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Report on the

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

Kuwait City, Kuwait
11–13 June 2005

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1. INTRODUCTION

The ninth intercountry meeting of directors of poliovirus laboratories in the WHO Eastern Mediterranean Region was held in Kuwait City, Kuwait from 11 to 13 June 2004. Directors of poliovirus 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 (EMRO).

Dr Faten Kamel, Medical Officer, WHO/EMRO, 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 Kuwait and Dr Ali Al Seif, Assistant Under-Secretary for Community Health and Environmental Affairs, for hosting the meeting. He expressed appreciation for the commitment and dedication with which the poliovirus laboratory network scientists had worked to provide timely and high quality virological investigation results, especially during wild poliovirus importations and outbreaks in Sudan and Yemen. He noted the efforts of laboratories to deal with the increases in workload due to sudden wild poliovirus outbreaks in Sudan and Yemen, and expressed appreciation for the support of CDC, RIVM and the poliomyelitis unit at the Regional Office for provision of logistic support to meet the extra workload. He highlighted the importance of coordination between EPI and poliovirus laboratories for accurate and timely action in the field to ensure early diagnosis and to limit the spread of outbreaks.

Dr Ali Al Saif, Assistant Under-Secretary for Community Health and Environmental Affairs, welcomed all the participants and highlighted the progress achieved in Kuwait in the fields of EPI and the poliomyelitis eradication initiative. He stressed his appreciation for the role of poliovirus laboratories in poliomyelitis eradication.

The chairmanship was shared on a rotating basis. Elected chairpersons were Dr Siham Al-Mufti (Kuwait), Dr Hinda Triki (Tunisia) and Dr Suleiman Al Bussaidy (Oman). The programme and list of participants are included as Annexes 1 and 2, respectively.

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

Dr Humayun Asghar, WHO/EMRO

Dr Humayun Asghar, Regional Laboratory Coordinator, reviewed the implementation and main achievements of the recommendations of the eighth intercountry meeting of directors of poliovirus laboratories in the Region.

No.	Recommendation	Implementation status
1.	National authorities should ensure that the budget of the poliovirus laboratory is sufficient to cover the costs of sustaining the basic laboratory facility, the staff and supplies and the maintenance of equipment.	None of the countries allocated a specific budget for their national poliovirus laboratory; however, WHO continues to support the laboratories and no shortage of supplies has occurred.
2.	Network laboratories should ensure that authenticated Sabin standard strains are used in cell sensitivity testing and other procedures. The poliovirus laboratories should also encourage other laboratories to replace Sabin standards of unknown origin with authenticated Sabin standards, and where possible, to provide authenticated Sabin strains to these laboratories. All wild polioviruses (WPVs) for which sequencing data are available should be destroyed or securely maintained in accordance with the containment guidelines. Inventory of all Sabin and wild polioviruses should be kept current and a copy of the inventory provided to the regional poliovirus laboratory coordinator.	All the network laboratories established the cell sensitivity testing with authenticated Sabin standard strains. All wild polioviruses for which sequencing data are available are destroyed under strict biosafety. The Tunisian regional reference laboratory (RRL) is still storing wild poliovirus and is planning to share the sequencing data with RIVM and later destroy these viruses. The inventory of all polioviruses is maintained and kept current.
3.	Protocol should be developed by network for implementation of authenticated Sabin standard use in all recommended procedures where live virus is used.	The protocol was finalized and is part of the fourth edition of the poliovirus laboratory manual 2004, which has been distributed to all network poliovirus laboratories.
4.	Recognizing the continued risks of wild poliovirus importation into polio-free areas from the remaining polio-endemic countries and the emergence of vaccine-derived polioviruses (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 wild poliovirus importations and vaccine-derived polioviruses.	The network laboratories are working hard to maintain laboratory performance indicators and sensitivity to detect any wild poliovirus importation or VDPV. Timely reporting of importations in Sudan, Yemen and Saudi Arabia were the proof of their efficiency. Dealing with high workload due to unforeseen outbreaks in Sudan and Yemen also showed the commitment and capacity to deal with the huge workload.
5.	All laboratories performing ITD tests should immediately report discordant ITD results in the recommended format to the Regional Poliovirus 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.	All laboratories are aware of the importance of reporting of intratypic differentiation (ITD) test discordant results.

No.	Recommendation	Implementation status
6.	All untypeable L20B positive isolates should be reported immediately to the Regional Poliovirus 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.	None was reported by any of the laboratories; however, all laboratories are aware of importance of sending such isolates to GSL.
7.	Poliovirus 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 wild polioviruses, and assure that all laboratory staff are fully immunized against polio according to national policy.	Strict BSL2/polio is implemented in all network poliovirus laboratories; however, some of the staff in the laboratories were not immunized against polio. Accordingly, they were followed up for vaccination according to national immunization 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 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.	New LABIFA software (Version 3) was installed in all network laboratories. Laboratories started using the Analysis option to monitor their performance indicators; however, its utility should be formalized as regular feature.
9.	Laboratories should update their standard operating procedures (SOPs) in accordance with the revised 2003 laboratory manual in the working language.	An outline of the standard operating procedures was provided to the laboratories and laboratories are in process of updating their standard operating procedures in light the fourth edition of the poliovirus laboratory manual 2004.
10.	Environmental surveillance activities in Egypt should be continued and efforts should be continued to restore the sensitivity of virus isolation to its past quality.	Environmental surveillance in Egypt continued to provide important information to supplement the acute flaccid paralysis (AFP) surveillance. The decline in its sensitivity was restored after changing the chloroform stabilized with alcohol.
11.	Overuse of "hot cases" designation can cause undue disruption to the smooth running of the laboratory. Laboratories should work with the country EPI team to refine the effective use of this designation.	In some countries large numbers of stool samples from 'hot cases' were collected, despite alarming the EPI that it may affect smooth functioning of the laboratory. All laboratories continued to provide timely results even after this increase in stool collection. The 'hot case' designation needs to be given due consideration to avoid waste of logistics and time.

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 by 2000. Effective strategies were designed (sensitive surveillance, routine and mass

immunization approaches), and aggressively implemented by polio-endemic countries. The impact on transmission was dramatic. Between 1988 and 2003, the incidence of poliomyelitis decreased from 350 000 estimated cases to 784 reported cases. During this period, the number of polio-endemic countries decreased from more than 125 to 6 (Nigeria, Niger, India, Afghanistan, Egypt and Pakistan). Towards the end of 2003, a huge outbreak occurred in Nigeria, which resulted in the importation of wild poliovirus into 16 countries in west, central and east Africa, the Arabian peninsula (Saudi Arabia, Yemen), and East Asia (Indonesia). Among these areas, six countries (Mali, Côte d'Ivoire, Burkina Faso, Sudan, Chad and Central African Republic) have re-established transmission (circulation for > 6 months). By the end of 2004, 1266 poliomyelitis cases were reported from 20 countries, out of which six countries were endemic and 14 countries were with importation. In 2005, the number of cases appears to be on the decline, and 448 poliomyelitis cases were reported from 10 countries, from which six countries were the same endemic countries, while three countries (Ethiopia, Indonesia and Yemen) were experiencing an outbreak due to importation. Factors contributing to the wild poliovirus spread included low oral poliovaccine (OPV) coverage (several countries), population movement, civil unrest and/or displaced populations, difficult access to populations, insufficient resources or commitment to implementing high quality poliomyelitis eradication activities.

