited circulation.

Although no paralytic cases have been detected in either of these environmental VDPV episodes, the virus strains isolated are neurovirulent in transgenic mice, and show antigenic divergence from the vaccine. Existence of this kind of viruses in OPV immunized populations is a challenge for controlled cessation of OPV use after eradication of the wild type polioviruses.

8.5 Decentralizing laboratory testing for timely reporting

Dr Mark Pallansch, Centers for Disease Control and Prevention, United States

As previously noted, there are considerable demands on the LabNet to achieve faster turnaround of results to facilitate rapid responses to virus detection. In the final stages of the program this need is particularly acute in the few remaining endemic countries where effective planning of supplementary immunization activities targeting remaining reservoirs is a critical need. Although the LabNet has repeatedly shown its ability to improve performance within the laboratory, many of the remaining delays in the timely reporting of results are directly related to delays in the shipment of specimens or isolates. One way to improve the timeliness of wild poliovirus identification is to shorten the time for obtaining final ITD results. At the same time it is necessary to address the programme priorities for ITD results: accuracy (maximize the percentage of correct answers); timeliness (increasing importance of rapid virus detection and characterization); and efficiency (reduce the resource demands of maintaining the laboratory network).

At present, limitations on timeliness are attributable to three main process steps. These include specimen transport, laboratory processes and information flow. Technologies can be implemented to address the latter two processes, but the primary potential solution to the first problem is to reduce the number of transport steps by decentralize the testing and bringing the tests closer to the patient. In addition to the ability to improve timeliness and reduce logistic costs, the solution also reflects the existing investment in the programme by the laboratories, the investment in people by the programme, addresses the future of the laboratory as a national resource and provides for the future as a network of public health laboratories for global priorities. At the same, this approach will create new challenges such as the need to maintain quality, providing critical resources, training and accreditation. If the programme wants to increase the number of ITD laboratories, it will be necessary to establish selection criteria, determine resource requirements, provide training and redirect specimen logistics. To assure success, the process will need to be monitored closely and assessed frequently during the early implementation phases.

8.6 New technologies for current and future stages of polio eradication programme

Dr Mark Pallanch, Centers for Disease Control and Prevention, United States

With the development of the Global Polio Eradication Initiative Strategic Plan 2004–2008, the Global Polio LabNet will need to assume new and changing roles in the future surveillance system. There are several critical phases in the programme that will likely begin in the near future and progress rapidly: a) interruption of poliovirus transmission; b) global (regional) certification; c) global OPV cessation; d) continued long-term poliovirus surveillance. In addition, the plan recommends that "By end 2005, develop and implement Network strategies and procedures to reduce by 50% the time taken to provide complete laboratory results (i.e. virus detection to VP1 sequence)." This improvement in timeliness is an essential component of the detection and response strategy that will be critical in the future to correctly identify source reservoirs and target mop-ups in these focal reservoir areas.

In the future following eradication, as OPV coverage rates decline, there will be a growing risk of circulating vaccine-derived polioviruses (cVDPVs). This will put additional demands on the LabNet to achieve faster turnaround of results to facilitate a rapid response. There are several ways to address this need, including improved logistics, forward positioning of ITD and sequencing capacities, and incorporation of more rapid laboratory procedures. Faster testing may also be possible through introduction of new laboratory methods. Using existing technologies it would be possible to bypass virus isolation through direct detection from stool specimen by PCR, to bypass conventional typing by using PCR, and direct identification by genotype-specific PCR. Although these technologies are already present within the network, additional development or refinement may be necessary and quality assurance steps need to be implemented. The advantages and disadvantages of all these approaches for specific technologies will have to be considered for each laboratory and the resource requirements for the network determined before any further implementation can be considered. It is clear that these approaches will make it possible to accelerate the detection phase in support of the future detection and response strategy, to incorporate new and powerful methods into the Polio LabNet, and to set new higher performance standard for future WHO laboratory networks.

