WHO-EM/POL/383/E

Report on the

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

Amman, Jordan
26–28 October 2009

World Health Organization
Regional Office for the Eastern Mediterranean

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1. INTRODUCTION

The thirteenth intercountry meeting of directors of poliovirus laboratories in the WHO Eastern Mediterranean Region was held in Amman, Jordan from 26 to 28 October 2009. Directors of poliovirus laboratories in Egypt, Islamic Republic of Iran, Iraq, Jordan, Morocco, Oman, Pakistan, Sudan and Syrian Arab Republic attended the meeting. Participants also included scientists from the National Institute of Public Health and the Environment (RIVM), the Netherlands, and the National Institute for Biological Standards and Control (NIBSC), United Kingdom, and staff from the World Health Organization (WHO) headquarters and regional offices for the Eastern Mediterranean (EMRO) and Africa (AFRO).

Dr Hashim Ali El Zein El Moussaad, WHO Representative of Jordan, 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 excellent work performed by the regional polio laboratory network. He expressed appreciation for the readiness of the polio laboratory network to adapt to changing technologies and approaches to meet the emerging needs of the polio eradication initiative. He acknowledged the network's support to surveillance and laboratory support to other vaccine-preventable diseases such as measles and rubella and, lately, H1N1 infection.

Dr Adil Belbeisi, Director Primary Health Care Administration, Ministry of Health, Jordan, welcomed the participants on behalf of His Excellency Dr Nayef Al Fayez, Minister of Health. He highlighted that routine immunization had been kept above 95% for more than two decades and that the government was fully committed to the poliomyelitis eradication initiative. He emphasized the need for the maintaining certification standard AFP surveillance, and expressed satisfaction with the performance of Jordan's national poliomyelitis laboratory in supporting virological analysis of AFP stool samples and in completing the first phase of survey and inventory of laboratory containment of wild polioviruses.

Dr Mustafa Karasneh (Jordan) was elected to Chair the meeting and Mr Shahzad Shaukat (Pakistan) was elected Rapporteur. The programme and list of participants are included as Annexes 1 and 2, respectively.

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

Dr Humayun Asghar, WHO/EMRO

It was shown that the recommendations of the twelfth intercountry meeting of directors of poliovirus laboratories in the Region addressed to national authorities and to WHO were implemented. It was also emphasized that many of these 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

Dr Faten Kamel, WHO/EMRO

The Eastern Mediterranean Region is making progress towards polio eradication and polio-free status is being maintained in 19 countries. Endemic transmission is continuing in Pakistan and Afghanistan, and Sudan suffered an outbreak that started in 2008 with the last case in June 2009.

The main reasons for continuation of endemic wild poliovirus transmission in parts of Pakistan and Afghanistan is impaired access to children in some areas due to insecurity, especially in the conflict-affected areas of southern Afghanistan and NWFP/FATA, or where managerial issues exists (Karachi and Quetta block). 21 of the 24 cases reported in 2009 in Afghanistan are from the insecure southern region (mainly Kandahar and Helmand provinces). Pakistan reported 72 cases so far in 2009, 41 of them from NWFP/FATA, mainly from Swat and Bajour districts. Interrupting virus transmission in the Pakistan and Afghanistan block depends on ensuring access to children in the security-compromised areas, continuing innovative approaches, pursuing efforts to enhance ownership and accountability, addressing managerial issues and preserving polio-free status in polio-free areas.

In Afghanistan, actions to improve access and coverage include a short interval additional dose, focused district strategy, high-risk cluster approach, working through nongovernmental organizations, community development committees of the Ministry of Rural Rehabilitation and Development and negotiating access and periods of tranquillity with ISAF/NATO, International Committee of the Red Cross, anti-government elements, *shuras* and access negotiators.