In response, several programmatic actions were taken to interrupt wild virus transmission. In January 2004, WHO headquarters hosted a ministerial meeting of remaining endemic countries and it was agreed that there should be a massive scale-up of supplementary immunization activities during the low season in the first six months of 2004, targeting 250 million children. In Kano, Nigeria, poliovirus immunization started after a 12-month suspension. The Regional Office for Africa expanded synchronized NIDs to 23 countries from October to November 2004. To further accelerate eradication, WHO, manufacturers, and regulatory authorities collaborated to make monovalent type 1 OPV (mOPV1) available as a supplementary tool to eliminate type 1 polioviruses in 2005.

As of June 2005, the impact of intensification of activities was evident as the absolute number of cases declined in Afghanistan, Pakistan and India, while in the African Region the number of cases is fewer as compared with 2004 and a large number of countries that reported importation in 2004 did not report cases in 2005. The situation in Egypt is encouraging, as for more than 12 months no wild poliovirus has been isolated from AFP cases. The last sewage sample was positive for wild poliovirus in early 2005. The genetic diversity of viruses has also decreased. There was a marked improvement in surveillance activities and number of supplementary immunization activities was increased.

Surveillance performance indicators, such as non-polio AFP rate and adequacy of stool collection were improved as compared with the same period in 2004, however, there is room for improvement. The adequate stool collection rate (surveillance indicator) in the Americas, western Europe, central Africa, western Africa and Australia is not up to standard. In 2004, some wild polioviruses were isolated after a gap of many years. The Khartoum wild poliovirus type 1 (WPV1) and West Kordofan wild poliovirus type 3 (WPV3) viruses isolated in 2004 were related to indigenous wild polioviruses (WPVs) circulating in 1999. The WPV3

isolated in Chad was related to a virus from 1996. The genetic data showed that these viruses were missed for some time as a result of surveillance gaps.

There was an overall increase in the laboratories' workload of about 20% in 2004, as compared to 2005. In some of the individual laboratories there was more than a 100% increase in the workload. The global network met the challenge of timely provision of results. Ninety seven percent (97%) of laboratories were accredited globally.

Good progress has been made in Asia and Egypt, although there are still substantial challenges facing Africa. There is a funding gap of US$ 50 million for 2005 and US$ 200 million for 2006.

3.2 Regional status of the poliomyelitis eradication initiative

Dr Faten Kamel, WHO/EMRO

Progress towards interruption of poliovirus transmission continued in the Eastern Mediterranean Region. Seventeen (17) countries have maintained their polio-free status for more than 3 years. The three endemic countries (Pakistan, Afghanistan and Egypt) in the Region have shown a decrease in the intensity of virus transmission and in its geographical extent. Intensification and quality supplementary immunization activities are the highest priority in endemic countries to ensure that each and every child under 5 years of age is vaccinated during immunization campaigns. Special efforts are made to avoid serious immunity gaps among children under 5 years in the polio-free countries. Therefore, supplementary immunization activities were planned for polio-free countries, in particular, those with low routine immunization coverage including Djibouti, Somalia and Yemen. Focus in these countries was on the identification of priority districts and areas to ensure highest quality technical support in these areas. Preparedness for importation in polio-free countries is a regional priority; efforts are continuing to improve and sustain certification standards surveillance and maintain high population immunity through routine and supplementary immunization activities.

Supplementary immunization activities were further intensified in endemic countries, each of them conducted more than six rounds of national immunization days (NIDs) and subnational immunization days (SNIDs). In addition, the strategy of mop-up activity in response to virus isolation was adopted taking into consideration epidemiologic developments and planned supplementary immunization activities.

During 2004, AFP surveillance was maintained at a high level of quality. The non-polio AFP rate was 2.68 at regional level and exceeded 1 per 100 000 population under 15 years in all countries except Bahrain, Djibouti and United Arab Emirates, due to the small number of expected AFP cases, and Palestine, with its difficult security situation. The percentage of AFP cases with adequate stool collection was also maintained at 89.2% in 2004 and in all countries except Djibouti (only 2 cases) and Jordan (77%). As of June 2005, the non-polio AFP rate was 3.22, and adequacy of stool collection was 89%. In 2004, surveillance reviews were conducted in Egypt, in the four provinces of Pakistan and in some parts of Afghanistan. In addition, reviews were conducted in polio-free countries (Lebanon, Libyan Arab Jamahiriya,

Syrian Arab Republic and Tunisia). Surveillance reviews are planned in 2005 for other countries in the Region.

The number of poliomyelitis cases in the Region decreased from an annual estimated 35 000 cases in 1988 to only 187 during 2004. This number is higher than 113 cases in 2003, this increase occurred due to wild poliovirus type 1 (WPV1) outbreak in Sudan (127 cases in 2004). The number of cases in the 3 endemic countries in 2004 decreased to about 50% than 2003 [Afghanistan (4), Egypt (1), Pakistan (53), Sudan (127)]. In Sudan, this epidemic started as importation from Nigeria via Chad into west Darfur in May 2004. The outbreak spread to 18 states in north and south Sudan and 127 cases had been detected by the end of 2004. The rapid spread of poliomyelitis in Sudan was facilitated by low population immunity, population movement and accumulation of large numbers of susceptible people due to suboptimal routine immunization activities, as well as the cessation of supplementary immunization activities since late 2002. Two imported cases were reported in Saudi Arabia and these were related to the Sudan virus. As of June 2005, 254 poliomyelitis cases were reported, out of which 220 cases were reported from Yemen. The outbreak in Yemen started as an importation from Sudan and infected 17 governorates of Yemen. As of June 2005, there has been further decrease in the number of poliomyelitis cases in endemic countries [Afghanistan (2), Egypt (0), Pakistan (7), and Sudan (25)]. Genomic sequencing results show a decline in the genetic diversity of the viruses isolated from Afghanistan, Pakistan and Egypt.

Technical support was provided through more than a 100 international and 800 national poliomyelitis eradication officers recruited by the WHO Regional Office and concentrated mainly in priority countries. These staff also support other programmes and in some places in south Sudan and Somalia they are the only form of health infrastructure currently operating.

The laboratory survey and inventory phase 1 of laboratory containment of wild polioviruses is in progress. As of June 2004, 9 of the 18 polio-free countries have reported completion of phase 1 containment activities. In addition, six countries are in the final stages with completion expected soon and three are in the process of writing their national plans to be implemented in 2005. A quality assurance tool was developed to document the quality of the first phase of the containment plan and countries are requested to perform self assessment of their activities.

The Regional Commission for Certification of Poliomyelitis Eradication continues its efforts in monitoring the process of poliomyelitis eradication and reviewing documentation submitted by the National Certification Committees. The Regional Certification Commission met twice in 2004 and reviewed the basic documentation from Somalia and Palestine to complete with them the list of polio-free countries. Annual updates were received from all the countries whose basic documentation was accepted before and preliminary reports were submitted by Afghanistan, Pakistan and Egypt.

The regional and country specific poliomyelitis eradication programmes are closely monitored by technical bodies. Technical advisory groups of endemic countries meet regularly and frequently to review the epidemiological situation and planned activities and to advise on the strategic directions of the programmes.

It is crucial to sustain political commitment at all levels in these countries and ensure that this commitment is translated into accountability and improved performance at grass root level. Advocacy efforts for poliomyelitis eradication in the Region are continuing. The Regional Director visited Pakistan and met with President General Pervez Musharraf, who promised to personally oversee the final push to eradicate poliomyelitis from Pakistan. Meetings were also held with Governors, Chief Ministers, Ministers and other senior officials and leaders in different provinces. The issue of poliomyelitis eradication was also discussed in the Organization of the Islamic Conference meeting whose 52 members include the remaining endemic countries in the Region.

