WHO-EM/POL/375/E

Report on the

Twelfth Intercountry meeting of directors of poliovirus laboratories in the Eastern Mediterranean Region

Damascus, Syrian Arab Republic
27-29 October 2008

World Health Organization
Regional Office for the Eastern Mediterranean

Document WHO-EM/POL/375/E/01.09/71

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1. INTRODUCTION

The twelfth intercountry meeting of directors of poliovirus laboratories in the World Health Organization (WHO) Eastern Mediterranean Region was held in Damascus, Syrian Arab Republic from 27 to 29 October 2008. 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 National Institute for Biological Standards and Control (NIBSC), United Kingdom; Kenya Medical Research Institute and staff from WHO headquarters and the Regional Office for the Eastern Mediterranean (EMRO).

Dr Betelmal, WHO Representative, Syrian Arab Republic, welcomed the participants and delivered a message on behalf of Dr Hussein A. Gezairy, WHO Regional Director for the Eastern Mediterranean. In his message, Dr Gezairy commended the work performed by the polio laboratory network in providing accurate information in a timely manner and meeting the emerging needs of the polio eradication programme. He also commended achievement of the targets of decreasing the reporting time of final virological investigation results and meeting the laboratory performance indicators. He urged the network laboratories to maintain the high standards of quality assurance, and use the polio laboratory network as a model for the development of laboratory services for other disease control and elimination programmes.

Dr Amira Arraj (Syrian Arab Republic) was elected as Chair of the meeting and Dr Shohreh Shah Mahmoodi (Islamic Republic of Iran) was elected Rapporteur. The programme and list of participants are included as Annexes 1 and 2, respectively.

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

Dr Humayun Asghar, WHO/EMRO

Review of the implementation and main achievements of the recommendations of the eleventh intercountry meeting of directors of poliovirus laboratories in the Region showed that all the recommendations addressed to national authorities and to WHO were implemented. It was emphasized that many of the recommendations continue to be valid and their implementation should be pursued by all concerned.

3. OVERVIEW

3.1 Overview of polio eradication in the Eastern Mediterranean Region (EMR)

Dr Faten Kamel, WHO/EMRO

The year 2007 witnessed intensification of polio eradication efforts and considerable progress towards the eradication goal, with the total number of poliomyelitis cases reported the lowest ever recorded in the Region (58). The majority (49 cases) were from the two endemic countries, namely Pakistan and Afghanistan, 8 were from Somalia, representing the tail of the outbreak that followed importation of poliovirus, and a single importation was reported in Sudan from Chad with no secondary cases. The total cases in 2007 represent a

46% reduction as compared to 2006 cases (107) and represent less than 5% of the global cases for the year 2007.

The reduction in case numbers and the restriction in geographic extent of virus transmission in Pakistan and Afghanistan in 2007 did not continue through 2008. Up to October, the number of polio cases reported from both countries reached 106 cases (84 in Pakistan and 22 in Afghanistan), which is three times what was reported for the same period in 2007. In addition, 7 cases were reported from Sudan, one P3 importation from Chad in West Darfur with no secondary spread and 6 P1 cases in south Sudan, representing ongoing P1 circulation in south Sudan and adjacent parts of Ethiopia.

In Afghanistan, the deteriorating security situation and active fighting is the main challenge hindering safe access to children. In addition to using windows of opportunities of improved access to immunize children, efforts continued to reach agreement with all partners to cease hostilities during Supplementary Immunization Activities to allow vaccinators to move safely and reach children. On 14 September, two of the national staff and a driver were killed in a brutal attack while on their way from Kandahar to Spinboldak.

In Pakistan, the increase in polio cases was mainly seen in the second half of 2008. The sudden increase in cases starting in July reflects the spread of virus to many districts in all provinces and the occurrence of P3 outbreak in Peshawar and P1 outbreak in Punjab. Several factors allowed the present epidemic spread of Wild Polio Virus in Pakistan to occur and the deteriorating security especially in NWFP and tribal areas resulted in extensive population movement facilitating the spread of poliovirus. The high risk approach did not succeed in completely interrupting transmission in the reservoir areas where different elements including insecurity, pockets of refusals, managerial issues and inadequate engagement of some of the authorities at provincial and district levels did not allow optimal quality and coverage. In addition, other areas not considered risky did not have enough campaigns or sufficient routine immunization coverage to ensure population immunity to protect against re-infection and spread.

A special plan was developed with advocacy efforts to address the managerial issues in Sindh and a special consultation was held in Karachi in June 2008 with participation of senior provincial and federal health authorities. Additionally, a comprehensive communication plan was developed and implemented especially in tribal areas and for different types of refusals based on field data. Coordination also continued with Afghanistan in order to optimize simultaneous comprehensive coverage of the border areas and of children on the move.

In October 2008, an urgent consultation was held in the Regional Office in response to the upsurge of cases in Pakistan. The group stressed the need for increased government oversight of eradication. It also emphasized that at this stage the whole country is considered at high risk and hence strategies should be adjusted accordingly, with more nationwide polio campaigns and more use of tOPV. In addition, new tactics including environmental sampling and seroprevalence surveys will be added to better understand the reasons for poliovirus persistence in some areas and to guide future strategy.

Importation of wild poliovirus to the region from remaining endemic countries especially for countries in the extended “Horn of Africa remains a major challenge. Special attention is given to ensure preparedness of countries to detect and respond to possible importations based on the two main pillars namely ensuring sensitive AFP surveillance and maintaining high population immunity.

The AFP surveillance system in the Region continues to perform at the accepted international standard and even exceed the required indicators in many priority countries. All endemic, infected or recently polio-free countries have maintained a non-polio AFP rate of at least 2/100,000 children under the age of 15. Many other countries particularly the ones at high risk of importation also exceeded the required level. Overall, the Region achieved a rate of 4.08 in 2007. The minimum required level of one case per 100 000 population under 15 years was reached by all individual countries except Palestine (0.93) with a difficult security situation and small population. Similarly the regional annualized rate up to 20 October 2008 is 4.42 and all countries are exceeding the rate of one case per 100 000 children under 15 years.

The second key quality indicator for surveillance is percentage of AFP cases with adequate stool collection. In 2007 this indicator was maintained above the target of 80% at the regional level (90.9%) and in all countries of the Region except in Bahrain (60.0%) and Lebanon (65.2%), both with a small number of cases. For 2008, the regional figure is 91.7%, with all countries except Djibouti, Kuwait and Syrian Arab Republic above the 80% target.

Priority attention continued to be given to implementing supplementary immunization activities, with the aim of ensuring that all children under 5 years are immunized against polio, especially in countries with low routine coverage. In 2007, more than 412 million doses of OPV were given in national and subnational immunization campaigns in the Region. Afghanistan, Pakistan and Somalia carried out supplementary immunization activities throughout the year at 4–6 week intervals. Mop-up activities were also implemented in response to wild poliovirus isolation in Afghanistan, Pakistan and Sudan using the appropriate monovalent OPV. To guard against spread after importation, some polio-free countries conducted campaigns addressing mainly high-risk areas and areas with low routine coverage (Djibouti, Egypt, Iraq, Lebanon, Jordan, Libyan Arab Jamahiriya, Saudi Arabia, Sudan, Syrian Arab Republic and Yemen)

Coordination is being extended to neighbouring countries of other WHO regions. Several coordination meetings for the Horn of Africa took place in 2007 and 2008; the Horn of Africa bulletin is being issued regularly with input of all relevant countries. As well, the Horn of Africa Technical Advisory Group met in April 2007 and July 2008. Synchronization of activities and exchange of information between countries has improved greatly. However, there is still room for improving direct coordination at local levels.

The RCC continued to review various national documents submitted by the National Certification Committees (NCC) of countries in the Region. Basic documents have been accepted from 19 countries, final reports from 14 countries and progress reports are regularly

submitted by Pakistan and Afghanistan. All countries continue to submit annual updates as well.

The main priorities in the Region are to: interrupt transmission in Pakistan and Afghanistan by ensuring high quality performance and ensuring access to children in the security compromised areas; interrupt the shared transmission of P1 virus in south Sudan and Ethiopia; maintain polio-free status in other countries through avoiding immunity gaps and maintaining certification-standard surveillance; maintain and further strengthen coordination activities between neighbouring countries; continue with containment and certification activities; avail the financial resources required to implement the regional plan for eradication; and optimize PEI/EPI collaboration.

3.2 Status of the global polio laboratory network

Dr Esther de Gourville, WHO/HQ

In 1988, the World Health Assembly resolved to eradicate poliomyelitis. Subsequently, the global polio eradication initiative reduced the worldwide incidence of poliomyelitis associated with wild polioviruses (WPVs) from an estimated 350 000 cases in 1988 to 1315 reported cases in 2007; it also reduced the number of countries that have never succeeded in interrupting WPV transmission from more than 125 to 4 (Afghanistan, India, Nigeria and Pakistan). From January 2007 to October 2008, 92% of cases were reported from these 4 endemic countries (Afghanistan 1.6%, India 35%, Nigeria 52% and Pakistan 5.8%). Circulation of type 2 WPV was last observed in October 1999.