8.7 Enhanced roles and responsibilities of polio laboratories in laboratory containment of WPVs

D H. Asghar, WHO/EMRO

In most of the countries of the Region where polio laboratories exist, the polio laboratory directors are either national containment coordinators or are involved as members of containment committees/task force. Good progress has been made in implementation of phase 1 of laboratory containment of wild poliovirus; 9 countries completed the phase 1 activities, another 9 are near completion and 3 countries have submitted the report on documentation of quality of phase 1 activities. The laboratory directors should play an active role in providing assistance to containment committees, including developing laboratory lists and inventories, helping in verification visits, data management, transfer, storage and discarding the poliovirus materials, and providing feedback on developments in containment activities.

After polio eradication, only source of virus infection will be the laboratories storing wild polioviruses. Reducing risk to communities will depend primarily on minimizing the number of laboratories with poliovirus material and minimizing operational risks in the laboratories. In this regard, the laboratory directors should ensure that laboratories are working under strict BSL2/polio and later enhanced BSL3/polio (post-OPV era) and staff working with poliovirus material are fully vaccinated against polio. The wild poliovirus material must be kept under lock and key with limited access. All inventories must be kept current. Any accident in the laboratory must be reported properly and standard operating procedures must be in place to deal all such emergencies.

8.8 Keeping LABIFA database updated

Dr Hala Safwat, WHO/EMRO

The modified LABIFA database in the Region has produced additional information on the workload in laboratories, hot cases, timeliness and other issues.

In 2004 to date, laboratories in Egypt, Oman, Pakistan, Saudi Arabia and Syrian Arab Republic were the most regular in updating the database. Other regional laboratories need to enhance the rate of weekly reporting as this affects the countries they support.

Data quality has improved, especially with regard to errors in dates and missing IDCODES. Surveillance officers and laboratory staff are encouraged to coordinate in standardizing the IDCODE formats and to link both databases for further analysis.

9. SUPPLEMENTAL SURVEILLANCE FOR POLIOVIRUSES

9.1 Environmental study in Egypt

Ms Iman Al Maamoun, VACSERA, Egypt

Egypt is the only country in the Eastern Mediterranean Region that is implementing environmental surveillance as supplementary tool beside AFP surveillance on a large scale. This work is in progress in collaboration with KTL, Finland. Since its start in July 2000, the objectives of the environmental surveillance were to:

- intensify polio surveillance in high-risk areas
- identify possibility of WPV circulation not picked through AFP surveillance
- identify reservoir communities and target them in OPV campaigns to interrupt indigenous wild PV transmission
- compare the genomic sequence of detected viruses from the environment and from the confirmed cases
- help in evaluation and impact of NIDs.

Sewage samples are collected from sewage systems serving urban populations, which are suspected of sustaining the WPV circulation, and have converging sewage networks. Samples are collected by the grab method. Extraction and concentration are done according to

KTL and WHO guidelines by 2-phase separation method. The concentrates are sent to KTL and CDC for parallel testing. WHO recommended methods are followed for sample inoculation, identification and characterization. Wild polioviruses are sent to KTL and CDC for molecular sequencing. The results of the three laboratories are complementary. The result is considered positive if one of the three laboratories has isolated a wild virus.

The number of sites sampled in provinces increased from 5 in 2000 to 33 in 2004, and to date 41 samples are collected per month. In 2000, only 2 provinces (Minya and Assiut), were included in the study; out of 54 samples only 2 specimens were positive for WPV type 1.

In 2001, in Upper Egypt 132 sewage samples were collected from 9 sites in 7 provinces (Fayoum, Beni Suef, Minya, Assiut, Qena, Sohag and Aswan); out of 132 samples, 72 (57%) were positive for WPV type 1. Two samples were collected from Lower Egypt (Gharbia, Tanta city), and both were positive for WPV type 1.

In 2002, 163 sewage samples were collected from 17 sites in 15 provinces in both Upper and Lower Egypt; out of 163 samples, 26 (16%) were positive for WPV type 1. In Lower Egypt, out of 7 provinces 5 were positive for WPV type 1: Alexandria (1), Menoufia (1), Sharkia (1), Beheira (1) and Cairo (6). In Upper Egypt, out of 8 provinces 6 were positive for WPV type 1: Giza (1), Fayoum (4), Beni Suef (3), Assiut (5), Sohag (1) and Qena (2).