In Pakistan, efforts are also being made to improve access through negotiations with local and religious leaders, conducting one-day campaigns with local teams, mapping and vaccinating internally displaced persons (IDPs) in camp exit points in the and in the community and utilizing the short interval aditional dose in some areas. High level political engagement is clear (inauguration of October national immunization day by H.E. Mr Asif Ali Zardari, President of Pakistan, and Ms Assefa Bhutto Zardari, Ambassador for Poliomyelitis Eradication), and a Prime Minister's Action Plan for Enhanced Inter-Sectoral Collaboration and Inter-provincial Ministerial Committee on Polio Eradication exist. Efforts are also being made to improve service delivery with better insight on campaign quality through improving monitoring data by using finger marking and independent monitor and focusing on high risk populations/areas. Innovative communication strategies were developed with polio control cells, jointly with 12 television channels, and include free text messages (12 million), weekly polio journal for journalists and 1st National Health Journalist Award (theme in 2009 is polio).

Efforts to improve performance of the district health management include engaging District Campaign Officers and post-campaign review with the Executive District Officers. However, performance of district management should be measured by immunization

indicators with re-vitalization of provincial steering committees chaired by the Chief Secretaries.

Importation of wild poliovirus to the Region from remaining endemic countries, especially into countries in the extended Horn of Africa, represents another major challenge. The outbreak in south Sudan resulted in 24 cases in 2008 and 40 cases in 2009 (last on 27 June) with spread to northern Sudan (5 cases) and neighbouring parts of Kenya and Uganda. Action was taken to curb the outbreak including provision of technical support, strengthening of country team, review/update plans for supplementary immunization activities, provision of logistic support, enhancing surveillance and enlisting local government commitment (Presidential Action Plan, establishment of an interministerial committee and involvement of top officials in support of campaigns) and ensuring coordination actions by all partners.

Sustaining polio-free status of other countries is done by avoiding large immunity gaps in polio-free countries; through improvement of routine immunization and implementation of supplementary immunization activities especially in foci of low population immunity. Population immunity is assessed through coverage data and regular review of vaccination profile of AFP cases 6–23 months of age. Maintaining certification-standard surveillance in all countries, both at national and sub-national levels and particularly among high risk areas/populations is another pillar of preparedness to importation. In addition to weekly analysis and monitoring of indicators, surveillance reviews are conducted regularly followed by training courses to address any gap observed during the reviews.

Coordination activities are being maintained and further strengthened between neighbouring countries, especially between Afghanistan and Pakistan and in the Horn of Africa including synchronization, exchange of information and local level planning and coordination. As well, certification activities are moving forward in parallel to the eradication activities.

3.2 Progress of the regional poliovirus laboratory network

The polio network laboratories continue to support AFP surveillance activities efficiently all over the Region. All network laboratories passed the WHO proficiency panel tests for both poliovirus isolation and intratypic differentiation (ITD) testing and all laboratories are fully accredited except the Kuwait laboratory, which is provisionally accredited.

The workload of the network laboratories is considerably high. During 2008 the polio network laboratories processed 32 210 specimens from cases and contacts, and the workload continues to be at the same level in 2009. The contact sampling of AFP cases is performed in 20 of 22 countries, in 2008 and 2009, 14 and 11 wild poliovirus (WPV) cases, respectively, were detected through positive contact of virus negative index cases. The average time between receipts of stool samples in the laboratory to reporting the result was 16 days. Overall, 94% of specimens had culture results within 14 days, 95% had ITD results within 7 days of virus culture positive referral and in 98% of AFP cases, the final laboratory testing results were provided within 45 days of paralysis onset.

The ongoing environmental surveillance in 21 districts with 34 collection sites in Egypt discovered two poliovirus type 1 were isolated, the first from Giza in September 2008 and second from Cairo in December 2008. The environmental surveillance was established in two districts (Karachi and Lahore) in July 2009.

The real-time PCR method for rapid characterization of polioviruses was fully implemented in three countries (Egypt, Islamic Republic of Iran and Pakistan), while standardization was in progress in remaining four laboratories (Kuwait, Morocco, Oman and Tunisia). New LABIFA version 4.1 is being developed to include changes due to rRT-PCR method and will be distributed in November 2009. High quality genomic sequencing of poliovirus has continued in the Pakistan laboratory, which is greatly helping surveillance activities in Pakistan.

Between January 2008 and October 2009, no vaccine-derived polioviruses (VDPV) were isolated from acute flaccid paralysis (AFP) cases. However, in the Islamic Republic of Iran, one type 2 VDPV was isolated from a follow-up stool sample collected in 2008 from an AFP case that had onset in 2007. One aVDPV type 1 was detected in April 2008 from a sewage sample in Behira governorate, Egypt.