The priority countries from the Region met again in early 2005 in the ministerial meetings held in Geneva as a follow up to last year's meeting. Ministers of health and their delegations presented the progress made in their countries and discussed the planned activities. They also reaffirmed their governments' commitment to achieving the target in 2005.

3.3 Regional status of the poliovirus laboratory network

Dr Humayun Asghar, WHO/EMRO

The performance of the WHO Eastern Mediterranean regional poliovirus laboratory network is sustained at certification standard indicators. The network laboratories supported poliomyelitis eradication activities in a timely manner. All except the Iraq national poliovirus laboratory, which could not be visited due to the security situation, were fully accredited. All the laboratories passed the WHO proficiency test (PT) panel of unknown viruses for both primary virus culture and intratypic differentiation (ITD) testing. There was a generalized increase in the workload due to improvements in AFP surveillance and two major epidemics in Sudan (2004) and Yemen (2005). Another factor in the increased workload was the collection of stool samples from contacts of cases and hot cases in Pakistan and Egypt.

All laboratory performance indicators are well above the set targets, except transportation of samples within 3 days, which was just 66%. As of June 2005, the timing of results from onset paralysis to final ITD testing improved from 40 days in 2004 to 33 days in 2005. The timing of ITD results within 14 days after serotyping improved from 88% in 2004 to 100% in 2005. The results of stool samples after receipt in laboratories to final ITD result was improved from 28 days in 2004 to 21 days in 2005. This high quality and rapid diagnosis was extremely helpful in dealing with critical situations during the Sudan and Yemen outbreaks, and importation into Saudi Arabia.

In 2004, 187 wild poliovirus cases were reported from countries of the Region: Afghanistan (4), Egypt (1), Pakistan (53), Sudan (127), Saudi Arabia (2 imported cases). As of June 2005, 254 cases due to wild polioviruses have been reported: Afghanistan (2), Pakistan (7), Sudan (25) and Yemen (220).

In Egypt, AFP surveillance is supplemented with environmental surveillance to increase sensitivity for detection of wild poliovirus. The percentage of environmental sites which tested positive for polioviruses decreased from 57% in 2001, 16% in 2002, 4% in 2003, 2.7%

in 2004, to 1.3% as of June 2005. The last wild poliovirus type 1 was isolated from Fayoum and Sohag in January 2005. The frequency and number of sewage sample collection sites were 41 samples per month from 33 sites, respectively. In Greater Cairo, a total of 13 sites are sampled each month. The decline in sensitivity of isolation of polioviruses and non-poliovirus enterovirus (NPEV) from sewage samples from September 2003 until early 2004 was resolved. After a long struggle it was found that chloroform used in the processing of sewage samples had not been stabilized with alcohol, which resulted in the loss of the virus.

The RRL in Pakistan established a sequencing laboratory with the support of WHO and Rotary International and has begun the sequencing of polioviruses. This is a major landmark and has set an example for self-reliance and the transfer of technology through the poliomyelitis eradication programme. The national poliovirus laboratory in Oman was upgraded to perform ITD testing of polioviruses and has successfully established all techniques and is now performing ITD testing independently.

The poliovirus laboratory network is faced with the challenge of sustaining the laboratories' performance, maintaining the quality assurance programme, and the specific budget allocation for poliovirus laboratories and with the provision of logistics.

4. VIRUS SURVEILLANCE AND MOLECULAR EPIDEMIOLOGY IN ENDEMIC COUNTRIES

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

Dr Olen Kew, Centers for Disease Control and Prevention (CDC), Atlanta

Analysis of VP1 sequences of wild poliovirus isolates obtained in the region and worldwide has shown:

- declining nucleotide diversity of wild poliovirus isolates in Pakistan and Afghanistan, India (in the WHO South-East Asia Region), and Egypt;

- spread of wild poliovirus type 1 from northern Nigeria to Chad to Sudan to Yemen and Saudi Arabia (and from Saudi Arabia to Indonesia);

- detection in 2004 of orphan lineages in Sudan (type 1 and type 3) and neighbouring Chad (type 3).

The sequence data indicate very high proficiency in several regional laboratories, including the national laboratories in Sudan and Oman, and the RRL in Egypt and Pakistan. This proficiency was maintained despite the very high workload experienced by these laboratories as they processed large numbers of specimens from the poliomyelitis outbreaks in Sudan and Yemen. VP1 sequences of polioviruses reisolated at CDC from the referred stool specimens matched those of the isolates originally obtained by the national laboratories. The recent sequence data show the:

- existence of a persistent reservoir for wild poliovirus type 1 in southern Punjab;
- continued circulation of wild poliovirus type 3 in southern Afghanistan;
- presence of two separate wild poliovirus type 1 reservoirs in Egypt (Minya–Assiut and Greater Cairo);
- explosive spread of wild poliovirus type 1 from Chad to Darfur to Khartoum to Port Sudan;
- separate importations of wild poliovirus type 1 from Sudan into Saudi Arabia;
- importations of wild poliovirus type 1 from Sudan into Hodeida, Yemen, with a subsequent explosive spread;
- importation of wild poliovirus type 1 from Mecca to Western Java, Indonesia, followed by an outbreak, which has remained largely localized.

The Sudan orphan lineages signal wild poliovirus type 1 and poliovirus type 3 circulation that continued undetected for at least 5 years. The recent isolates are related to polioviruses previously found in Sudan (PV3) and central Africa (PV1), but the immediate source reservoirs are unknown. Another wild poliovirus type 3 orphan lineage was found in areas of Chad bordering Sudan. These findings indicate that severe surveillance gaps occurred in areas around (and possibly within) Sudan in recent years. Sequence data from India document the steady progress toward eliminating the last reservoirs of WPV1 and WPV3 in the states of Uttar Pradesh and Bihar.

A type 2 VDPV (1.5% VP1 divergence; Sab2/Sab3 recombinant) was isolated from an 11-month old boy from Riyadh, Saudi Arabia, who contracted poliomyelitis in February 2005. The isolate had genetic properties consistent with an iVDPV, and the child died of septic shock from a bacterial infection, possibly indicating an underlying immunodeficiency.

4.2 Egypt: virus surveillance

Dr Iman Al Maamoun, VACSERA, Egypt

The VACSERA RRL supported poliomyelitis eradication activities in six countries in the Region. There was a large increase in workload due to improvements in AFP surveillance and also due to sudden outbreaks in Sudan and Yemen. The RRL was fully accredited and scored 100% in PT panel for both primary culture and ITD. In 2004, the RRL processed 1799 stool samples from 768 AFP cases, 142 hot cases and 269 contacts. The laboratory performance indicators were sustained at a high standard; 100% of stool sample results reported within 28 days, 100% ITD results within 14 days, non-polio enterovirus (NPEV) rate 20%. As of May 2005, 566 stool samples were processed from 216 AFP cases, 53 hot cases and 131 contacts.

In 2004, one WPV 1 was isolated from a case and its two contacts in Assiyut–Dairut, Egypt, which was closely related to environmental isolates from Qena and Cairo. One contact of virus negative clinical case from Fayoum was also positive for WPV1, and was related to environmental isolates from Fayoum, Giza and Cairo.

The RRL tested stool samples and isolates from other countries (Iraq, Lebanon, Saudi Arabia, Syrian Arab Republic, Sudan and Yemen). Since May 2003, all AFP cases stool

samples from Iraq are tested at VACSERA. In 2004, a total of 718 stool samples and 256 isolates were referred for testing, out of which wild poliovirus type 1 was isolated from Saudi Arabia, Sudan and Yemen. In 2005, a total of 506 stool sample and 66 isolates were referred for testing, from these wild poliovirus type 1 was isolated from Sudan and Yemen.