In 2007, a high standard of performance was maintained in all regions, and the accreditation status of the 145 network laboratories was 141 fully accredited, 2 provisionally accredited, and 2 pending accreditation. Performance review has not been completed for 2008.

Between January 2007 and June 2008, the Global Polio Laboratory Network (GPLN) analysed 234 521 stool samples from AFP cases, representing an overall increase of 12% in workload compared with the previous 18 months. In 2007 alone, 156 795 stool samples from AFP cases and 10 555 from non-AFP sources were tested. In 2007, the percentage of virus-isolation results reported within 14 days of sample receipt was 83% in African Region, 81% in the Eastern Mediterranean and 36% in South-East Asia; in 2008, the percentage reported within 14 days remained 83% in the African Region and increased to 95% in the Eastern Mediterranean and 84% in South-East Asia. There was a 50% reduction in laboratory reporting time in polio-endemic regions; this was done through combination of implementing new test algorithms and increasing intratypic differentiation (ITD) testing capacity.

Between January 2007 and June 2008, laboratory results confirmed the circulation of WPV serotype-1 (WPV1) and serotype-3 (WPV3) in the WHO regions of Africa, Eastern Mediterranean and South East Asia. The WPV detected belonged to 4 genotypes: South Asia wild poliovirus type 1 and 3 (SOAS WPV1 and SOAS 3) and West Africa B type 1 and 3 (WEAF-B WPV1 and WEAF-B WPV3). The 2 SOAS genotypes are endemic to Afghanistan, India and Pakistan; the 2 WEAF-B genotypes are endemic to Nigeria. The WPV were isolated from AFP cases in 12 non-endemic countries (Angola, Australia, Benin, Central African Republic, Chad, Democratic Republic of the Congo, Ethiopia, Myanmar, Nepal, Niger,

Somalia and Sudan), and in all instances the viruses were genetically linked though VP1 viral nucleotide sequences to genotypes found in India or Nigeria. In July 2007, SOAS WPV1 was isolated from an adult Pakistani patient in Australia, who had onset paralysis in Pakistan before entering Australia. The GPLN detected WPVs from non-AFP sources: WPV1 and WPV3 in sewage water of Mumbai, India; Geneva, Switzerland; Giza, Egypt, and from contacts of an AFP case in Sudan.

The GPLN screens for programmatically important viruses among Sabin-related isolates. The screening algorithm flags isolates for VP1 nucleotide sequencing if discordant results are revealed in ITD tests based on genetic and antigenic principles. Between January and October 2008, the GPLN screened >8500 Sabin-related strains from AFP cases and found circulating Vaccine Derived Polioviruses (cVDPVs) in Myanmar (8 serotype-1 from 4 cases) and Nigeria (207 serotype-2 isolates from 105 cases, 4 of whom had mixed WPV-cVDPV infections). In Russian Federation, iVDPVs were found (1 case of serotype-2). In the Islamic Republic of Iran 2 iVDPV cases were detected: a case was co-infected with both serotype-1 and serotype-2 and the other had only serotype-2 viruses. In China, 4 serotype-1 aVDPVs were isolated from AFP cases in Guangxi (1 case), Shandong (2 cases) and Shanxi (1 case) provinces; these were independent events with no evidence of circulation. Serotype-2 VDPV was isolated from a single case of AFP in the Russian Federation in 2008. A single serotype-2 aVDPV was found in the Democratic Republic of the Congo in 2007, and a serotype-3 aVDPV was detected in single child in Malawi in 2008. The GPLN and collaborating laboratories also found aVDPV in the following non-AFP samples: serotype-1 aVDPVs in a single sewage sample collected in Zurich, Switzerland in 2008; 2 serotype-2 aVDPVs in sewage samples in Egypt in 2007 and 2008; in multiple sewage samples collected in Israel in 2007 and 2008, and in a single sewage sample collected in Geneva, Switzerland in 2008.

There are concerns about communication between laboratories and regional laboratory coordinators, which should be addressed to develop better communication for problem solving and follow-up on gaps in performance e.g. timely reporting and follow-up of results of cell sensitivity testing, sharing of frequent problem of invalid ELISA tests, and occasional invalid P3 PCR tests not shared with test providers or WHO for several months, follow-up of incidents of reporting of inaccurate results. There is need to establish accreditation criteria for laboratories performing sequencing: timeliness of sequence results are not evaluated for accreditation, no criteria for evaluating technical performance of some low workload laboratories that contribute data for programme use, and no proficiency testing. A few other issues related to nucleotide sequencing that need to be resolved are inconsistent approaches to dealing with increasing genetic diversity and defining genetic sub-clusters in all sequencing laboratories.

The following were the new developments in the GPLN

- New accreditation checklists introduced in January 2008
- New indicators for reporting times for laboratories using new test algorithm
- Proficiency test panel for new algorithm evaluated in 3 network laboratories (Egypt, Senegal and Mumbai-India) in 2007

- Real time PCR assays for ITD and VDPV screening being evaluated in 3 Regional Reference Laboratories (RRLs) (NIH-Pakistan, ERC-Mumbai-India and NICD-South Africa) and 6 GSLs

- Work started on development of audiovisual training materials for a bio-risk campaign in GPLN

- GPLN contributions to polio research agenda

3.3 Regional progress of the regional poliovirus laboratory network

Dr Humayun Asghar, WHO/EMRO

The laboratory network continues to efficiently support AFP surveillance activities and high quality of performance is maintained. All network laboratories passed the WHO proficiency tests (PT) for both poliovirus isolation and intratypic differentiation testing and all laboratories are fully accredited, except Kuwait, which is provisionally accredited.

Since 2005, the workload of the network laboratories has increased substantially due to an increase in the number of reported AFP cases and in samples taken from contacts. During 2007 and as of October 2008, the polio network laboratories in the Region processed 24 215 and 21 266 samples from cases and contacts, respectively. The contact sampling of AFP cases is performed in 20 of 22 countries and six WPV (3 in Pakistan, 1 in Afghanistan, and 2 in south Sudan) cases have been detected through positive contact of virus negative index cases. In 3 countries (Saudi Arabia, Sudan and Iraq), which have polio laboratories, the average time from collection of first specimen to receipt in laboratory was more than 3 days. With the introduction of the new testing algorithm, 97% of samples had culture results within 14 days and 86% had ITD results within 7 days. Overall, in 98% of cases of AFP, the final laboratory testing results were provided within 45 days of paralysis onset.

The polio laboratories network successfully implemented the new testing algorithms for virus isolation and ITD. The average reporting time from receipt in laboratory to final ITD results decreased from 13 days in 2007 to 11 days in 2008. The main challenge in implementing the new algorithm has been the increase in workload resulting in increase resource needs for sample testing supplies and ITD reagents. New LabIFA4 was also successfully implemented to adjust to changes resulting from implementation of the new algorithm.

Egypt continues to collect sewage samples from 34 sites, once per month. VACSERA performance is continuously monitored through NPEV and Sabin virus isolation. Two serotype-2 aVDPVs were isolated from sewage in Behira governorate in Egypt, one in December 2007 and the other in April 2008. In September 2008, one WPV1 was detected in a sewage sample collected from Al-Haram, in Egypt, which has been sent to KTL, Finland for characterization by nucleotide sequencing.

Between January 2007 and October 2008, VDPVs were isolated from acute flaccid paralysis (AFP) cases, who were subsequently confirmed as immunodeficient patients. These included two children in the Islamic Republic of Iran (one with mixture of type 1 and 2, and another with type 2), and one child in Egypt (type 3). Isolates from these cases were classified

as iVDPVs. There was no evidence of secondary spread of VDPVs from any of the immunodeficient persons.

There are plans to establish environmental surveillance in Karachi and Lahore, Pakistan, for better understanding of circulation of WPVs and targeting the areas for immunization activities. Real-time PCR method for rapid characterization of polioviruses will be established in polio intratypic laboratories in the Region, and a training workshop is scheduled to be held in January 2009 at the National Polio Laboratory in Muscat and Oman.

4. VIRUS SURVEILLANCE

4.1 Laboratory performance indicators

Pakistan and Afghanistan

Mr Sohail Zaidi, National Institute of Health, Islamabad, Pakistan

1..1 Pakistan:

The Regional Reference Laboratory, Pakistan, continued testing of stool samples of AFP and non-AFP cases collected from Pakistan and Afghanistan. The new test algorithm was fully established in 2007. The laboratory passed the proficiency testing (PT) panel and is fully accredited for 2008. The laboratory performance indicators showed good quality laboratory performance indicators. The virus isolation and ITD results of 99% of specimens were completed and reported within 14 days and 7 days, respectively. The laboratory received specimens from 4087 AFP cases (1684 Punjab; 1016 Sindh; 1129 NWFP; 177 Baluchistan; 23 Islamabad; 19 FANA; 39 Azad Jammu and Kashmir), 1788 from contacts and 108 from excluded cases. To date in 2008, 87 cases of wild polioviruses were reported (63 due to WPV1 and 24 due to WPV3).