In 2003, 312 sewage samples were collected from 27 sites in 18 provinces in both Upper and Lower Egypt; out of 312 samples, 12 (4%) were positive for WPV type 1. In Lower Egypt, 2 out of 10 provinces were positive for WPV type 1: Cairo (3) and Sharkia (2). In Upper Egypt, 3 out of 8 provinces were WPV1 positive: Giza (1) Minya (5) and Qena (1).

In 2004, sewage sample collection sites and frequency of sampling was increased in Greater Cairo and a few sites in Lower Egypt. As of May 2004, 184 sewage samples are collected from 33 sites in 18 provinces in both Upper and Lower Egypt; out of 184 samples only one was positive for WPV type 1. Only Minya province was positive for WPV type 1. This year it was found that there was some technical problem in processing the sewage concentrate. The virological information was not truly representative of the situation. The isolation rate of both SLPV and NPEV was very high in 2001 and 2002 and until September 2003. It began to slightly decrease after that and it began to clearly decline from the beginning of 2004. The problem is being addressed and scientific investigation is in progress.

9.2 Problems in maintaining polio surveillance (AFP and non-AFP) after regional certification

Dr Tapani Hovi, WHO Temporary Adviser

Success in the elimination of indigenous poliovirus circulation contains an inherent potential danger to polio surveillance: a false sense of safety. Consequent shifting of priorities, reallocation of human and material resources, and simply tiring of zero reporting may compromise sensitivity of poliovirus surveillance after certification of a region, and already before that in countries which have been free of poliomyelitis for years. Maintaining high

sensitivity is a special challenge because of the necessary contribution of several different parties in optimal functioning of surveillance.

Successful AFP surveillance can be seen as a joint activity of at least four different parties, each of which could also be considered a potential weak point in the system after certification. 1) Clinicians and other health care and community workers remain the key persons to suspect and notify AFP patients. They require repeated reminders of the necessity to keep eyes open in spite of possible long history of absence of documented polio cases. 2) Epidemiological investigation of suspected cases and organization of stool sample collection might become a problem if the relevant resources are targeted to other important diseases. Polio surveillance should remain among the priorities until and beyond the global eradication of poliomyelitis. 3) The laboratories must continue high performance but again, this may be challenged by a possible decrease or reallocation of the necessary resources. Extending the working field outside polioviruses is a current trend but this may not be realistic for all laboratories. A national plan to guarantee continuing reliable laboratory analysis of stool samples from AFP patients after certification is desirable. 4) Analysis of collected surveillance data must be undertaken, with intervention when necessary. A detailed national plan of action for potential importation or re-emergence of poliovirus circulation should be prepared.

It has been often stated that closer to the global eradication of wild type poliovirus, supplementary surveillance activities will have relatively greater roles. It is true that with decreasing sensitivity of AFP surveillance, there will be a growing need for supplementary approaches. It should be remembered, however, that all supplementary approaches have serious limitations and can only partially counteract possible weaknesses of AFP surveillance. No non-AFP clinical case driven surveillance principle has been developed, while three possibilities exist that are targeted directly to circulating poliovirus. 1) Environmental surveillance has the advantage of rapidly providing information on populations potentially including poliovirus excretors, by examining downstream samples of a converging sewage network. However, this approach is relatively laborious and cannot be applied for populations not served by sewage networks. 2) Enterovirus surveillance means scrutinizing virus isolation results of routine virological diagnostics and analysing all poliovirus strains isolated from any clinical entity. Although this approach is useful at least in some developed countries, the samples rarely cover the entire population evenly and the clinical laboratories responsible for the primary isolation work tend to have varying sensitivity to detect poliovirus. 3) Stool surveys may reveal the persons excreting poliovirus in a suspected population but the obvious inherent workload prevents surveys on larger populations.