4.3 Pakistan: virus surveillance

Mr Sohail Zahoor Zaidi, National Institute of Health, Pakistan

The RRL Pakistan processed stools collected from 3258 AFP cases and contacts during 2004; as of June 2005, the RRL processed specimens from 2605 cases. Transportation time also improved; 95% of specimens were received in laboratories in less than 72 hours and NPEV rate was 22%.

In 2004, 53 wild polioviruses were isolated from 31 districts (47 WPV1 and 7 WPV3). As of June 2005, 8 WPV1 were isolated from 7 districts, and no WPV3 has been isolated to date. From Afghanistan, two WPV1 and two WPV3 were isolated in 2004; in 2005, only two wild polioviruses, bothWPV3, have been isolated.

RRL Pakistan has achieved a technological landmark by sequencing two recent wild viruses from Pakistan and one recent case from Afghanistan. In future the laboratory will start poliovirus sequencing in parallel with CDC, Atlanta, as the routine test.

4.4 Sudan: virus surveillance

Mr Hatim Babiker, National Poliovirus Laboratory, Sudan

In the period from May 2004 to May 2005, the total number of samples received and investigated was 1426, collected from 582 AFP cases and 218 AFP contacts. During this period the laboratory dealt with an explosive WPV1 outbreak in Sudan, in which 318 WPV1 and 2 WPV3 were isolated. The national poliovirus laboratory performed all its activities well, despite the increased workload, and timeliness and accuracy of results were not compromised.

4.5 Somalia and south Sudan: virus surveillance

Mr Peter Borous, Kenya Medical Research Institute, Kenya

The national poliovirus laboratory based at the Kenya Medical Research Institute (KEMRI), Nairobi, supports poliovirus surveillance in Somalia and south Sudan. The laboratory serves Kenya, Somalia, south Sudan, Eritrea and Djibouti. The laboratory had scored 100% in the PT panel since 2001, but in 2004 the results from the first PT panel were unsatisfactory due to contamination. The laboratory will be doing a repeat PT panel after reviving the new cells and adjusting the laboratory practices to avoid future contamination.

In 2004, KEMRI tested 190 stool samples of 95 AFP cases reported from Somalia and 224 of 112 AFP cases from south Sudan. No wild poliovirus was isolated. In 2004, 86 stool samples of 43 AFP cases from Somalia and 64 of 32 AFP cases from south Sudan were tested in the laboratory. In south Sudan, WPV1 was isolated from six cases and WPV3 from two

cases. In 2005, again WPV 1 was isolated from four cases in south Sudan. The NPEV rate decreased both in Somalia and south Sudan as compared with the previous year. Stool samples from Somalia and south Sudan do not generally reach the laboratory within three days (due to delayed flights), resulting in the general lowering of the overall 3-day indicator.

5. LABORATORY EXPERIENCES IN DEALING WITH SPECIFIC PROBLEMS

5.1 Egypt RRL: increased workload and logistics demand

Dr Laila El Bassiouni, VACSERA, Egypt

The workload increased due to a variety of reasons: a general improvement in AFP surveillance, collection of samples from contacts and hot cases, parallel testing of Iraq AFP samples, primary culture of Yemen outbreak samples, ITD testing of Sudan and Saudi Arabia positive isolates. During same period of time, the number of environmental sampling sites and frequency of sampling was also increased. VACSERA was testing each environmental sample using 2 L20B and 1 RD flask, but after satisfactory performance the National Public Health Institute advised them to use 4 L20B and 2 RD flasks for inoculation of each sewage sample.

The increase in workload led to increased demand for logistics, personnel and time, to handle the volume of samples. Due to this unforeseen increase in workload, difficulties arose in maintaining the timely reporting of results. Delays in supplies also added to difficulties, but all these were overcome by the support from WHO, by provision of supplies through local purchase and expediting the supplies through suppliers. The staff devoted more time to meet the results deadline.

The most important lesson learnt is that there should be consultation between the laboratory and EPI before instituting any strategy, and calculation of logistics should be rationalized in accordance with the expected workload.

5.2 Kuwait RRL: implementing the quality assurance programme in RRL Kuwait

Dr Siham Al Mufti, Regional Reference Laboratory, Kuwait

The quality assurance programme was implemented in a systematic way in accordance with the WHO poliovirus laboratory manual. Quality checks were made on the equipment and reagents and documentation was made mandatory for all laboratory procedures. In tissue culture laboratory, scrupulous attention was paid to work with more sensitive cells. ITD testing was carefully monitored through the use of proper controls. Full implementation of quality assurance led to improvements in laboratory performance.

5.3 Oman NPL: report of the Yemen outbreak and handling cell culture, demands of network laboratories providing fresh and sensitive cells

Dr Sulieman Al-Busaidy, National Poliovirus Laboratory, Oman

The Yemen outbreak response was a well-coordinated effort involving many stakeholders and the remarkable spirit of teamwork of national poliovirus laboratory (NPL) staff, VACSERA, CDC and WHO. The Oman national poliovirus laboratory and CDC

provided timely results of WPV isolation and sequencing, respectively. After the reported cluster of AFP cases, it took 19 days from the time the sample was received in the laboratory to final sequencing; this included the time taken for the transportation of the sample from Oman to VACSERA, Egypt and CDC. This creates confidence that the poliovirus laboratory network has the capacity to react quickly in crises situations, such as during outbreaks or importations. The impact of this outbreak on the laboratory was the depletion of stocks of reagents and consumables, as a large number of samples were processed. However, all supplies were replenished quickly.

The regional poliovirus laboratories were provided with fresh sensitive cell lines. The cell bank is maintained under strict quality assurance and cells are stored in liquid nitrogen tanks and in ultra freezers.

5.4 Pakistan RRL: keeping balance between demands of AFP surveillance and laboratory performance

Mr A. Naeem, Regional Reference Laboratory, Pakistan

Reasons for increased demand were due mainly to the increase in collection of the number of AFP cases, contacts associated with the hot cases and AFP excluded cases. In addition, the Yemen outbreak led to a dramatic increase in the number of cases. The RRL responded well to this situation. The extra need for supplies was met from other sources and the WHO Regional Office. This enabled the laboratory to maintain timeliness and accuracy of results. Utilization of reagents for ITD testing increased following more positive cases in the Yemen outbreak; however, future needs were communicated to the Regional Office and headquarters in plenty of time to avoid any shortage of reagents.

Storage of stool specimens was a problem faced by RRL, due to the large number of samples and the shortage of deep freezers. This problem has been resolved by procuring more deep freezers.

5.5 Saudi Arabia NPL: why the NPEV rate is low

Ms Moghram Al Amri, National Poliovirus Laboratory, Saudi Arabia

The national poliovirus laboratory in Saudi Arabia is fully accredited by WHO. The NPL is following the procedure in accordance with the WHO poliovirus laboratory manual. Quality assurance is fully implemented to ensure that no virus isolation is missed. Regular cell sensitivity testing is performed with WHO Sabin standards, and satisfactory titres of laboratory quality control are observed on both RD and L20B cells. The continuous low non-polio enterovirus (NPEV) rate is a major concern to the national poliovirus laboratory and all efforts are made to use sensitive cells, good quality cell growth media, correct processing of stool samples, and proper temperature of incubators. It was noted that there was some delay in the transportation of stool samples from the field to the laboratory. This was discussed with EPI to help avoid future such delays and to ensure maintenance of reverse cold chain during storage and transportation of samples, after collection from the field.