The quality assurance programme is fully established and cell sensitivity testing is performed regularly on both L20B and RD cell lines. The results are within an acceptable range of $\pm$ 0.5 of given titre of LQCs for each serotype. The increase in workload is met with high efficiency; however, delay in supplies in 2008 is a concern. Another problem is frequent power cuts and non-availability of heavy duty generators and UPS, as back up.

1..2 Afghanistan:

1102 AFP and 502 contact cases were received from Afghanistan. Laboratory testing showed the non-polio enterovirus rate as 23%. The virus isolation and ITD results were provided with in 14 days and 7 days for 100% of specimens, respectively. Sabin-like viruses were isolated from 75 cases, and 22 wild polioviruses from AFP cases (17 WPV1 and 5 WPV3).

1..3 Egypt

Dr Iman Al Maamoun, VACSERA, Egypt

The laboratory at VACSERA continued to test stool samples and virus isolates for Egypt, Iraq, Lebanon, Syrian Arab Republic, Sudan and Yemen. VACSERA is dealing with a

large workload with good accuracy and efficiency. Between January 2007 and October 2008, a total of 5093 samples (3965 from AFP cases and 1128 from contacts) were tested. A total of 292 samples (206 from AFP and 86 from contacts) were tested from countries other than Egypt. The performance indicators were sustained at high level: in 2008, 84% of culture results were provided within 14 days: 96% of ITD results were provided within 7 days; 100% of specimens were referred for sequencing within 7 days; the NPEV rate was 13%; and 100% score was achieved in PT panel testing of virus isolation, polymerase chain reaction (PCR) and nucleic acid probe hybridization (NAPH) test.

The laboratory performance is monitored regularly in general and in particular by performing cell sensitivity testing as direct evidence of quality of cell culture. Data shows Sabin-like viruses and NPEVs were isolated throughout the year from almost all governorates. One iVDPV3 from Kafr El-Sheikh was detected in July 2007.

Environmental surveillance continued with collection of sewage samples from 34 sites once per month in 19 provinces. Almost all sewage samples collected yielded either NPEV or Sabin-like viruses or a combination. One WPV1 was isolated in September 2008 from a sewage collection station in Al-Haram, Giza. Two aVDPVs were isolated from Behira sewage collection station in December 2007 and April 2008. In accordance with WHO recommendations only problematic poliovirus isolates and sample concentrates were sent to KTL; however, after isolation of aVDPVs from Behira, all positive Sabin-like viruses and sewage sample concentrates are sent for parallel testing to KTL, starting from April 2008 onwards. Another change was the collection of 4 samples per month from 4 branches draining into the main Behira sewage collection station. There is high concordance of results of KTL and VACSERA; discrepancies in some of the samples are due to competition of viruses' size of inoculum or surface area of inoculated cell culture.

1..4 Sudan

Mr Hatim Babiker, WHO Sudan

The laboratory continued to test stool samples from AFP cases and contacts. Between January 2007 and October 2008, a total of 1932 samples (1310 from AFP cases and 628 from contacts) were tested. In 2008, the good quality of performance indicators was sustained at high level: 93% of culture results within 14 days, 100% referred for ITD within 7 days, NPEV rate of 15% and 100% score in virus isolation PT panel. One isolate positive on L20B cell line was reported to the EPI as potential poliovirus immediately and sent for ITD to VACSERA, Egypt, where it was confirmed as WPV3. The laboratory is successfully dealing with the current workload.

1..4.1 KEMRI: Somalia and South Sudan

Mr Peter Borus, KEMRI, Nairobi, Kenya

The polio laboratory at the Kenya Medical Research Institute (KEMRI) serves as the intercountry laboratory for Kenya and Eritrea in the WHO African Region and for Somalia, South Sudan and Djibouti in the Eastern Mediterranean Region. The laboratory was fully

accredited for 2008-2009 using the new accreditation checklist and new proficiency test panels scheme.

In 2008, the laboratory implemented the new virus isolation algorithm. As of October 2008, it has tested 2044 stool samples from both AFP cases and contacts. Of these, 792 and 588 were from Somalia and south Sudan respectively. From Somalia, 326 samples were from AFP cases whereas 466 were from contacts. The samples from Sudan consisted of 256 and 332 stools from AFP cases and contacts, respectively.

The laboratory handled more samples from the Eastern Mediterranean Region countries in 2008 compared to the corresponding period in 2007. There were no major logistical difficulties in receiving and processing the samples. However, due to the progressive incremental increase in samples over the years, financial support from the Regional Office to support shipment of isolates and other recurrent expenditures continues to be stretched.

The laboratory met all its performance indicators based on the new isolation algorithm. Results of virological investigation are provided for 93.5% of samples within 14 days. The accuracy of confirmation of suspected poliovirus isolates at the regional reference laboratory was 98.6%. The laboratory referred 97.5% of isolates to the RRL within 7 days of the isolation result.

The cell sensitivity assay were done on both RD and L20B cell lines and trends of virus titre were plotted for all 3 serotypes in each cell line, and these were within +/- 0.5 of the laboratory quality control standard. All assays were valid and cells were found to be sensitive.

The overall non-polio enterovirus isolation rate for all samples tested in the laboratory was 12.4%. The rate for Somalia and south Sudan was 15.1% and 16.6% respectively. There was no definite monthly trend in the non-polio enterovirus isolation rates. However, no enteroviruses were isolated from south Sudan in January and February 2008. A detailed evaluation of reasons for this zero rate showed no contribution of laboratory factors, as both cell sensitivity and propagation of RD cells were good during this period. The total number of poliovirus isolates from stools tested in the laboratory was 128. These included 17 wild poliovirus type 1 isolates from south Sudan that originated from 5 AFP cases and 7 contacts. In addition, 88 Sabin-like viruses were isolated consisting of 30 type 1, 48 type 2 and 38 type 3 from Somalia. Fifteen Sabin-like viruses were isolated from south Sudan, which consisted of 7 type 1, 3 type 2 and 5 type 3.

The laboratory is implementing antigenic intratypic differentiation (ITD) method, i.e. enzyme-linked immunosorbent assay (ELISA). After initial challenges, the results from the assays have been consistently good for all three serotypes, and proficiency test panels are being awaited for external quality assurance. The concordance of the laboratory's ELISA results to the RRL's is being monitored to ensure consistency in the test assays.

Regarding introduction of PCR assays, the laboratory is discussing the training and implementation timelines with the laboratory coordinators. A thermal cycler and other equipment have already been purchased and delivered to the laboratory.

4.2 Characteristics of wild polioviruses in Afghanistan, Pakistan, Somalia and Sudan

Mr Sohail Zahoor Zaidi, National Institute of Health, Pakistan, and

Dr Humayun Asghar, WHO/EMRO

Nucleotide sequence analysis (VP1 region, ~900 nt) of isolates from Pakistan and Afghanistan is performed by the regional reference laboratory at the National Institute of Health Pakistan. Sequencing of isolates from Somalia and Sudan is performed by the Cenetres for Disease Control and Prevention (CDC), Atlanta and the regional reference laboratory at the National Institute for Communicable Diseases (NICD), Johannesburg, South Africa.

Pakistan and Afghanistan: Wild poliovirus type 1 (WPV1) isolates from Pakistan and Afghanistan were distributed into three main clusters in 2007–2008: A-3A, A-3D, and B-4A. Twenty clusters (A-1, A-2, A-3B, A-3C, B-1, B-2, B-3,B-4B, C-1, C-2, D, E-1, E-2, F, G-1, G-2, G-3, H, I, and J) were inactive over the past two years. The declining number of clusters is an indication that endemic reservoirs continue to be cleared of WPV1. Within the active clusters, independent chains of transmission could be resolved.

WPV1 Cluster A-3A isolates signalled continued endemic circulation in Kandahar, Helmand and Oruzgan, with spread to Farah and Baluchistan. Karachi does not appear to be an endemic reservoir, but rather an indicator community. There is no evidence of sustained local circulation of the same lineages in Karachi. Instead, the Karachi lineages can be traced to an external reservoir. Karachi may be a high season transit point and transient amplifier of imported viruses. Central Sindh remains a key reservoir area within Pakistan. WPV1 spreads from Sindh to NWFP and Baluchistan.

WPV1 Cluster A-3D isolates underscore the point that Sindh continues to be an independent reservoir in 2008.

WPV1 Cluster B-4A isolates reveal that NWFP continues to be a reservoir area for WPV1. B-4A virus from NWFP reservoirs spread into Punjab in 2008, causing high-season cases.

WPV3 chains of transmission are at historic lows for Pakistan and Afghanistan. Clusters A, C, D, and E appear to have been eradicated. Within the Cluster B group, only B-1C has been found to be active. WPV3 B-1C has two main sub clusters of lineages.