The expertise of the polio laboratory network in surveillance is being offered to other vaccine-preventable programmes; at present 4 laboratories (Iraq, Kuwait, Oman and Pakistan) are involved in H1N1 diagnostic work.

3.3 Status of the global polio laboratory network

Dr Esther de Gourville, WHO/HQ

Between January 2008 and October 2009, the global polio laboratory network detected WPVs in specimens from 2849 cases of AFP in 22 countries. The majority of all WPVs reported globally were found in the African Region, where 17 countries had WPVs, compared with 3 countries in the Eastern Mediterranean Region and 2 countries in the South-East Asia Region.

Comparative analysis of the nucleotide sequence of the VPI region of the viral genome allows for identification of the genotype and determination of transmission links based on genetic relatedness. Only 4 WPV genotypes have been detected globally since 2005. One genotype each of type 1 wild poliovirus (WPV1) and type 3 wild poliovirus (WPV3), designated West Africa B (WEAF-B), are endemic to Nigeria. One other genotype each of WPV1 and WPV3, designated South Asia (SOAS), is endemic to Afghanistan, India and Pakistan. Transmission of endemic genotypes continued in all 4 countries during 2008–2009. The WPVs from AFP cases detected in 18 countries where polio is not endemic were WEAF-B (14 countries) or SOAS (in 3 countries: Angola, Democratic Republic of the Congo and Nepal) genotypes only, or both (in the Central African Republic). Both WEAF-B WPV1 and WEAF-B WPV3 genotype viruses were found in Benin, Chad, Niger and Sudan; in all but Sudan, this was the result of importation of WPV1 and WPV3 during 2007–2009, originating from Nigeria. WPV1 detected in Sudan represented the continuation of an outbreak from 2004. WPV3 detected in the Central African Republic and Sudan represented importations

from Chad. In 10 countries (Burkina Faso, Côte d'Ivoire, Ethiopia, Ghana, Guinea, Kenya, Liberia, Mali, Togo and Uganda), only WEAF-B WPV1 was detected. SOAS WPVI and WPV3 genotypes were both found in Angola and the Democratic Republic of the Congo; SOAS WPV1 was found in the Central African Republic; and SOAS WPV3 was found in Nepal. There have been 3 importations of SOAS WPV into Angola. Circulation of SOAS WPV1 from the first importation in 2005 was interrupted in Angola but continued in the Democratic Republic of the Congo in 2008 after being introduced there in 2006, with subsequent spread to the Central African Republic in 2008. However, in 2008–2009, WPV1 circulation in Angola represented continuation of transmission that had started with a second importation of virus from India in 2007. The third importation was a SOAS WPV3 in 2008 and resulted in continued circulation and exportation to the Democratic Republic of the Congo in 2008.

Importations of WEAF-B WPVI and SOAS WPVI viruses into Egypt were detected once each in 2008 through testing of separate sewage samples. The WEAF-B WPV1 was genetically most closely related to WPV1 in Sudan, and the SOAS WPV1 was genetically related to WPV1 in Uttar Pradesh, India, both from the same year. The WPVs were detected in Mumbai sewage genetically linked to virus found in AFP cases in Bihar in 2007.

Between January 2008 and June 2009, 229 cases due to circulating vaccine derived polioviruses (cVDPV) were detected; out of which 209 were type 2 cVDPV from Nigeria and remaining from Democratic Republic of Congo (16) and Ethiopia (4). A total of 42 iVDPV cases have been reported from middle and high income countries and most of these are type 2 iVDPV. Between January 2008 and as of October 2009, 21 ambiguous VDPV, 17 from AFP cases and 14 cases from other sources have been detected.

Between January 2008 and June 2009, there was an 8% overall increase in workload compared with the preceding 18 months. In regions where polio is endemic, workload increased by 18% in the African Region, and by 10% in the South-East Asia Region and decreased by 14% in the Eastern Mediterranean Region. In polio-free regions workload decreased by 1% in the Western Pacific Region, while it there was not significant increase in the regions of the Americas and Europe.