5.6 Syrian Arab Republic NPL: use of sensitive cells and effect on virus isolation

Ms Hala Saba, National Poliovirus Laboratory, Syrian Arab Republic

In 2002, a significant decline was noted in the NPEV rate, and it was also noted that no poliovirus was isolated in 2002–2003. Both EPI and the laboratory worked together so as to rule out the possibility of problems in cold chain and sensitivity of cell lines. There was no problem in the PT panel and the laboratory was obtaining high scores. Old cell stocks were destroyed and were replaced with more sensitive cells, and it was also ensured that cells were replaced after 15 passages. Cell sensitivity testing was established and monitored. After all these changes, the NPEV and poliovirus isolation rate has increased.

5.7 Sudan national poliovirus laboratory, isolation and identification of wild polioviruses after four years of silence

Ms Rehab Alaib, National Poliovirus Laboratory, Sudan

In Sudan, the last wild poliovirus was isolated in 2000. From this time up to May 2004, more than 1600 stool samples were processed and no wild virus was isolated, although Sabin-like viruses were isolated and confirmed by VACSERA RRL. During this period some of the stool samples were sent to VACSERA for cross-checking, and showed 100% concurrence of results with the Sudan national poliovirus laboratory.

In May 2004 a fast growth of virus on cell culture was noted in L20B and RD. The EPI and WHO were informed immediately, and laboratory procedures were expedited. Upon the advice of WHO, the stools and isolates were sent to VACSERA for immediate ITD testing. The ITD results and genomic sequencing confirmed a wild poliovirus outbreak. Following this, a total of 284 WPV1 and 2 WPV3 were isolated.

The promptness with which the results were obtained was a result of close coordination with EPI, regional and global specialized laboratories, and WHO.

5.8 Tunisia RRL, dealing with referred samples from national poliovirus laboratories: isolation, identification and ITD

Dr Hinda Triki, Regional Reference Laboratory, Tunisia

The RRL Tunisia works with countries in the an Region for primary investigation of AFP cases from the Libyan Arab Jamahiriya, double-check of AFP and contact specimens from Jordan and ITD of poliovirus strains isolated in the RRL in Tunis (from Tunisia, the Libyan Arab Jamahiriya and Jordan) or referred from other national poliovirus laboratories mainly from Morocco. The main concern with the Libyan Arab Jamahiriya was the low enterovirus isolation rate due to long delays in transportation of specimen to the laboratory: from 1996 to 2000, the length of time for specimen transportation ranged from 1 to 210 days (24 days average) and no NPEV was isolated. The time for specimen transportation improved gradually to reach an average of 6 days and 2 days in 2004 and 2005, respectively. This led to improvement in the NPEV isolation rate.

Double checking of specimens from Jordan was initiated in April 2002. During 2002 and 2003, some enteroviruses were not detected in the national poliovirus laboratory Jordan but it now appears that this problem has been resolved, as during the last previous 12 months the number of discordant results was very low.

Finally, very few poliovirus isolates are being forwarded from the Region to the RRL in Tunisia for ITD. Improving referral of such isolates would reduce the workload in the other RRLs of the Region and help the RRL in Tunisia to have constant activities in this field and maintain performance.

6. QUALITY ASSURANCE

6.1 Accreditation status of regional poliovirus laboratories

Dr H. Asghar, WHO/EMRO

In 2004, 11 of 12 regional network laboratories were fully accredited by WHO. The Iraq national poliovirus laboratory could not be visited due to security problems. As of June 2005, the national poliovirus laboratories in Sudan and the Syrian Arab Republic were fully accredited by WHO. All national poliovirus laboratories implemented recommendations made during accreditation visits.

There is sustained good quality performance of all laboratories of the network. All network laboratories were able to maintain the timeliness of reporting of the virology results. There was uniform implementation of quality assurance programme. Performance of the cell culture laboratories needs continuous monitoring for accurate results.

6.2 Evaluation of proficiency testing: virus isolation identification and ITD by ELISA for and dilemma of P3 ELISA performance and supply

Dr H. van der Avoort, WHO Temporary Adviser

Regional laboratories have proven to be very proficient in the tests for isolation and typing throughout the last 5 years, with mean scores of more than 90%, far above the accepted scores of 80% for national poliovirus laboratories and 90% for RRLs. The PT score for 2004–2005 was no exception (mean score 92%). However, two laboratories (IRN/IRQ) did not initially pass the test this year, most likely because of problems of cell culture sensitivity, as the sample with the lowest virus concentration was reported as negative by both laboratories. Intensive study of worksheets and laboratory procedures identified some weaknesses that were addressed immediately. Afterwards both laboratories passed a new PT, hence all laboratories have passed the PT, with a mean score for the regional laboratory network of 98.8%.

The PT for intratypic differentiation (ITD) by ELISA showed excellent performance by all six laboratories that have implemented this technique. All scored 100% with excellent optical density (OD) values and ratios.

The global need for ELISA reagents has led to a backlog in the production of P3 NSL serum at RIVM in 2003 and 2004. A new antiserum was produced in rabbits that proved to be a more suitable starting point for the preparation of P3 NSL specific antiserum. For 2005, the production of P3 ELISA kits can equal the global needs of the laboratory network. Efficient use of the available antisera and good planning based on real needs remain essential to prevent backlogs in the future.

6.3 Evaluation of proficiency testing molecular methods for ITD, and why PCR will remain the method of choice

Dr Olen Kew, CDC

Regional network laboratories have mastered diagnostic polymerase chain reaction (PCR): four laboratories scored 100% and one laboratory scored 90% in the 2004 testing. Turnaround times for proficiency testing have been excellent. The Oman national laboratory quickly mastered diagnostic PCR, and accurately applied it to the identification of isolates from the Yemen outbreak. The goals now are to:

- maintain current levels of high proficiency;
- consider the introduction of new technologies into the regional laboratory network; and
- consider further technology transfers to at least one other regional network laboratory.

In addition, and significantly, the Pakistan RRL is now showing excellent proficiency in poliovirus sequencing.

6.4 Evaluation of cell sensitivity for poliovirus infection

Dr Javier Martin, WHO Temporary Adviser

Since the global poliomyelitis eradication programme started, poliovirus laboratories have played a crucial role in the surveillance of poliovirus, and have providing information essential for the programme to move forward. Assessment of the quality of the cells used for poliovirus isolation remains a central step in the quality assurance process of the work carried out in the laboratories. Laboratories are reminded that the virus titre of the laboratory quality control standards prepared in house should be validated using the National Institute for Biological Standards and Control (NIBSC) standards in three independent virus titre determinations done in parallel. The validated laboratory quality control (LQC) standards will then be used to evaluate the sensitivity to poliovirus infection of all newly resuscitated cells.

It is recommended that cells are evaluated approximately midway through their expected use of 15 passages. It is proposed that laboratories with a heavy workload perform a second virus titre determination just before the cells are discarded to provide additional reassurance. Reports on standard forms should be sent to NIBSC through the Regional Office and their assessment will be part of the laboratory accreditation process. Strict follow-up and a plan of action to correct any detected malfunction in laboratories with low sensitivity performance should be implemented by the regional laboratory co-coordinator.

7. GROUP DISCUSSION: CELL SENSITIVITY TESTING AND ITS INTERPRETATION

The group discussion was focused on the technical aspects of cell sensitivity testing. The following are the salient points.