WPV3 Cluster B-1C virus spread northward in 2007–2008 from the Kandahar reservoir to northern insecure areas in Pakistan and Afghanistan. The virus then spread from Nangarhar/NWFP to Islamabad. In addition, local WPV3 circulation in Helmand spilled over to Nowshera in NWFP between 28 July 2007 and 27 April 2008. A single 2008 isolate from Kandahar signalled continued activity in Kandahar/Baluchistan corridor in 2008. For unknown reasons, the Helmand lineages were more restricted than the main Kandahar WPV3 lineages

South Sudan and Western Ethiopia. In March 2008, orphan WPV1 was isolated from AFP patients in south Sudan who were living in western Ethiopia. Closely related viruses were isolated from Western Ethiopia cases on 8 April 2008 and 14 May 2008. Two additional closely related isolates were from 9 August 2008 and 20 August 2008 cases in south Sudan. The viruses originally spread from northern Nigeria in 2004. This I-1C5B

cluster signalled previously undetected local circulation of WPV1 in the adjoining areas of the two countries, and indicates the need for more sensitive surveillance in both countries.

Somalia. The last Somalia WPV1 isolate was from March 2007. The virus originated in Kano, Nigeria in 2004, and spread to at least 20 other countries, including Ethiopia, Kenya, Saudi Arabia, Sudan and Yemen. Cross-border transmission with Ethiopia was frequent.

4.3 Meeting the laboratory performance indicators in crisis situation – Iraq

Dr Fiasal Al-Hamadani, National Poliovirus Laboratory, Iraq

In spite of the fact that Iraq has been in an ongoing complex emergency situation since 2003, the laboratory continues to perform good quality work, while facing a variety of problems from personal safety to frequent electric power shortage/cuts and difficulty in transportation etc. The laboratory was accredited by WHO for 2008, with the onsite visit waived due to security situation.

The NPEV isolation rate during the first 40 weeks of 2008 is 6%. However, the sustained drop of this rate through 2008 from an annual average of 16% in previous years raises doubts regarding the quality of the stool specimen collection, storage and transport. The stool adequacy as reported by the routine immunization programme is around 90%. Only 7 out of 19 provinces were able to send 80% of samples within 3 days after collection of first specimen in the field.

The NPL is facing a lot of problems: non-receipt of laboratory supplies, acute shortage of reagents; frequent power cuts which may lead to loss of reagents, interruption of work, difficulty in accessing the internet; and high turnover of laboratory staff.

5. LABORATORY QUALITY ASSURANCE

5.1 Accreditation status of regional polio laboratories

Dr Humayun Asghar, WHO/EMRO

In 2007, 11 of 12 regional network laboratories were fully accredited. As of October 2008, 10 of 12 regional network laboratories are fully accredited by WHO, except the national poliovirus laboratories in Sudan, which is pending accreditation visits. The Kuwait regional reference laboratory was provisionally accredited in 2007 and 2008. 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 new timeliness of reporting of virological investigation results i.e. within 14 days from receipt of sample in the laboratory. In a few laboratories some gaps were found in implementation of cell sensitivity testing which were corrected after accreditation visits, otherwise, all laboratories were implementing quality assurance programme satisfactorily.

5.2 Report on proficiency testing (PT) – virus isolation and typing

Dr Esther de Gourville, WHO/HQ

All polio laboratories in the region reached 100% score in the 2007 proficiency test for isolation and typing. This remarkable achievement illustrated the reliability of the Eastern Mediterranean Region laboratory network.

For 2008, regional polio network laboratories will test a new type of Proficiency Testing panel for isolation of polioviruses and enteroviruses in stool samples, consisting of 10 stool specimens to be analysed according to the isolation part of the new algorithm. The new panel has been successfully field tested in 2007; VACSERA, Egypt, the Regional Reference Laboratory for Eastern Mediterranean was among three laboratories which participated in the field testing.

5.3 Proficiency Testing for isolation of polioviruses according to the new algorithm: results of field study

Dr Esther de Gourville, WHO/HQ

The new PT panel of stool samples has been evaluated in the field and will be implemented in the polio network laboratories of the Eastern Mediterranean Region in 2008. It will consist of 10 samples, mimicking real life situations and may contain single poliovirus (PV) or mixture of PV, enteroviruses (EV) which may grow only on HEp2 cells, other enteric viruses or a combination of three viruses. Possible final results reported per sample would be: negative or L20B-positive or NPEV or L20B-positive and NPEV. The results should be presented per arm in worksheets, and should be sent to reviewer within 14 days.

The basic principle of the scoring system is an optimal score for correct detection of virus-negative samples, for correct detection of poliovirus in poliovirus-positive samples, for correct detection of NPEV in NPEV-positive (only NPEVs growing on RD are scored as NPEV; NPEV growing on other cell lines only are scored as other enteric viruses)/poliovirus-negative samples, for correct interpretation of results obtained for samples with other enteric viruses, and no deduction of score for missing NPEV in polio-positive samples.

The scheme for scoring of results in PT for isolation of polioviruses according to the new algorithm is as follows:

- Correct analysis of poliovirus-positive sample: 20 points
- Correct analysis of poliovirus-negative sample: 5 points
- Contamination of polio-negative sample with poliovirus: 20 points
- Contamination of any sample with NPEV or other enteric virus: 10 points
- Incorrect interpretation of worksheets: 10 points

Penalties are subtracted from maximum points for a sample. If the final result is not according to the interpretation of rules in the new algorithm, then a penalty of 5 points is given. Final score is percentage of maximum number of points that can be scored. All laboratories should have 90% as a passing score

The new PT panel was tested in three laboratories in 2007: VACSERA (EMR), Institute Pasteur, Dakar, Senegal (AFR), ERC, Mumbai, India (SEAR). All polioviruses-positive samples were correctly reported as L20B-positive, and both negative samples were reported as negative by all three laboratories. None of the laboratories reported a contamination in one of the samples. (Poliovirus in polio-negative samples or NPEV in virus-negative samples). Coxsackie B1 virus in one sample which caused cytopathic effect (CPE) was reported in all three labs, but interpretation and reporting of final result was different: term non-entero virus (NEV) is accepted, but avoid using it, proper is to use NPEV.

5.4 Experience with using the new accreditation checklist in the Region

Dr Humayun Asghar, WHO/EMRO

The revised WHO accreditation checklist was introduced in January 2008 for evaluation of laboratories. Changes have been made to include new targets, for timeliness of reporting, evaluation of managerial functions, and evaluation of cell sensitivity for virus isolation. Nine of 12 polio network laboratories (Egypt, Pakistan, Kuwait, Islamic Republic of Iran, Iraq, Morocco, Oman, Saudi Arabia and Tunisia) were evaluated using the revised checklist. The checklist was very well understood, but laboratories faced a few problems in implementing the changes suggested in some sections of the checklist: organization chart, terms of reference of staff, cell sensitivity testing, supervision documentation and display of biosafety signs, recording of equipment calibration and maintenance, and development of guidelines for emergency procedures in case of fire or other emergency.

It was perceived that the revised checklist helped both reviewer and laboratory director to understand the laboratory organization and management issues. This in turn, helped them to address the weaknesses and gaps for smooth functioning of the laboratory.

5.5 Update on cell sensitivity testing in EMR network laboratories

Dr Javier Martin, WHO/EMRO

All laboratories in the Region have successfully implemented cell sensitivity testing.

The timely and critical evaluation of the results is crucial for the laboratory to fully benefit from this test. It is the responsibility of the laboratory director to critically evaluate the results and to implement immediate corrective measures if necessary (in coordination with the Regional Coordinator and NIBSC, if needed). The extent/impact of failed sensitivity tests (duration in time, cell line/s affected, serotype/s involved, whether failure is due to high or low titters, etc.) should be carefully assessed in order to decide if/what stool samples would need to be re-processed.

The availability of case study examples and troubleshooting guidelines would be very helpful to be aware of the type of corrective actions to be taken. Evaluation of trend results

including historical data would help the early detection of decreasing cell sensitivity for poliovirus infection contributing to the rapid implementation of corrective measures. Reports should be sent to the regional coordinator within the next 48 hours after results are available and should include all historical results, data showing the correct validation of laboratory quality controls (LQCs) and any relevant information concerning change/s in cell culture procedures. Reports should be sent in standard form saved as a file name that includes the name of the country and the date of the report. Examples using the standard form could be provided on request.

5.6 Follow-up on establishing cell sensitivity testing

1..5 Syrian Arab Republic

Dr Arraj Amira, NPL, Syria

New NIBSC standards were received in 2007, and new LQC standards were prepared in both L20B and RD cells, according to the WHO polio laboratory manual. The LQCs were tested three times for each poliovirus serotype in parallel with NIBSC standards. The virus titre for NIBSC standards on both RD and L20B cell lines were within expected range for each serotype, and also valid results were obtained of LQCs for each serotype on both cell lines.