There was remarkable progress in improving the efficiency of testing and reporting times, both in virus isolation and ITD results. However, there were some challenges related to staff and laboratory capacity in SEARO to meet the target of ITD timeliness. To improve timeliness, the lTD testing capacity was increased in the 44 laboratories in endemic regions: from 17 laboratories in mid-2006 to 27 in 2008; 4 more laboratories are in the process of being upgraded. Overall, 136 (94%) of 144 network laboratories were fully accredited by WHO in 2008. Six laboratories that were provisionally accredited generally met required standards for accurate results, but had some other performance deficiency. Two non-accredited laboratories failed the proficiency test and are testing samples in parallel with accredited reference laboratories while they resolve performance concerns. In 2008, revised accreditation checklist was introduced, and it was initially implemented in 52 laboratories in the WHO regions of Africa, the Americas, the Eastern Mediterranean and South-East Asia. A

new proficiency testing panel for the new algorithm was also used in these four WHO regions.

A new real-time reverse transcriptase polymerase chain reaction (rRT-PCR) assay has been developed at the United States Centers for Disease Control and Prevention (CDC). Field evaluation was done in 9 Global Specialized Laboratories and it included retrospective testing of known VDPVs as well as previously reported Sabin-like viruses. As of March 2009, 2819 isolates were screened and 598 isolated were flagged as potential VDPV (all VDPVs were detected by NICD, South Africa). Out of 598, 228 were sequenced and 46 were found with > 1% nucleotide divergence in their VP1 region of the poliovirus genome. Based on field testing adjustments were made in rRT-PCR reagents.

The global polio laboratory network is making its contribution to polio research product development (OPV and IPV evaluation, iVDPVs surveys, new polio vaccine research, IPV fractional dose and interadermal studies etc.), and to H1N1 pandemic response. As part of continuous development, a few more protocols are under development: safe (non-infectious) referral of isolates for ITD and sequencing; validation protocol for developing algorithm for poliovirus detection using only molecular tests; validation of IgM ELISA quality assurance programme for sequence laboratories; and training modules on interpreting sequence data.

3.4 Regional progress of the regional poliovirus laboratory network

Dr Francis Kasolo, WHO/AFRO

The African regional polio laboratory network consists of 3 regional reference laboratories, located in Ghana, South Africa and Central African Republic, and 13 national laboratories found in Algeria, Cameroon, Cote d'Ivoire, Ethiopia, Nigeria (Ibadan and Maiduguri), Kenya, Madagascar, Democratic Republic Congo, Senegal, Uganda, Zambia and Zimbabwe. Five of the national laboratories have also capacity to perform intratypic differentiation (ITD) testing.

The African regional laboratory network has over the past two years recorded significant achievements, namely:

- Coping with an 18% increase in stool specimen workload in 2008
- Providing confirmed results to the polio eradication programme within 14 days on average
- As of September 2009, 15/16 of the laboratories had been fully accredited, with only Zimbabwe being provisionally accredited. All laboratories have passed their isolation and ITD proficiency testing where applicable
- The regional genomic sequencing facilities and dendogram-generating capacity at the regional reference laboratory in South Africa and CDC have generated genetic information on all wild poliovirus isolated over the period 2008–2009. This information suggests that there are four genetically linked epidemiological blocks in the region: west Nigeria–Chad, Angola–Democratic Republic of Congo–Burundi, Chad–Central African Republic and south Sudan–Uganda–Kenya circulation.

Major constraints are improving the overall results turnaround in an environment of: increasing workload and competing demands (H1N1 work is ongoing in 10 polio laboratories); pressure from programme for timely and accurate reporting of results; most laboratories are now working weekend schedules in order to meet programme needs; continued loss of staff at the National Institute of Communicable Diseases molecular laboratory, which threatens to disrupt sequencing work for the entire region.

Remaining challenges are:

- continued closer supervision and support to all laboratories in order to sustain the current performance
- Successful introduction of ITD testing in Ethiopia, Kenya and Algeria laboratories: onsite support is planned for first week of January 2010
- scaling up support to countries on issues of laboratory containment as the region moves towards interruption of wild poliovirus.