10. CONCLUSIONS

The polio laboratory network in the WHO Eastern Mediterranean Region has maintained its performance and continues to provide a high standard of technical support to the poliomyelitis eradication programme. All network laboratories were fully accredited except the Iraq NPL. The Iraq NPL is operational, but the accreditation visit could not be made due to security situation. Currently, stool samples are tested in parallel at VACSERA. Noteworthy is an increase in collection of adequate stool samples and timely and accurate

reporting of virological investigation. The network laboratories continue to show very good capability to detect WPVs and are reporting the results quickly to the EPI. The surveillance system and laboratories have reduced the time of reporting to a mean time of 37 days from onset paralysis. All WPV isolates are subjected to genomic sequencing and results are interpreted for planning polio eradication activities.

There is strong coordination between laboratories and national polio eradication programmes. Consistent with the Global Polio Eradication Initiative Strategic Plan, polio laboratory staff increasingly are pursuing opportunities to work with other disease surveillance programmes to continue to apply their experience to other priority public health programmes. The polio laboratory network also continues to provide guidance and technical support to achieve the goal of laboratory containment of WPVs.

WHO/EMRO is continuing to encourage collaboration of the polio laboratory network in the development of laboratory support for other priority disease surveillance programmes, while providing continuing strong support to the polio eradication programme in order to maximize the benefits of resources, strengthen national laboratory capacity and ensure sustainability.

Future challenges to the laboratory network include sustaining high level laboratory performance, logistic support and equipment maintenance, and ensuring national support to the national polio laboratories.

11. RECOMMENDATIONS

1. National authorities should ensure that the budget of the national polio laboratory is sufficient to cover the costs of sustaining the basic laboratory facility, staff, supplies and equipment maintenance.

2. Network laboratories should ensure that authenticated Sabin standard strains are used in cell sensitivity testing and other procedures. The polio laboratories should also encourage other laboratories to replace Sabin standards of unknown origin with authenticated Sabin standards, and where possible, should provide authenticated Sabin strains to these laboratories. All WPVs for which sequencing data are available should be destroyed or securely maintained in accordance with the Containment Guidelines. An inventory of all Sabin and WPVs should be kept current and a copy of the inventory provided to the Regional Polio Laboratory Coordinator.

3. A protocol should be developed by the network for implementation of authenticated Sabin standard use in all recommended procedures where live virus is used.

4. Recognizing the continued risks of WPV importation into polio free areas from the remaining polio endemic countries and the emergence of VDPVs in areas of low immunization coverage, high standards must be maintained in all aspects of surveillance and laboratory investigations to ensure the rapid detection and reporting of WPV importations and VDPVs.

5. All laboratories performing ITD tests should immediately report discordant ITD results in the recommended format to the Regional Polio Laboratory Coordinator. This information is further disseminated to WHO/HQ and sequencing laboratories for systematic collection of complete data on isolates eventually designated as VDPV and those with between 0.5% and 1.0% sequence divergence in VP1.

6. All untypeable L20B positive isolates should be reported immediately to the regional polio laboratory coordinator and referred to a global specialized laboratory (GSL) for identification. WHO/EMRO should provide assistance to laboratories for shipment of such viruses to GSL.

7. Polio laboratories should ensure that all laboratory practices are performed under strict BSL2/polio as described in the WHO Global Action Plan for Laboratory Containment of WPVs, and assure that all laboratory staff are fully immunized against polio according to the national policy.

8. Laboratories should ensure the complete entry and revision of data for all variables in the LABIFA system. The data analysis option in LABIFA should be used to monitor performance indicators. All laboratories should produce a weekly line list of cases received and also of the work in progress and notify EPI of significant items to take necessary action, such as fast growing viruses, missing EPID no or dates.

9. Laboratories should update their standard operating procedures (SOPs) in accordance with the revised 2003 laboratory manual in the working language.

10. The environmental surveillance activities in Egypt should be continued and efforts should be made to restore the sensitivity of virus isolation to its past quality.

11. Over-use of the "hot case" designation can cause undue disruption to the smooth running of the laboratory. Laboratories should work with the national EPI team to refine the effective use of this designation.