After validation test, LQCs are used for routine cell sensitivity testing on both cell lines, half way through 15 passage cycles and before discarding the cells. On all occasions, except once when incubator temperature increased accidentally, the cell sensitivity test results on both L20B and RD cells have been within expected range (virus titre)

1..6 Morocco

Mr M. Benhafid, National Institute of Hygiene, Morocco

The NPL received NIBSC standards in 2007 in good condition. The LQC and NIBSC Sabin standards were tested on both RD and L20B cells received from VACSERA and Kuwait; the results showed lower titre than expected on both L20B and RD cells lines. This may be due to poor sensitivity of cells available in the laboratory or condition of the NIBSC standards or technical problems.

In 2008, fresh RD and L20B cell were received from CDC. The cell sensitivity testing method was reviewed carefully with NIBSC and laboratory coordinator, the validation of test was re-done, and good within range virus titre for all poliovirus serotypes was obtained on both RD and L20B cell lines. The NPL continues to perform the cell sensitivity test for both RD and L20B, mid-way during the recommended 15 passage of cell lines and a second test just before cells are discarded, to reassure that cells have maintained sensitivity during their use. Original quality control data sheets and summaries of corrective action are retained for documentation and discussion with reviewer.

5.7 Strengthening managerial and supervisory practices in the network laboratories

Dr Esther de Gourville, WHO/HQ

The expected outcome of laboratory activities is to provide the polio eradication initiative with consistent, high quality results in a timely manner. To achieve the cost-effective utilization of available resources and sustain activities, the managers should engage in good planning, supervision and give priority to quality assurance programmes. As an example, cell sensitivity testing helps to maintain good quality cells and it leads to better results.

Most problems encountered in the laboratory network can be traced to limitations in laboratory management and supervision, and this needs more emphasis within the network. Significant delays in identification and resolution of technical problems, individually and collectively, derive from ineffective communication and limits in tapping the extended resources of the network. There are a number of examples of poor management and supervision: i.e. Planning and monitoring, the laboratory informs the programme about procurement of reagent when it is about to finish; use of resources, inappropriate participant is sent to training workshop or keeps on trying to solve technical problems in laboratory without using the help of coordinator and losing the training or learning opportunity; supervision, standard operating procedure is not followed by the technician and modifications are made in the procedure.

Improvement in laboratory management and supervision can be achieved through good laboratory practices, adhering to quality assurance programmes, continuous or refresher training of staff, better resource management (both human and financial), and using a constructive approach towards problem solving. Improvements in laboratory management represent another opportunity to contribute to the legacy of the polio eradication initiative.

6. NEW TEST ALGORITHM

6.1 Progress and impact on introduction of new test algorithm in the remaining endemic countries

Dr Esther de Gourville, WHO/HQ

According to 2006 GPLN recommendations, a new test algorithm was implemented with the objective of reducing reporting time, and it was implemented in polio endemic *regions as a priority. It was also recommended that 75% of the workload in polio endemic regions should be tested in laboratories with capacity for both virus isolation and ITD.*

A number of activities were conducted to implement the new test algorithm: briefing of laboratory directors on technical issues during laboratory network meetings, ITD training workshops were conducted (Uganda, November 2006), establishment of 9 new ITD labs (UGA, MAD, KEN, CAE, MOR, SYR, Kasauli and Ahmadabad in India, DPRK), switching from probe hybridization to PCR in 6 laboratories (IBD-NIE & GHA, Lucknow and Chennai in India, BAN-Indonesia, and Sri Lanka), cell culture training workshop in South Africa in

April 2008 as follow up to implementation. In parallel other activities were also conducted: Revision of Standard Operating Procedures (SOP) within individual laboratories, follow up on ITD training workshops, laboratories to do ITD PT tests, new ITD laboratories tested polioviruses in parallel with RRL until accuracy in routine work achieved, procurement of equipment and reagents for new ITD and high workload laboratories, orientation of surveillance and data management personnel, revision of databases and format of reporting within laboratories and for regional bulletins (EMRO and AFRO completed). It took around two years for the full implementation of new algorithm.

There was remarkable impact of implementation of new test algorithm. The number of laboratories with appropriate virus isolation and ITD capacity increased from 17 (in January 2006) to 26 laboratories in 2008. The workload of laboratories in endemic regions with appropriate capacity increase from 59% (in January 2006) to 68%: from 22% to 62% increase in AFR, remained at ~ 90% in EMR, remained at ~63% in SEAR. In non-endemic regions, AMRO adopted the new algorithm, and workload tested in laboratories with appropriate capacity increased from 85% (in January 2006) to 90%. EURO and WPRO have not implemented the new test Algorithm. There was significant impact on reporting time of cell culture and ITD results: percent virus isolation within 14 days and ITD within 7 days improved in all the regions, except in SEARO for the reason being 300 times more workload and slow pace of increasing the number of labs with ITD capacity. There was also improvement in mean time (in days) for WPV confirmation. It was noted that some of the laboratories had confusion in calculation the reporting times, which was corrected.

It is concluded that implementation of new test algorithm is on track in 2 endemic regions, except in SEARO. Multiple factors contributing to slower progress in SEARO need to be addressed: make new ITD laboratories fully functional, re-distribute workload, give attention to improving efficiency and management and use the correct variables in calculating laboratory indicators. Generally, there was significant documented improvement in reporting times. With this progress and continuous support of the network laboratories, it is evident that new reporting times are achievable. For the implementation of the new test algorithm in European and Western Pacific Regions, new ITD labs should be established to increase the capacity.

6.2 Experiences with addressing factors affecting implementation of new test algorithms

1..7 Cell culture

Mr Mohamed Masroor, WHO/Pakistan

Pakistan has implemented the new testing algorithm since May, 2006 after a successful pre-implementation evaluation. Implementation of new testing algorithm significantly reduced the time of virus culture and ITD results. This new strategy of testing resulted in improved the timeliness for Sabin viruses from 18 to 11 days and for wild viruses from 12 to just 5 days, in Pakistan RRL.

Implementation of the new algorithm generally resulted in increase in workload and specifically in cell culture laboratory. Despite this increase the laboratory performance indicators have been sustained at certification standard without compromising performance. There was an increase in the consumption of tissue culture flasks, tubes and reagents' use of the new testing algorithm decreased the amount of ITD testing due to setting up of PCR for most of the isolates positive on L-arm of the new algorithm. There was a significant decrease in poliovirus neutralization testing, which is only performed on monotypic Sabin-like viruses or mixtures of Sabin-like viruses detected by PCR.

In conclusion, the new testing algorithm significantly meets the programme needs for immediate reporting of poliovirus isolates. It also emphasizes strict monitoring of the cell sensitivity testing data. Stringent attention should be given to logistics and supplies stocks/demand to avoid any unnecessary delay in laboratory working.

1..8 Intratypic differentiation (ITD)

Dr L. El-Baissouni, VACSERA, Egypt

ITD tests were performed according to the new algorithm in 2007. During that period all samples positive on both L-arm and/or R-arm were tested for ITD by PCR method as primary test, followed by ELISA if viruses are Sabin-like (both monotypic and mixtures). Later in October 2007, as per recommendation L-arm positive samples were preferably tested by PCR. To switch from the old to new algorithm, changes were made in worksheets for virus isolation and ITD methods; similarly, changes were made in the database.

From January 2007 to as of October 2008, through ITD testing methods 7 samples were found positive for WPVs, 238 samples Sabin Like viruses, 40 mixtures of Sabin Like viruses and 7 mixtures of Sabin Like and NPEVs. The 13 samples were discordant by ITD methods, out of which two were confirmed as VDPVs by nucleotide sequencing. There was 7 fold increases in workload in PCR with new algorithm comparing with last year. There was also significant increase in neutralization test and ELISA testing due to isolation of large number of Sabin Like monotypic and mixtures. These increases in workload proportionate the increase in logistic demand.

1..9 Sabin mixtures and discordants

Dr S. Shahmahmoodi, National Poliovirus Laboratory, Islamic Republic of Iran

The new test algorithm has reduced the time for final ITD result from 14 to 7 days. However, this is not easy to achieve, especially when PCR shows mixed Sabins in the specimen, and so neutralization (NT) prior to ELISA is necessary.

In the NPL of the IRAN, PCR is often performed on the same day of virus isolation, and coating for ELISA is done as soon as the PCR result is available. Therefore, the final ITD result for monotypic viruses is reported within 2 days after reporting the culture result.

When PCR shows a mixture of Sabins, the specimen is sent for serotyping and separation of mixtures. Serotyping by neutralization test usually takes approximately 3 or 4 days, and if the virus titre is not enough for ELISA, re-inoculation of the monotype virus is necessary which requires an additional 1 or 2 days. There is a definite need for re-inoculation if the virus is Polio3 because 400 micro-litre of monotype virus is not enough for chloroform treatment, and it takes 1 or 2 days more. Overall, the time needed for NT is 3- 6 days; more if there is a 2 day weekend or a national holiday.

Considering 0 to 1 day for PCR, 3 to 6 days for NT and 1 to 2 days for ELISA, it is obvious that final ITD result sometimes cannot be obtained within 7 days, creating great pressure to the laboratory.