- The laboratories should determine the titre of LQC in parallel with NIBSC standards and three valid tests with LQC should be performed.
- Rresults of all cell sensitivity tests should be reported to NIBSC through the laboratory coordinator.
- Reports from NIBSC should be taken into account by the reviewer during accreditation visits.
- A plan of action to implement correct procedures should be set up with strict follow-up.
- The cell sensitivity should be performed halfway through the 15 passages, and cell passage history should be maintained. For those laboratories processing a large number of samples, second tests should be performed just before cells are discarded.
- An Excel sheet should be prepared by NIBSC to see the trend of data.

8. GROUP DISCUSSION: ACCELERATION OF IDENTIFICATION OF POLIOVIRUSES BY MOLECULAR METHODS

The main emphasis of this group discussion was on rapid, accurate and timely reporting of virus investigation of stool samples. The following were the main points:

- Most of the time delays occur in the field: delay in collection of sample and transportation;
- EPI should be more proactive in prediction of potential outbreaks;
- The time required to ship positive isolate/stool from NPL to RRL is the main factor for delay in obtaining the final ITD results;
- Increasing the number of ITD testing laboratories can resolve the problem of delay in shipment of positive isolate/stool. Good performing laboratories may be upgraded to perform ITD tests. Selection of national poliovirus laboratories should be based on performance and neighbouring countries to which services will be provided.

9. FUTURE THINKING FOR THE POLIOMYELITIS ERADICATION PROGRAMME

9.1 Experience with RT-PCR in identification of polioviruses

Dr Hinda Triki, Regional Reference Laboratory, Tunisia

Acceleration of polioviruses identification using molecular methods is one of the main concerns at this stage and for the later stages of the poliomyelitis eradication programme. The proposed methodologies aim at rapid detection by polymerase chain reaction (PCR) testing of the viruses, directly in stool samples or after a short passage on cell culture, and rapid serotype identification by PCR of virus isolates. Direct detection of enteroviruses in

pathological samples by PCR was introduced in Tunis laboratory since 2000; it is mainly used for diagnostic of aseptic meningitis and viral conjunctivitis.

A study was recently conducted to evaluate its usefulness for enterovirus (EV) detection in stool specimens. The 81 stool samples previously found EV positives and kept stored in the laboratory were tested by PCR and retested in parallel in cell culture. The EV genome could be detected in all specimens, the virus could be re-isolated from only 72 samples.

A prospective study was conducted from May 2004 to April 2005. Three hundred and twenty-one (321) stool samples were tested in parallel upon reception by the two methods. The 51 specimens were found EV positives by PCR verses 35 samples only by virus isolation on cell culture; 32 samples were positive by the two methods, three samples were PCR(-)/cell culture (+) and 16 samples PCR (+)/cell culture (-). Serotype identification by sequencing the PCR product is ongoing, three samples were tested up-to-date in which CAV20, EV71 and ECV3 were identified.

The ability of sequencing the PCR product to identify the serotype was also studied on the EV sequences published in GenBank. The main conclusions are that the PCR test is of high sensitivity and combined to a less laborious cell culture protocol, it can accelerate the final result and reduce the workload of the laboratories. Direct detection of EV genome in stool samples has two main advantages as compared to PCR techniques using preliminary rapid cell culture. It can detect viruses that do not cultivate on L20B and RD cell lines and, thus, would be more convenient for laboratories conducting other diagnostics in clinical virology (associated or not with poliovirus surveillance). It is very important at later stages of the poliomyelitis eradication programme to extend surveillance outside the poliovirus laboratory network and to other EV-related diseases such as aseptic meningitis. It can detect viruses with altered infectivity which will not grow on cell culture; this happens mainly in the case of inadequate storage and transportation of specimens, and detection of the virus genome in stools would be a valuable tool to initiate more extensive epidemiological and virological investigation around the case.

9.2 Programmatic screening of VDPV

Dr Esther de Gourville, WHO/HQ

All poliovirus isolates identified through AFP surveillance undergo two methods of ITD testing. Isolates with dissimilar results in the ITD tests are sequenced, and viruses with > 1% sequence divergence from the Sabin virus in VP1 region of poliovirus genome are considered to be VDPV. All such isolates are further subjected to sequencing of non-capsid region of poliovirus genome. The VDPVs are categorized as cVDPV (1 paralytic case with isolation of related but non-identical viruses), iVDPV (immunodeficiency and long-term excretion of the virus from the same patient) and "other" VDPV (single isolate with no immune deficiency in patient; environmental source without cases; small number of isolates with no evidence of wide community spread cVDPV).

During 1999–2004, a total of 16 573 polioviruses viruses were characterized globally in poliovirus network laboratories; only 48 (< 0.5%) of 9860 Sabin related viruses were

identified as VDPVs. The first known cVDPV outbreak occurred in Hispaniola (22 cases) in 2000, and later in 2001 in Philippines (3 cases), in 2002 in Madagascar (4 cases), and most recently in 2004 in China (2 cases). VDPV has also been isolated from non-AFP sources: contacts, healthy children and sewage samples. Most of the VDPVs have been reported from polio-free countries. There is improved collection of epidemiological and test data on all VDPVs. Further research is in progress for detection of VDPVs, and a recombinant-PCR (targeting 3D and 2C regions of the genome) test is under development with potential for use in network laboratories.

9.3 Efforts in providing home made ITD reagents: rationalized demands based on workload and expected number of tests per kit

Dr Olen M. Kew, CDC, and Harrie van der Avoort, WHO Temporary Adviser

The demonstrated high proficiency of the Institut Pasteur in Tunis, the NIH in Pakistan, the VACSERA regional reference laboratories in Cairo, and the Islamic Republic of Iran and Oman national laboratories in diagnostic PCR sets the stage for possible introduction into the global and regional laboratory net of newly-developed, rapid techniques based on real-time PCR (rtPCR).

A progress report was given on the development at CDC of rtPCR methods for rapid identification of poliovirus isolates. The rtPCR methods are based upon the existing diagnostic PCR primers to be used in conjunction with new probes developed to specifically detect the poliovirus amplicons. All components of the current standard diagnostic PCR kits (except the panEntero primers and the Sabin 3 primers) have been adapted to the rtPCR format. Full development is ongoing. The use of rtPCR opens the way for development of wild genotype-specific PCR primer/probe sets that allow direct identification of wild poliovirus isolates by genotype, with negligible risk of false positives by carryover.

The ELISA is an antigenic method for ITD testing and is used in 31 laboratories globally, and annually an estimated 7100 tests are performed in these laboratories, which amount to about 225 ELISA kits per year. The reagents are very precious and should be used carefully and more economical way. The ELISA should not be wasted by doing serotyping. OD values of anti-total sera and controls should be monitored regularly and any sudden change in value or trend will point to problem in quality. All ideas in improvement of methodologies are welcome, but no change should be brought in protocol without documentation of data and sharing with RIVM. Feedback on field experience of test performance is crucial for optimal functioning of the network. The yearly needs of kits should be discussed with specialized laboratories well beforehand, and the laboratory coordinator should demand kits based on the expected workload.

10. SUPPLEMENTAL SURVEILLANCE FOR POLIOVIRUSES

10.1 Progress in environmental study in Egypt during 2004–2005

Dr Laila El Bassiouni, VACSERA, Egypt

Egypt implemented environmental surveillance in September 2000 to assist AFP surveillance. Testing of sewage samples in 2001 and 2002 showed widespread circulation of wild type 1 polioviruses, identified gaps in AFP surveillance, and allowed targeting of efforts to improve surveillance and immunization performance.