4. VIRUS SURVEILLANCE

4.1 Laboratory performance indicators

4.1.1 Pakistan

Mr Sohail Zaidi

The WHO regional reference laboratory for polio in Pakistan continued supporting the AFP surveillance with excellent performance. Between January 2008 and October 2009, stool specimens of 9373 and 2581 AFP cases from Pakistan and Afghanistan, respectively, were tested. The laboratory also tested contact samples from Afghanistan (1451) and Pakistan (3806). This shows the generalized increase in workload as compared to the previous 18 months. All laboratory performance indicators were maintained at certification standard.

The laboratory participated in rRT-PCR field evaluation and successfully established the method and tested all Sabin-like viruses during 2007–2009. The environmental surveillance system for sewage testing for isolation and characterization of polioviruses was estalished in Karachi and Lahore; at the same time an environmental surveillance laboratory was also established at RRL, Islamabad. WPVs have been isolated from sewage samples which are supplementing the AFP surveillance for more targeted polio eradication activities.

4.1.2 Egypt

Dr Iman Al Maamoun, VACSERA, Egypt

The laboratory at VACSERA continued to support the polio eradication initiative by testing stool specimens and virus isolates for Egypt, Iraq, Lebanon, Syrian Arab Republic and Sudan. Between January 2008 and October 2009, VACSERA tested 5525 samples (4269 from AFP cases and 1256 from contacts) from Egypt and other countries. The quality of performance indicators was sustained at certification standard. In 2009, a score of 95%was

obtained in proficiency test (PT) panel testing for virus isolation, 100% in polymerase chain reaction (PCR) and 92.5% in nucleic acid probe hybridization (NAPH) test. Most recently, rRT-PCR was established and a 100% score was obtained in PT panel of rRT-PCR.

4.1.3 Sudan

Mr Hatim Babiker, WHO Sudan

Between January 2008 and October 2009, the national polio laboratory tested 2198 specimens from AFP cases (1406) and contacts (792). The laboratory performance indicators were maintained at certification standard. The lab scored 100% in PT panel testing. In 2009, 5 WPV1 were isolated from AFP cases, and 3 from contacts. Additionally, one WPV3 was isolated from a Chadian AFP case reported from South Darfur, Sudan.

4.2 Molecular characteristics of wild polioviruses in Afghanistan, Pakistan and Sudan

Mr Sohail Zahoor Zaidi, National Institute of Health, Pakistan
Dr Humayun Asghar, WHO/EMRO

In Pakistan and Afghanistan both SOAS WPV1 and WPV3 genotypes are circulating. In 2009 new genetic sub-clustering was introduced, according to which WPV1 genotype has 5 sub-clusters (A3A1A, A3A1B, A3A2, A3D2) active in Pakistan and Afghanistan. Out of these sub-clusters, A3A1A is widely circulating in Pakistan and Afghanistan. Previously, it was mainly circulating in southern Afghanistan but now has spread to adjacent northwestern Baluchistan (Quetta–Pishin–Kila Abdullah area) and Sindh. Most recently it has been found in NWFP, mainly in Swat, causing an outbreak. In addition, highly divergent WPV1 isolated in September from the sewage water sample from Lahore also belongs to the same sub-cluster. Sub-luster A3A1B appears to be circulating only in southern Afghanistan (Kandahar). A3A2 have been circulating in the southern Afghanistan (Helmand and Kandahar) in 2008 and 2009, but also shared circulation with Sindh and Baluchistan provinces in 2008. Local circulation continued in Swat in 2008 and 2009. Sub-cluster A3D2 is circulating in Punjab and NWFP. Sub-cluster B4A1 circulation was primarily in central Punjab and NWFP/FATA, while during 2009 it was found in Bajour with local circulation in Lahore, Multan and TT Singh and shared circulation with bordering eastern Afghanistan (Kapisa).

The WPV3 genotype has one active B1C cluster, which has been further divided in to 4 sub-clusters (B1C5, B1C6A, B1C6B, and B1C7), which remain active during 2009. Cluster B1C5 found in Sindh in 2008 (Hyderabad and Karachi Gulshan Iqbal) and was associated with cases in several places in NWFP (Bajour [two separate lineages], Mohmand, and Charsada). Virus also found in Baluchistan (Killa Abdullah) and with several high-season cases in southern Afghanistan (Kandahar). Sub-cluster B1C6B is mainly active in NWFP/FATA and Bajour. Sub-cluster B1C7 is primarily found in NWFP/FATA in 2008 (outbreak in Peshawar), with some spread to eastern Afghanistan and Punjab, it continued to circulate in NWFP (Charsada, Bajour and Dir Upper) in 2009 with early detection in Punjab (Sialkot).