NPL-Iran has been able to meet the 7-day deadline for Sabin mixtures by minimizing the time for PCR and ELISA, so that these tests are done as soon as the result of the previous tests are available (for PCR the same day the culture result is reported; for ELISA the same day NT result is reported). Furthermore, if ITD testing is near the weekend, there is every chance that deadline of days may not be met, unless the process is completed during the weekend. So far there are no problems with the discordant isolates, because they were mainly fast growing monotypic viruses.

1..10 Reporting

Dr S. El-Busaidy, Ministry of Health, Oman

The LABIFA software has evolved through the years from version 1 to the current version 4. The software is comprehensive and is constantly being updated according to many individual requirements. Although LABIFA4 requires entry of large number of variables and validations, which is time consuming, it is comprehensive and a good fit for the programme. There are a few minor issues:

- Due to many validations of data, fields sometimes block, which cannot be edited
- Editing of information has to be done from the bottom up
- There are many date fields, which can sometimes be confusing for the user, and for new users it is not a "friendly" database.

The inclusion of districts in LABIFA4 has simplified data entry. The automated reports are transmitted to the EPI in Oman, and to Saudi Arabia and Yemen. For Yemen and Saudi Arabia reports are sent weekly; this will be changed to same day reporting for all positive samples.

6.3 Group discussion: new testing algorithm

Group discussion 1: Virus isolation

***Moderators**: Dr Esther de Gourville, WHO/HQ; Dr Humayun Asghar, WHO/EMRO; Dr Javier Martin, WHO/EMRO*

For this group discussion the countries were divided into two groups. Group 1: Egypt, Islamic Republic of Iran, Iraq, Oman, Pakistan and Sudan and KEMRI, Kenya. Group 2: Jordan, Morocco, Saudi Arabia, Syrian Arab Republic and Tunisia.

The discussion began by reviewing the laboratory procedures for virus isolation adapted by the countries included in the discussion.

All laboratories are using both L20B and RD for virus isolation. The cell passage cycle is continued without any change, and 10% and 2% filterable minimum essential media (MEM) is in use for the propagation and maintenance of both cell lines, i.e. RD and L20B. There is an increase in workload due to the passaging of cells twice a week. Regarding the composition of MEM, it is available in two different formulations based on the salt included i.e., Hank's salt and Earle's salt. Hank's salt is mostly suitable for working with non-CO_2 incubations while Earle's salt is preferred for CO_2 incubators. MEM with Hank's salt is being used at polio laboratories but manufactured by three different companies: Gibco, Sigma and Euro-Bio. These media were given the best results without any problem in cell culture. The water added to the media should be deionized double distilled water with p^h 7. The final p^h of the medium should be in range of 7.2 to 7.4.

The cell culture medium preparation involves the addition of L-glutamine, HEPES buffer, fetal calf serum (FCS), sodium bicarbonate and antibiotics. The quantity of each component of the medium is followed as per recommended in the Polio Laboratory Manual 2004. Sterility testing is performed by inoculating in thioglycate broth against all reagents and each batch of media prepared, in duplicate and incubated both at room temperature and 36 °C to rule out any contamination of reagents or during working. L-glutamine preparations have a short half-life and retain optimum working for three to four weeks when refrigerated. Also, HEPES buffer should be kept in dark as it generates some toxic products when exposed to light. It was noted that some of the laboratories were using a low concentration of FCS to slow the growth of cell culture. It was agreed that seeding density should be optimized with 10% and 2% MEM, for the confluence growth of cell monolayer to be completed during the working day. The cells prepared should be kept incubated at 36 °C for 48 hours for the optimum growth of the cells in the cell culture tubes followed by replacement 2% growth medium before sample inoculation. There is no need to change the media during cell maintenance. All laboratory procedure should be documented and change or deviation in standard procedure should be documented.

The cell growth and sensitivity data should be regularly monitored for any new reagent or new batch of cells. The supervision of cell sensitivity testing should include cross checking by at least one other experienced staff member, and test plates should not be thrown away by the technician until validated by the supervisor. If any problem in cell sensitivity is found, the supervisor should immediately take action to solve the problem, and in the meantime take advice of the laboratory coordinator and NIBSC.

While reporting results the consideration should be given to the results obtained from both the L-arm and R-arm of the new algorithm, the final result should be a combination of both arms results. Any sample giving non-specific CPE should be reported as negative; however, the sample should be referred to CDC for further characterization of virus.

Group discussion 2: Intratypic differentiation

***Moderators**: Dr Esther de Gourville, WHO/HQ; Dr Humayun Asghar, WHO/EMRO; Dr Javier Martin, WHO/EMRO*

For this group discussion the countries were divided into two groups Group 1: Egypt, Islamic Republic of Iran, Morocco, Oman, Pakistan, Syrian Arab Republic, Tunisia, and KEMRI, Kenya. Group 2: Jordan, Iraq, Saudi Arabia and Sudan. A number of issues were raised and recommended actions were made.

All the laboratories are following the new testing algorithm for poliovirus intratypic differentiation by PCR and obtaining excellent results. The Iran NPL experienced low titre values in ELISA for polio type 3 viruses but improved with Chloroform treatment of the isolates after consultation with the regional coordinator. The Morocco laboratory recently established PCR and scored 100% in the proficiency panel. The major concern of all the laboratories is related to the timely provision of supplies and reagents. The regional coordinator explained the adaptation of a new procurement system by WHO for purchase of reagents and advised to keep a keen eye on the stock of reagents and laboratory equipment to avoid any delay in the routine work.

The transportation of samples from NPL to ITD laboratories is a chronic problem and needs to be fixed. It was suggested that laboratory directors should search for the best courier service in their respective country, preferably those with door to door services. The infectious material should be labelled properly according to IATA regulations, and it should be packed in a triple envelope at proper temperature.

The Tunis RRL informed the group about the protocol for the production of Sabin-like control to be used in ITD. She will share this protocol with the polio laboratory coordinator, who after review will distribute to ITD laboratories.

7. DEVELOPMENT AND EVALUATION OF REAL TIME PCR ASSAYS FOR ITD AND VDPV SCREENING

7.1 Overview of real-time PCR assay for ITD and VDPV screening

Dr Javier Martin, WHO/EMRO

The PCR technique, long established in the network, can now be used as the primary method for discriminating wild from vaccine-related isolates as implemented in the new ITD algorithm. The possibility of adapting this conceptual approach to a real-time PCR platform has been explored. The use of real-time PCR offers a number of advantages such as its increased sensitivity and the fact that there are no gels to run which would result in important savings in time and money. There is also a decreased risk of cross-contamination since sample tubes need not to be opened between reaction steps. All data are obtained in electronic form which gives laboratories the opportunity for integration in diagnostic databases.

A real-time prospective assay developed at CDC was evaluated to demonstrate sensitivity and specificity using panels of known characterized viruses. The assay was evaluated at CDC and three pilot sites on retrospective collections of viruses and in parallel testing with the established PCR ITD. The test was validated, completed initial evaluations, and its pilot testing showing very promising results. Technical, training and implementation issues were identified and used to refine assay training and implementation plans, which will require resource mobilization for equipment, training and supplies.

The possibility of using the real-time method for VDPV screening has also been evaluated. The assay targets specific nucleotides in the VP1 region known to mutate in VDPV strains and the 3D region to search for intertypic recombination. Assay development and pilot studies have also shown promising results. The VDPV real-time test is currently being evaluated by Global Specialized Laboratories to include all known VDPV strains. After full validation, a new real-time algorithm will be adopted, which would eliminate the requirement for serotype separation and ELISA-ITD that has failed to identify some VDPV strains. The new test will most likely result in faster and more accurate results but would require some changes in equipment, expertise and proficiency testing.

7.2 Preliminary results of pilot testing of real-time PCR assay in Pakistan

Mr S. Zaidi, National Institute of Health, Pakistan

A retrospective evaluation for real-time PCR (rRT-PCR) was performed in Pakistan during early 2008. The evaluation testing was performed on a total of 200 isolates including wild type from Pakistan and Afghanistan for three years from 2006 to 2008. The assay targets were successfully achieved with the following findings:

Preparation of PCR reaction mix is similar to traditional assay. The data analysis after test run is based on a kind of software. Amplification graph and cycle threshold (Ct) values are used together to decide positive or negative results. Also, the biosafety risk is less with qRT-PCR in comparison to traditional assays. Use of extracted RNA as template significantly improved the quality of results. Training of personnel for result interpretation and troubleshooting should be managed prior to the implementation of the assay. Relative operational cost of RT-PCR is less than traditional assays. A universal report format for reporting the result is needed which can be used in the network.

In conclusion, real-time PCR has improved the sensitivity of poliovirus detection and facilitated the timeliness of reporting of ITD results. The assay results were found to be 100% in concordance with results obtained previously.