As of 2005, sewage samples are collected and analysed from 33 locations in 18 Egyptian provinces, with 42 samples per month. The genetic diversity of the wild type 1 isolates declined sharply from 2000 to 2004, with only two (Cluster E and H) of the original 11 clusters active in 2005. Cluster E represents repeated introduction of WPV1 into Fayoum from high-risk areas in Cairo and Giza. Cluster H is closely related to Upper Egypt.

Between September 2003 and July 2004, a gradual decline in NPEV and Sabin virus isolation occurred due to chloroform stabilized with amylene. It was replaced with chloroform stabilized with alcohol and the problem was immediately resolved.

10.2 Principles and practices of environmental surveillance

Dr Tapani Hovi, WHO Temporary Adviser

Environmental surveillance has the potential to detect poliovirus circulation even in the absence of reported cases of poliomyelitis, provided that representative samples are collected and examined in a proper way. Because of the significant resource demands inherent, environmental surveillance should be applied for carefully selected targets only.

Sabin strain-derived moderately drifted polioviruses are frequently isolated from environmental specimens. Environmental surveillance could be used to monitor the drift level in populations of interest. Additional value for environmental surveillance could be obtained by nucleotide sequence analysis of poliovirus Sabin-like isolates to monitor genetic drift as a potential risk of cVDPV generation; however, development and validation of suitable selective procedures for VDPV detection is desirable to reduce the necessary resource investment.

10.3 Environmental sampling in the United Kingdom, in search for viruses from the world's longest poliovirus excreter

Dr Javier Martin, WHO Temporary Adviser

Vaccine-derived polioviruses from long-term immunodeficient poliovirus excreters (iVDPV) represent a challenge for the programme and a potential threat for the post-eradication era. The prevalence of such cases in the population is not really known although several studies suggest that it may be relatively low (0.01%–0.1% of individuals with B-cell deficiencies).

The longest known poliovirus excreter lives in the United Kingdom and has been excreting iVDPV strains for an estimated 18 years. Isolates from this individual showed full reversion to wild-type properties and consequently high levels of neurovirulence in animal models. This individual is however protected from developing paralytic paralysis by regular intake of intra-venous immunoglobulin. Studies with monoclonal antibodies demonstrated extensive changes in the antigenic structure of these iVDPVs strains that however did not result in failure of human sera to neutralize the virus in laboratory assays. As expected, viruses of this kind do not seem to represent a serious risk to well-immunized populations. However, they could be a serious threat to non-immunized children when or if polio vaccination is interrupted following global poliomyelitis eradication.

Since environmental surveillance has proven to be very useful and sensitive in detecting both VDPV and wild polioviruses even in the absence of paralytic poliomyelitis cases, it was decided to implement this technique in the United Kingdom. This would provide valuable information on how easy is to detect iVDPV strains when the location of the excreter is known, and it also came at a time when the United Kingdom was about to switch from the use of a live-attenuated OPV to inactivated poliovaccine so these studies could also provide some additional information on how long the OPV strains last in the environment. The methodology has been established in the laboratory and studies samples from two different locations in the United Kingdom have been initiated.

10.4 Status of survey and inventory phase (phase 1) of laboratory containment of wild poliovirus

Dr H. Asghar, WHO/EMRO

Out of the 18 currently polio-free countries, nine have reported completion of the laboratory survey and inventory phase (phase 1) of activities (Bahrain, Djibouti, Islamic Republic of Iran, Jordan, Lebanon, Libyan Arab Jamahiriya, Oman, Qatar and Saudi Arabia), six are in the final stages with completion expected before the 2005 meeting of the Regional Certification Commission (Iraq, Kuwait, Morocco, Syrian Arab Republic, Tunisia, United Arab Emirates). Sudan was considered among the countries that had completed the laboratory survey and inventory phase activities, but it has been dropped from the list due to current re-infection with wild poliovirus. Yemen has written its national plan, but its implementation has been delayed due to a recent wild poliovirus outbreak. National containment coordinators have not been nominated in three countries (Afghanistan, Pakistan and Somalia). Efforts are continuing to resolve this issue with the governments.

As of 2004, 19 060 laboratories have been surveyed and only six laboratories have been identified as storing wild poliovirus material. Most of these belong to the regional network of national poliovirus laboratories.

Guidelines for documenting the quality of phase 1 of containment activities were pilot tested in three countries (Islamic Republic of Iran, Oman and Saudi Arabia), and reports were submitted to Regional Certification Commission thorough National Certification Committee. The Regional Certification Commission accepted the format, and suggested adding data tables in the reports. Other polio-free countries have been requested to submit their report to

Regional Certification Commission. In this regard, WHO provided technical assistance through visits and consultation by email and telephone. There is strong political commitment for containment activities.

Future plans are to support countries in the completion and documentation of phase 1 of containment activities, to encourage still endemic countries (Pakistan and Egypt) to start preparing the list of laboratories, and to prepare for global certification containment activities.

10.5 Characteristics of the new LABIFA and solutions to problems

Dr Hala Safwat, WHO/EMRO

LABIFA is being implemented efficiently in all laboratories of the Region. Data quality has improved. Laboratories are encouraged to make a great use of the system reports and to perform regular backups.

In 2005 to date, laboratories in Egypt, Pakistan, Islamic Republic of Iran, Oman, and the Syrian Arab Republic were the most regular in updating the Regional Office on a weekly basis. Other laboratories of the Region are urged to improve the frequency of their reporting.

Information on contacts in the Regional Office is mainly produced from LABIFA. During the period 2004–2005 to date, three districts were identified as infected from contacts data.

11. CONCLUSIONS

The regional poliovirus laboratory network faced extraordinary challenges in 2004–2005, because of the large outbreaks in Sudan and Yemen and continued circulation of wild polioviruses in endemic countries: Afghanistan, Egypt and Pakistan. Poliovirus network laboratories responded to these challenges by increasing their efficiency and improving cooperation and efforts towards continued improvement. Throughout this period the regional laboratories demonstrated high technical proficiency and capacity to assimilate and quickly apply new technologies, to cooperate in a Region-wide manner to manage human and material resources effectively, and to maintain and even improve performance in the face of the major public health crises. The regional laboratory network is poised to provide the programme with the comprehensive virological support essential to successful transition to polio-free status.

The poliovirus laboratory network in the Region is maintaining a high standard of performance. Its timely and accurate reporting of virological investigation results to the poliomyelitis eradication programme has helped to target actions in the right direction. All network laboratories were fully accredited except for the Iraq national poliovirus laboratory, which could not be visited for accreditation due to the security situation. Increases in the workload brought improvements in AFP surveillance and wild poliovirus outbreak, as a result of importation, in Sudan and Yemen was dealt by the concerned laboratories efficiently. Excellent timeliness and accuracy of the results was seen. Despite the increase in workload, the mean time of final ITD results from the date of onset paralysis was 33 days, and from the

date received in laboratories was 21 days. All wild poliovirus isolates are referred to global specialized laboratories and genomic sequencing results are transmitted to the poliomyelitis eradication programme for precise targeted action in the field.

Although the poliovirus laboratory network has shown its commitment to meeting the rising programme demands, the current high level of performance is not sustainable without an increase in resources proportionate to the increasing workload.

Polio-free countries of the Region remain at risk of wild poliovirus importation from polio-endemic countries and the emergence of VDPVs in areas of low immunization coverage. Further challenges to the laboratory network include sustaining high level laboratory performance, logistic and human resources support, and ensuring national support to the national poliovirus laboratory.

12. RECOMMENDATIONS

1. Poliovirus laboratories are essential national public health resources. National authorities should support the poliovirus laboratory through the provision of a sufficient budget and personnel. WHO should continue its advocacy with governments to meet the unmet needs.