The WPV1 originating from northern Nigeria spread to more than 20 other countries beginning in 2003, and later it moved from Nigeria through Chad to Sudan in 2004 and 2007.

The WPV1 discovered in south Sudan in July 2008 continued to circulate in southern Sudan in 2009; the most recent case is with date paralysis onset on 27 June 2009. Genetic data indicated a shared reservoir between southern Sudan and adjacent areas of western Ethiopia. WPV1 spread from southern Sudan to adjacent areas of Uganda and Kenya, and also spread to northern Sudan (Red Sea State and Khartoum). The WPV1 was introduced into Western Darfur, Sudan from Chad.

The WPV3 was independently introduced into Western Darfur, Sudan from Chad in 2008 and no further cases were reported. This virus also originated from northern Nigeria

5. LABORATORY QUALITY ASSURANCE

5.1 Accreditation status of regional polio laboratories

Dr Humayun Asghar, WHO/EMRO

In 2008, 11 of 12 regional network laboratories were fully accredited. The Kuwait regional reference laboratory was provisionally accredited in 2007 and 2008. As of October 2009, 10 of 12 regional network laboratories were visited and are fully accredited by WHO, except the national poliovirus laboratories in Kuwait and Saudi Arabia which are pending accreditation visits in 2009. All national poliovirus laboratories implemented recommendations made during accreditation visits.

There is sustained good quality performance of all network laboratories. All laboratories were able to maintain the new timeliness targets for reporting of virological investigation results, i.e. within 14 days from receipt of sample in the laboratory and 7 days for ITD results. 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 for virus isolation in cell culture and ELISA

5.2.1 Proficiency testing for isolation of polioviruses according to the new algorithm: experience in 2008 and 2009 in 4 WHO regions

Dr Harrie van der Avoort, WHO Temporary Adviser

The new algorithm for isolation of polioviruses was implemented in 2008 and 2009 in 4 WHO regions: the three endemic regions (African, Eastern Mediterranean and South-East Asia regions); and the Region of the Americas.

The successful implementation of the cell culture part of the new algorithm is monitored by a newly developed proficiency test, consisting of 10 faecal samples with 0, 1 or 2 polioviruses and/or non-polio-enteroviruses. This type of proficiency test was successfully piloted in a field study in three WHO network laboratories in three WHO regions and the outcome of this pilot as well as a scoring system for the evaluation of reported results were extensively discussed at the annual global laboratory network meeting in Geneva in 2008.

High scores (mean of 94%) were obtained in the three endemic regions, 38 of the 43 participating laboratories in the endemic regions passed the test with a score > 90%. In the Eastern Mediterranean Region one laboratory did not pass because of three basic mistakes: misunderstanding of the new algorithm, late reporting and missing a NPEV in a polio-negative sample. After corrective measures were taken, a new proficiency test was performed by the lab with a 100% score. All laboratories received suggestions for improvement of their performance, and for optimal reporting of data and for more clear documentation of performance in newly designed worksheets.

In contrast to the results in the three endemic regions, the overall results (mean score of 81%) for the Region of the Americas were lower, and 3 of the 9 participating laboratories did not pass. At regional level the consequences of introducing the new algorithm were clearly not completely understood: there was no adjustment in reporting system yet, and limited guidance was available for the laboratories during implementation. At the laboratory level, the rationale for the various steps of the new algorithm was not understood and some laboratories thought local variants were allowed.

The Region of the Americas has decided not to use the proficiency test for accreditation purposes, but rather to see the 2008–2009 test as an exercise and aim at accreditation with the new proficiency test in 2009.

Overall conclusions: the proficiency test for isolation of polioviruses according to the new algorithm has proven to be an excellent tool to document the implementation status of the new algorithm in 4 WHO regions, to document real deficiencies in cell culture practices in various laboratories in these regions, and had documented deficiencies in the understanding of the new algorithm and in the rationale of all its various steps.