Annex 1

PROGRAMME

Monday 26 July 2004

08:00–08:30	Registration
08:30–09:30	Opening session - Address by H.E. Dr Mohamed Cheikh Biadillah, Minister of Health, Morocco - Message from Dr Hussein A. Gezairy, WHO Regional Director for the Eastern Mediterranean Region - Election of the Chairman and Rapporteur Implementation status of the recommendation of the seventh intercountry meeting of directors of poliovirus laboratories/ Dr H. Asghar
Session 1	*Overview*
09:30–09:50	Global status of polio eradication initiative/ Dr E. de Gourville
09:50–10:10	Regional status of polio eradication initiative/ Dr F. Kamel
10:10–10:30	Regional status of polio laboratories network/Dr H. Asghar
10:30–11:30	Discussion
Session 2	*Virus surveillance and molecular epidemiology in endemic countries*
11:30–11:45	Molecular epidemiology of polio endemic countries in EMR/ Dr O. Kew
11:45–12:00	Egypt, virus surveillance/Dr E. El Maamoun
12:00–12:15	Pakistan, virus surveillance/ Mr S. Zaidi
12:15–12:30	Somalia and south Sudan virus surveillance/Mr P. King'ori
12:30–14:00	Discussion
Session 3	*Laboratories experience in dealing with specific problems*
14:00–14:10	Egypt RRL, decline in NPEV/SLPV in environmental sampling/Dr L. Bassioni
14:10–14:20	Iran NPL, Maintaining sensitive cell culture/Dr H. Tabatabai
14:20–14:30	Iraq NPL, Re-establishment of polio laboratory/Dr F. Al Hamdani
14:30–14:45	Jordan NPL, Meeting accreditation standard performance/ Dr N. Al Najar
14:45–15:30	Kuwait RRL, Implementing the QA programme/Dr S. AL-Mufti
15:30–15:45	Morocco NPL – Molecular sequencing of old stocks of polioviruses/ Ms Hayat Caidi
15:45–16:00	Oman NPL – Establishment of ITD testing capabilities/Dr S. Al-Busaidy
16:00–16:30	Discussion

Tuesday 27 July 2004

Session 4	*Quality assurance*
09:00–9:20	Accreditation status of regional polio laboratories/Dr H. Asghar
09:20–09:40	Evaluation of cell sensitivity for poliovirus infection/Dr J. Martin
09:40–10:00	Responding to increased laboratory workload to produce certification standard quality virological investigation results/Dr H.van der Avoort
10:00–11:00	Discussion

11:00–12:30	Group discussion: Quality assurance and performance indicators Group 1 – NPLs. Moderators: Dr E. De Gourville, Dr.T. Hovi, Dr. J Martin. Dr. H. Asghar Group 2 – RRLs, NPLs (Iran, Oman). Moderators: Dr M. Pallansch, Dr O. Kew, Dr H. van der Avoort
12:30–14:00	Group presentation and discussion
Session 5	*Challenges to polio eradication programme*
14:00–14:20	Risk of decline in AFP surveillance activities/Dr F. Kamel
14:20–14:40	Long-term poliovirus excretion by immuno-deficient individuals – The UK experience/Dr J. Martin
14:40–15:30	Acceleration of OPV cessation/Dr H. van der Avoort
15:30–15:45	Decentralizing laboratory testing for timely reporting/Dr M. Pallansch
15:45–16:00	New technologies for current and future stages of polio eradication programme/ Dr M. Pallansch
16:00–16:20	Enhanced role and responsibilities of polio laboratories in laboratory containment of wild polioviruses/ Dr H. Asghar
16:20–16:40	Keeping LABIFA database updated/Dr H. Safwat
16:40–17:00	Discussion

Wednesday 28 July 2004

Session 6	*Supplemental surveillance for polioviruses*
09:00–09:20	Environmental study in Egypt/Dr E. Al Maamoun
09:20–09:40	Strategies for maintaining polio surveillance (AFP and Non-AFP) post-regional certification/Dr T. Hovi
09:40–10:30	Discussion
10:30–11:30	Open discussion on remaining issues
11:30–13:00	Closing session Discussion on conclusions and recommendations

Annex 2

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