2. The EPI should work with the laboratory to assess the incremental surveillance sensitivity obtained by collection of contact stools (and other specimens from excluded cases) in the context of increased workloads in the laboratory and the requirements for additional human resources and logistics support.

3. Cell culture sensitivity testing should be standardized by using the NIBSC standard Sabin strains and laboratory quality control standards, according to WHO protocol. All tests should be documented and the worksheets shared with NIBSC in the United Kingdom and the WHO Regional Office. All laboratories should perform routine sensitivity testing of cell cultures halfway during the recommended 15 passage of cell lines and a second test just before cells are discarded, to be reassured that cells have maintained sensitivity during their use.

4. Supplies and resource needs should be planned based on the increased workload of laboratories due to increase in AFP rates and any added strategy such as the collection of contact stool samples. In addition, adjustments should be made in case of an unexpected crisis e.g. an outbreak. Efforts should be continued to mobilize resources to cover supplies, personnel and logistic needs and provide budget through specific allocations for laboratory support. WHO should continue to coordinate with national authorities and partners in poliomyelitis eradication to mobilize resources.

5. In the light of the recent Sudan and Yemen wild poliovirus outbreak experiences, which set an example of efficiency, laboratories are advised that in such situations they should work closely with WHO for prompt response to expedite the transportation, testing and reporting of samples.

6. Day-to-day performance (sensitivity and specificity) of test controls in ELISA-ITD should be monitored continuously. Strict documentation of exact dilutions used, incubation time, as well as registered optical densities (ODs) values of anti-total reactions, will demonstrate decreasing quality of reagents, should this occur.

7. All polioviruses isolated from clinical specimens should be referred to global specialized laboratories for further characterization to monitor their genetic diversity for VDPV detection. The referred viruses should be accompanied by referral form.

8. Environmental surveillance in Egypt is supplementing the AFP surveillance at high sensitivity. The National Public Health Institute, Helsinki (KTL) should continue to characterize all poliovirus isolates of programmatic importance from environmental samples. At the present high level of workload, all poliovirus isolates from the environment surveillance in Egypt should be tested by one molecular method (e.g. probe hybridization). All candidates for wild polioviruses isolates should be flagged and confirmed by ITD-ELISA, reported to the national poliomyelitis eradication programme, and at the same time should be sent to KTL for characterization.

9. WHO annual accreditation helps the laboratory to overcome the technical and managerial problems. A completed accreditation checklist should be sent to concerned laboratories for their records and to facilitate their follow-up.

10. All poliovirus network laboratories should ensure full immunization of staff against poliomyelitis according to the national immunization policy and document the vaccination of staff in accordance with the national policy. This documentation should be submitted to the regional poliovirus laboratory network coordinator at the WHO Regional Office for the Eastern Mediterranean.

11. Poliomyelitis eradication is progressing towards its final stages and there are increasing demands to provide timely virological investigation results of AFP cases. To achieve this target, the national poliovirus laboratories, as appropriate, should be upgraded to perform ITD testing.

Annex 1

PROGRAMME

Saturday, 11 June 2005

08:00–08:30	Registration
08:30–09:30	Opening session Address by H.E. Dr Ali Al Seif, Assistant Undersecretary for Public Health, Ministry of Health, Kuwait Message from Dr Hussein A. Gezairy, WHO Regional Director for the Eastern Mediterranean Election of the Chairman and Rapporteur Implementation status of the recommendation of the eighth Intercountry Meeting of Directors of Poliovirus Laboratories/Dr H. Asghar
Session 1	*Overview*
09:30–09:50	Global overview of the poliomyelitis eradication initiative/Dr E. de Gourville
09:50–10:10	Regional overview of the poliomyelitis eradication initiative/Dr F. Kamel
10:10–10:30	Regional performance of poliovirus 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 the Region/Dr O. Kew
11:45–12:00	Egypt, virus surveillance/Ms E. El Maamoun
12:00–12:15	Pakistan, virus surveillance/Mr S. Zaidi
12:15–12:30	Sudan, virus surveillance/Mr H. Babikar
12:30–12:45	Somalia and South Sudan, virus surveillance/Mr P. Borus
12:45–14:00	Discussion
Session 3	*Laboratories' experience in dealing with specific problems*
14:00–14:15	Egypt RRL, increase in workload and logistics demand/Dr L. Bassioni
14:15–14:30	Kuwait RRL, implementing the quality assurance programme in RRL Kuwait/Dr S. Al-Mufti
14:30–14:45	Oman NPL, Report of the Yemen outbreak and handling cell culture demands of network laboratories: providing fresh and sensitive cells/Dr S. Al-Busaidy
14:45–15:30	Pakistan RRL, keeping balance between demands of AFP surveillance and laboratory performance/Mr A. Naeem
15:30–15:45	Saudi Arabia: why the NPEV rate is low/Mr M. Al Amri
15:45–16:30	Syrian NPL, use of sensitive cells and effect on virus isolation/Ms H. Saba
16:30–16:45	Sudan NPL, isolation and identification of wild polioviruses after 4 years of silence/Ms R. Sideeg
16:45–17:00	Tunisia RRL, dealing with referred samples from NPLs: isolation, identification and ITD/Dr H. Triki
17:00–17:30	Discussion

Sunday, 12 June 2005

Session 4	*Quality assurance*
08:30–8:40	Accreditation status of regional poliovirus laboratories/Dr H. Asghar
08:40–09:00	Report on proficiency testing- virus isolation, identification and intratypic differentiation by ELISA, and dilemma of P3 ELISA performance and supply/Dr H. Avoort
09:00–09:20	Report on proficiency testing- molecular methods for intratypic differentiation, and why PCR will remain the method of choice for ITD? Dr O. Kew
09:20–09:40	Evaluation of cell sensitivity for poliovirus infection/Dr J. Martin
09:40–10:00	Efforts in providing home made ITD reagents: rationalized demands based on workload/Drs O. Kew and H. Avoort
10:00–11:00	Discussion
11:00–13:00	Group discussion: Cell culture sensitivity testing and its interpretation Moderators: Dr J Martin, Dr T. Hovi
13:00–14:00	RRLs (Egypt and Pakistan) Side discussion during lunch: Management of increase workload, how to make use of limited funds and rationalizing the supplies needs Moderators: Dr E. De Gourville, Dr H. Asghar
14:00–16:30	Group discussion: Acceleration of identification of polioviruses by molecular methods Moderators: Drs O. Kew, H. Avoort, T. Hovi, J. Martin, E. de Gourville, H. Asghar
Session 5	*Future thinking for the poliomyelitis eradication programme*
16:30–17:00	Experience with RT-PCR in identification of polioviruses/Dr H. Triki
17:00–17:30	VDPVs detected through programmatic screening/Dr de Gourville
17:30–18:00	Discussion

Monday, 13 June 2005

Session 6	*Supplemental surveillance for polioviruses*
09:00–09:20	Progress in environmental study in Egypt during 2004–2005/ Ms E. Al Maamoun
09:20–09:40	Principles and practices of environmental surveillance: an update/Dr T. Hovi
09:40–10:00	Environmental sampling in the UK, in search for viruses from the world's longest poliovirus excreter/Dr J. Martin
10:00–10:20	Status of survey and inventory phase 1 of laboratory containment of wild polioviruses/Dr H. Asghar
10:20–10:40	Characteristic of new LABIFA, and solution to problems/Dr H. Safwat, WHO/EMRO
10:40–11:30	Discussion
11:30–12:00	Open discussion on remaining issues
12:00–13:00	Closing session Discussion on conclusions and recommendations

Annex 2

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