5.2.2 Report on proficiency testing programme: RIVM ELISA for ITD

In 2008 and 2009, 29 laboratories in the WHO polio laboratory network participated in the ELISA ITD, proficiency test programme. Six of these laboratories are from the Eastern Mediterranean Region. Five of these laboratories passed the proficiency test with scores of 90% or more. Results reported by the Kuwait laboratory showed a series of technical mistakes as well as misinterpretation of results. All suboptimal scores were followed up, in line with recommendations given by RIVM.

With the recent introduction of the rRT-PCR for VDPV screening the need for laboratories in the Region to show proficiency in ELISA decreases in importance. Laboratories doing environmental surveillance will still do the ELISA, as mixtures of Sabin and wild viruses from the same serotype might escape detection by molecular methods in these samples.

5.3 Report on proficiency testing of PCR (both conventional and real time)

Dr Humayun Asghar, WHO/EMRO

The molecular diagnostic proficiency test panel consists of non-infectious in-vitro RNA transcripts containing sequences targeted by PCR. Transcripts are of positive (genome "sense") polarity, and contain 5′-UTR and VP1 sequences. Format for the real-time RT-PCR follows 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). Unknowns used with real-time VDPV kits include: Sabin-strain-VDPV specific primer pair + specific probes, three sets targeting VP1 and three sets targeting 3Dpol.

With the exception of Kuwait, all ITD polio laboratories in the Region (Egypt, Oman, Pakistan and Tunisia) achieved a passing score of >90% in 2008 proficiency test panel for polymerase chain reaction (PCR) method. This achievement reflects the reliability of the regional laboratory network.

In 2009, regional polio network laboratories (Egypt, Islamic Republic of Iran and Pakistan) that successfully established the rRT-PCR method were given the proficiency testing panel comprising of two sets of unknown (Sabin-like and VDPVs) viruses. All these laboratories achieved a passing score of >90%.

5.4 Update on cell sensitivity testing in regional network laboratories

Dr Javier Martin, WHO/EMRO

Cell sensitivity testing is well established in laboratories of the Region. Cells are tested periodically and observed titres of LQC standards are within the specified range. However, some concerns have been identified in three laboratories. In all three cases, high out-of-specification titre values were reported for laboratory quality control standards for a number of tests. After investigation, the root causes appeared to be due to procedural errors, lack of awareness of validity criteria and misreading of test worksheets. In neither of the cases can it be concluded that the cells used in the laboratory were of inadequate cell sensitivity for poliovirus. Managerial intervention should help correcting these errors.

There are also minor mistakes regarding the reporting of results. As previously established, results should be sent to the Regional Coordinator within 48 hours including all historical data and using a standardized spreadsheet form. It is very important to include any relevant changes and any action that was taken when low or high titres were observed. Laboratories are also reminded that when LQC standards are exhausted, new LQC standards have to be prepared from NIBSC standards and validated as indicated in the polio manual.

5.5 Training module on interpreting genetic sequencing data

Dr Harrie van der Avoort, RIVM, the Netherlands

There is need for a course in the interpretation of molecular epidemiology data. The course should be flexible in content and format as the audience might vary: laboratory staff, epidemiologist, programme staff or scientific community. Depending on participants' background in molecular biology, the content might vary in breadth (theory, process, assumptions and caveats), in depth (qualitative and or quantitative approach) and of course in the examples given.

The input (number and origin of sequences) and the algorithms used to generate genetic trees determines the outcome and may give rise to misunderstandings by persons that do not know assumptions and limitations of the methodology used. Dendrograms are graphic tools to represent the sequence relationships among a collection of virus isolates. Interpretations on reservoirs of virus, importations, geographical extent of transmission, consequences of identical sequences and of orphan sequences and on programme progress should be drawn with great care. The graphical representation should illustrate the message and should always be accompanied with clear statements on the conclusions and the programmatic impact.

Background information on the completeness or selection of datasets, and the algorithm used are essential in the translation process of molecular epidemiology data into programmatic relevant statements with implications for action in the field. There is ongoing need for education in the interpretation of dendrogrammes for various audiences within the polio eradication initiative.