7.3 Proficiency test for Real time PCR assay

Dr Javier Martin, WHO/EMRO

The molecular diagnostic PT panels consist of unknowns of in vitro RNA transcripts containing sequences targeted by probe hybridization and PCR. Transcripts are non-infectious, are of positive (genome “sense”) polarity, and contain 5′-UTR and VP1 sequences. Format for the real-time RT-PCR will follow standard diagnostic PCR PT Panels. Unknowns to be used with real-time ITD kits include: 1) panEnterovirus primer pair + probe (target: 5′-UTR), 2) panPoliovirus primer pairs + probe (target: VP1), 3) Serotype-specific primer pairs + specific probes (target: VP1; three sets), and 4) Sabin-strain-specific primer pair + specific

probes (target: VP1; three sets). We propose to prepare separate panels of Sabin-related unknowns (VDPVs and non-VDPVs). Unknowns to be used with real-time VDPV kits will include: Sabin-strain-VDPV specific primer pair + specific probes, three sets targeting VP1 and three sets targeting 3Dpol. Preparation of the appropriate VDPV control RNAs is in progress. Regional laboratories have performed with very high proficiency with the molecular PT panels. All but one of the seven regional reference laboratories scored ≥95% with excellent turnaround times with the last PT panel (9) for the standard diagnostic RT-PCR assays.

7.4 Introduction to the revised LABIFA 4.0, and problems encountered in data management and their solutions

Dr H. Safwat, WHO/EMRO

Revised LABIFA version 4.0 is now being implemented in the 12 laboratories of the region and includes the polio surveillance data of 2007 and 2008 according to the new test Algorithm. At country level shifting to new system started in April 2008 and at the Regional Office in September 2008. The Polio Fax was issued in the new format on 14 September (week 37/2008).

New variables and codes were added to LABIFA version 4.0 to integrate additional requested information and validations were used to ensure high quality during the data entry. A large number of ready-made reports were developed in the new system including reports on errors in data entry, performance indicators, accreditation of laboratories, timeliness of specimen processing and VDPVs.

To ensure sustainability of data quality and avoid problems and errors in data entry it is recommended to run regularly error reports retrospectively from January 2007 up to date, run system reports regularly and interpret the outcomes, and do backups.

8. OTHER ISSUES

8.1 Integrated laboratory services for surveillance of vaccine preventable disease

Dr Esther de Gourville, WHO/HQ

As the AFP surveillance system has matured, it has been applied to the detection of other high-priority vaccine-preventable diseases. The concept for the integrated laboratory support for surveillance is a global network of laboratories based on the Global Polio Laboratory Network (GPLN). The Regional Laboratory Network is an excellent example of the broad benefits of laboratory network building. This will be part of the mainstreaming of the polio eradication infrastructure. Despite the compelling need to rapidly expand to broader VPD surveillance, the current capacity for expansion is limited, and there is serious risk of overstretching the existing laboratory network coordinators. The Global Framework on Immunization Monitoring and Surveillance (GFIMS) was developed by WHO in collaboration with global partners, and was endorsed by the SAGE. The GFIMS was based on the four guiding principles to link with other surveillance and monitoring systems: with other VPD surveillance and monitoring, with non-VPD surveillance and monitoring, with the

private sector, and links where appropriate and feasible. The most important aspect is that it should be done without any compromise in quality.

While attempting to integrate laboratory networks, questions to be addressed are about the kind of integration, rationale, opportunities, limitations and structure. The success of the GPLN was due to effective management, excellent communications, clear and broadly agreed upon goals, demonstrable impact of intervention, standardized procedures, continued evolution of diagnostic tools and opportunity for technological growth.

The advantage of integrating the network for VPD is that it is a highly cost-effective intervention and there is ability to monitor impact of intervention. There are many partners who are interested and examples are extension of the polio and measles/rubella laboratory networks. Factors to be considered when setting up VPD surveillance are disease control objectives, disease burden, properties of the infectious agent, clinical presentation/attack rates, antigenic types/variability, specimen transport requirements, local context/infrastructure, and resource needs. Care should be taken as it may be trying to do too much too fast and underestimating the complexities of network building. The global resources are still far too limited and at the present time there is a limited pool of well-trained staff.

The benefits of the integration of laboratory networks can lead to facility improvements, equipment, supplies and an efficient specimen transport and referral system. There will be more ease of staff training, standardization of techniques, data management and reporting. Above all there will be better coordination between laboratories and between the laboratory networks and the immunization programme. However, there are obstacles to complete and smooth integration, as not all VPDs are in the routine immunization programme, and programmes have different objectives and needs. It is possible and desirable to expand and integrate laboratory networks for broader VPD surveillance, but will require additional resources, including more laboratory network coordinators.

8.2 Status of survey and inventory of Phase 1 of laboratory containment of wild polioviruses

Dr H. Asghar, WHO/EMRO

The polio laboratory network coordinators were informed of their important role in laboratory containment of polioviruses and other potential infectious material, because in EMR most of the poliovirus materials are stored in polio laboratories and/or they are national containment coordinators or member of national containment committees.

Eighteen countries (Bahrain, Djibouti, Egypt, Islamic Republic of Iran, Iraq, Jordan, Kuwait, Lebanon, Libyan Arab Jamahiriya, Morocco, Oman, Palestine, Qatar, Saudi Arabia, Syrian Arab Republic, Sudan, Tunisia and United Arab Emirates) have reported completion of 1 of laboratory survey and inventory activities. National plans of action have been developed by Afghanistan and Yemen and were submitted for the approval of their ministries of health. To date no information has been sent regarding their status. As regards Pakistan, despite several efforts, it has not been possible to initiate preparations for Phase 1 of laboratory survey and inventory of laboratories for containment of wild poliovirus and potentially

infectious material. To date, 19 775 laboratories have been surveyed and only 9 laboratories have been identified to be storing WPV material.

All countries that have completed Phase 1 of containment activities were required to submit quality assurance report. Documentation of the quality of Phase 1 of containment activities was submitted by fifteen countries (Bahrain, Djibouti, Islamic Republic of Iran, Iraq, Jordan, Libyan Arab Jamahiriya, Morocco, Oman, Qatar, Saudi Arabia, Sudan, Syrian Arab Republic, Tunisia and United Arab of Emirates). The original or revised report has not been submitted by 6 countries (Djibouti, Egypt, Kuwait, Lebanon, Palestine and Syrian Arab Republic). Egypt and Palestine recently completed Phase 1 of containment.

9. CONCLUSIONS

Countries of the Eastern Mediterranean Region have made considerable progress towards the polio eradication goal. Poliovirus transmission has been interrupted in 20 of the 22 countries in the Region and only Afghanistan and Pakistan are still considered to be endemic. In 2007, the total number of reported cases was the lowest ever recorded in the Region (58), and an outbreak due to imported virus in Somalia was controlled with the last cases detected in March 2007. In 2007, there was evidence suggesting geographic restriction and decreasing genetic diversity among isolates from Afghanistan and Pakistan, although there was upsurge in the number of confirmed wild polio cases in 2008 with 86 cases reported in Pakistan and 22 in Afghanistan up to October. Additionally Sudan, which had been polio free since 2005, reported 8 polio cases in 2008, 7 of them due to wild poliovirus type 1 (WPV1) reported from southern Sudan, and one confirmed polio case due to importation of wild poliovirus type 3 (WPV3) in west Darfur. Egypt, similarly, has not reported WPV cases since May 2004, but is continuing testing of sewage samples as a supplement to AFP surveillance activities. Two aVDPVs of poliovirus type 1 were isolated from sewage in Behira governorate in Egypt, one in December 2007 and the other in April 2008. In September 2008, WPV1 was detected in a sewage sample collected from Al-Haram, in Egypt, which is to be characterized by nucleotide sequencing of VP1 region of the poliovirus genome.

Between January 2007 and October 2008, vaccine derived polioviruses (VDPV) were isolated from acute flaccid paralysis (AFP) cases, who were subsequently confirmed to be from immunodeficient patients. These included two children in the Islamic Republic of Iran (one with mixture of type 1 and 2, and another with type 2), and one child in Egypt (type 3). Isolates from these cases were classified as iVDPVs. There was no evidence of secondary spread of VDPVs from any of the immunodeficient persons.

The laboratory network continues to support AFP surveillance activities efficiently and high quality of performance continues to be maintained. All network laboratories passed the WHO proficiency panel tests for both poliovirus isolation and intratypic differentiation testing and all laboratories are fully accredited except Kuwait, which is provisionally accredited.

A remarkable achievement of the polio laboratory network in 2007 was the successful implementation of the new testing algorithms for virus isolation and ITD. The average

reporting time from sample receipt in laboratory to final ITD results has decreased from 13 days in 2007 to 11 days in 2008. The main challenge in implementing the new algorithm has been the increase in workload resulting in increased resource needs for sample testing supplies and ITD reagents. Adjustments were also required to the laboratory database.

Laboratories are now being evaluated according to a revised WHO accreditation checklist that was introduced in January 2008. Changes have been made to include new targets, for timeliness of reporting, evaluation of managerial functions and evaluation of cell sensitivity for virus isolation. Laboratories faced a few problems in implementing the changes suggested in some sections of the checklist: organization chart, terms of reference of staff, cell sensitivity testing, supervision documentation and display of biosafety signs, recording of equipment calibration and maintenance, and development of guidelines for emergency procedures in case of fire or other emergency.

The meeting concluded that the regional laboratory network was successfully overcoming challenges presented by the increasing workload, and logistic and infrastructural challenges brought about by insecurity (Iraq) or deteriorating electricity supply (Pakistan), without compromising quality, adequacy or timeliness of reporting of laboratory results.

10. RECOMMENDATIONS

1. WHO should continue to advocate with national authorities in Member States and partner agencies for continued support of polio network laboratories.

2. All network laboratories should work in close collaboration with the regional laboratory network coordinator to further improve laboratory performance. This should include fully implementing quality assurance, management and supervision, improving bio-safety and security, helping to ensure electricity supply, developing guidelines for emergency procedures like fire or any other emergency, and proper inventory of laboratory supplies. The national authorities should be sensitized for preventive maintenance of equipment, and onsite visits to laboratories should be performed to assess the status of equipment.

3. The ITD laboratories testing samples for other countries should report results as they become available and not delay reporting in order to submit weekly batch reports.

4. Cell sensitivity testing should continue to be performed in all laboratories halfway through the recommended 15 cell passage cycle and again before discarding the cells, using Laboratory Quality Control (LQC) standards for the three polio serotypes. The laboratory director should critically evaluate results and implement immediate corrective measures, if needed. Reports should be sent to the regional laboratory network coordinator and NIBSC within 48 hours of completing tests and should include ALL historical results, data showing the correct validation of LQCs and any relevant information concerning change/s in cell culture procedures. The EMRO

laboratory network coordinator will send appropriate feedback to laboratories within the subsequent 72 hours.

5. The global polio laboratory network envisages implementing real-time PCR assays for ITD and VDPV screening in 2009 pending mobilization of resources. In anticipation of this change, WHO/EMRO should update LABIFA4 software in accordance with the interpretation of real-time PCR tests, data entry and automated reporting.

6. All laboratories should continue to work closely with the regional laboratory network coordinator and the data manager to identify gaps and problems of data entry and analysis to improve the functioning of the new LABIFA4 management system software. WHO/EMRO should support laboratory data managers, when required, through visits and training.

7. New ITD laboratories in Syrian Arab Republic and Morocco, should continue to share ITD test information (including completed worksheets) with the regional laboratory network coordinator for assistance with troubleshooting, until their laboratories are fully accredited by WHO to perform ITD methods.

8. The expansion of laboratory support to include other vaccine-preventable disease programmes should be pursued in ways that do not divert resources specified for polio eradication and there must be allocation of adequate resources for coordination of any additional activities.

Annex 1

Monday, 27 October 2008

08:00-08:30	**Registration**	
08:30-09:30	**Opening Session**	
	– Welcome and Opening Remarks	
	– Message from Dr Hussein A. Gezairy, WHO Regional Director for the Eastern Mediterranean Region	Dr Ibrahim Betelmal, WR SYRIA
	– Message from H.E MOH Syria	Dr Maysoun Nasri Deputy Minister of Health for Pharmaceutical and Laboratory Affairs
	– Election of the Chairman and Rapporteur	
	– Implementation status of recommendations of the 11th Intercountry Meeting of Directors of Poliovirus Laboratories	Dr H. Asghar, WHO/EMRO

Session 1: Overview

09:30-09:45	Overview of polio eradication in EMR	Dr F. Kamel, WHO/EMRO
09:45-10:00	Status of global polio laboratory network	Dr E. de Gourville, WHO/HQ
10:00-10:15	Progress of the regional polio laboratory network	Dr H. Asghar, WHO/EMRO
10:15-11:00	Discussions	

Session 2: Virus surveillance

11:30-12:10	**Laboratory performance indicators**	
	Pakistan and Afghanistan	Mr S. Zaidi
	Egypt	Dr E. Al Maamoun
	Sudan	Mr H. Babikar
	Somalia and South Sudan	Mr P. Borus, KEMRI
12:10-12:30	Molecular characteristics of wild polioviruses in Pakistan, Afghanistan, Sudan and Somalia	Mr S. Zaidi/ Dr H. Asghar
12:30-12:45	Meeting the laboratory performance indicators in crisis situation – Iraq	Dr F. Al Hamdani
12:45-13:15	Discussion	

Session 3: Laboratory quality assurance

14:00-14:15	Accreditation status of EMR polio laboratories	Dr H. Asghar, WHO/EMRO
14:15–14:30	Proficiency testing (PT), for isolation of poliovirus according to the new algorithm results of field study	Dr E. de Gourville, WHO/EMRO
14:30-14:45	Virus Isolation Typing	…….
14:45-15:15	Discussion	
15:15–15:30	Experience with using new accreditation checklist in EMR	Dr H. Asghar, WHO/EMRO

16:00–16:20	Update on cell sensitivity testing in EMR laboratories, and discuss the re-testing of LQCs which are in storage for more than 3 years	Dr J. Martin, WHO/EMRO
16:20-16:40	Follow-up on establishing cell sensitivity testing	
	– Syrian Arab Republic	Dr A. Arraj
	– Morocco	Mr M. Ben Hafid
16.40–17:00	Strengthening Managerial and supervisory practices in the network laboratories	Dr E. de Gourville, WHO/HQ
17:00 – 17:30	Discussion	

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Session 4: New test algorithm

09:00-09:30	Progress and impact of introducing of new test algorithm testing in the remaining endemic countries	Dr E. de Gourville, WHO/HQ
09:30-10:30	Experiences with addressing factors affecting implementation of new test algorithms	
	Cell culture	Mr M. Masroor
	ITD	Dr L. El Bassioni
	Sabin mixtures and discordant	Dr S. Shahmahmoodi
	Reporting	Dr S. Al Busaidy

10:30–10:45	Introduction to the group discussion: highlighting the challenges faced by the labs in implementing the virus isolation and intratypic differentiation (ITD) testing algorithm, and introduction to group discussion	Dr H. Asghar, WHO/EMRO
11:15-13:15	Challenges in meeting timeline for virus isolation and ITD	

Group discussions: <u>Virus isolation:</u>

Discuss cell culture cycles, propagation, quality assurance, logistics, supplies, results reporting and problems encountered etc.

<u>Group 1:</u>

Egypt, Iran, Iraq, Oman, Pakistan, Sudan

Moderators:
Dr E.de Gourville, WHO/HQ
Dr H.Asghar, WHO/EMRO

<u>Group 2:</u>

Jordan, Kuwait, Morocco, Saudi Arabia, Syria, Tunisia

Moderators:
Dr J. Martin, WHO/EMRO

NOTE: each lab will discuss its individual problems

Discussion

14:15-16:15	**Group discussions: Intratypic differentiation** **<u>Group 1:</u>** Egypt, Iran, Kuwait, Morocco, Oman, Pakistan, Tunisia, Syria	Moderators: Dr E. de Gourville, WHO/HQ Dr H. Asghar, WHO/EMRO
	<u>Group 2:</u> Jordan, Iraq, Saudi Arabia, Sudan,	Moderators: Dr J. Martin, WHO/EMRO
16:15-17:00	Group presentation and discussion	

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Session 5: Development and evaluation of real time PCR assays for ITD and VDPV screening

09:00-09:20	Overview of real-time PCR assay for ITD and VDPV screening	Dr Javier Martin, WHO/EMRO
09:20-09:40	Preliminary results of pilot testing of real-time PCR assay in Pakistan	Mr S. Zaidi, Pakistan
09:40-10:00	Proficiency test for real-time PCR assay	Dr Javier Martin, WHO/EMRO

10-10:30	Discussion	
11:00-11:10	Introduction to the revised LABIFA 4.0 and problems encountered in data management and their solutions	Dr H. Safwat, WHO/EMRO
11:10-12:00	Discussion	

Session 6: Other Issues

12:00-12:20	Integrated laboratory services for surveillance of vaccine preventable disease	Dr E. de Gourville, WHO/HQ
12:20-12:30	Status of survey and inventory required in Phase 1 of laboratory containment of wild polioviruses	Dr H. Asghar, WHO/EMRO
12:30-13:00	Discussion	
14:00-15:00	Discussion on conclusion and recommendations	
15:00	*Closing of meeting*	

Annex 2

LIST OF PARTICIPANTS

EGYPT
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Dr Humayun Asghar, Regional Poliovirus Laboratory Network Coordinator, WHO/EMRO
Dr Esther de Gourville, Global Poliovirus Laboratory Network Coordinator, WHO/HQ
Dr Hala Safwat, Technical Officer, WHO/EMRO
Dr Javier Martin, Temporary Adviser, WHO/EMRO
Mr Mohamed Masroor, Virologist, WHO Pakistan
Mr Hatim Babiker, Laboratory Technician, WHO Sudan
Mr Karim Al Hadary, IT Assistant, WHO/EMRO
Ms Abir Hassan, Senior Administrative Clerk, WHO/EMRO