5.6 Issues of quality assurance in Kuwait laboratory and their follow-up

Since the last accreditation in 2008 many improvements have been made in the light of recommendations of the reviewer. To achieve the minimum of 150 stool samples per year for accreditation, a letter was sent by the Ministry of Public Health to all hospitals and health facilities for the collection of more stool samples from patients other than AFP cases, with paralysis or with infections related to enteroviruses, e.g. aseptic meningitis, encephalitis, etc. As a result of this the numbers of samples have gradually started increasing, and to date 34 samples from AFP cases, 31 from contacts, and 28 from clinical cases have been received in the laboratory. All stool specimens from AFP cases were received within 14 days from paralysis onset, received in the laboratory within 24 hours, processed and inoculated in both cell lines within 24 hours of receipt in the laboratory. The virus isolation results were sent within 14 days for more than 80% of samples, and also the ITD results were sent out within 7 days. All isolates are sent to RIVM, The Netherlands, for confirmation and sequencing.

A problem with ELISA testing could not be resolved for poliovirus type 3, while results of PV1 and PV2 were improved. The results were sent to RIVM and WHO headquarters and Regional Office for comment and evaluation. The problem with conventional PCR was resolved and better results were obtained after changing and preparing fresh reagents. Suggestions of the reviewer with regard to physical re-arrangement of equipment and maintenance have been implemented.

6. MANAGEMENT AND SUPERVISION

6.1 Management and supervision in the network laboratories

Dr Esther de Gourville, WHO/HQ

The laboratory is a highly complex work environment with many managerial challenges. In laboratories with performance problems, the root causes are frequently managerial rather than technical in nature. It is common for most scientists in managerial positions to acquire management skills through on-the-job experience rather than formal management training and as a result, some may focus on technical work to the exclusion of the multiple other managerial responsibilities or have difficulty delegating scientific work. The expected outcome of laboratory activities is to provide high quality results in a timely manner. To achieve this, managers should engage in good planning, management and supervision.

The manager is the person who is ultimately accountable and responsible for the functioning of the polio laboratory. A manager must manage directly or in collaboration with others: personnel and financial resources; development of documentation such as standard operating procedures (SOPs); ensuring compliance with SOPs; data management and reporting of results; bio safety and biosecurity; and customer relations, e.g. reporting to clients, institute, ministry of health or partner agencies.

For human resources management, certain selection criteria should be used in hiring: verify professional qualifications through review of transcripts; perform background checks, e.g. contact referee; and ensure medical evaluation, including immunization as needed. Job descriptions should be readily available, as well as an organogram clearly demonstrating hierarchy, reporting relationships and accountability. For new staff, ensure orientation on safety, SOPs, task-specific training, evaluation of competency before deployment. Annual performance evaluation should be conducted for all staff, and continuing education opportunities must be made available for all, e.g. through training courses, seminars, journal clubs, other approaches. Frequent challenges in managing staff, such as attitudinal issues, inter-personal conflicts, time management, inequity of work distribution, arrangements during staff absence should be addressed appropriately.

In supervision, manager should provides critical oversight to ensure appropriate documentation of work, ensures timely completion of work, critical reviews of relevant documentation of work and bring problems to the attention of next level in the management hierarchy and assists with finding solutions. The manager should review the technical work through traceability and accuracy; ensure that validity criteria are met for test run; interpretations are correct; and ensure results are accurately entered into database and accurately reported.

6.2 Group discussion on management and supervision in the network laboratories

The laboratory directors were divided into two discussion groups as follows:
Group 1: Egypt, Islamic Republic of Iran, Iraq, Pakistan, Sudan

Group 2: Jordan, Kuwait, Morocco, Oman, Syrian Arab Republic

The groups were asked to highlight the management and supervision issues faced in their laboratories in sustaining the good quality laboratory performance.

The following are the salient points which were mentioned by the groups.

- Proper management of laboratory stocks and availability of reagents and laboratory supplies is important because any shortage or non-availability of reagents or disposables can affect the laboratory performance. Laboratory directors should always revise the inventory and develop a mechanism whereby any decrease or shortage of stock can be pointed out well before time. Another issue related to supplies is customs clearance of items which are donated by WHO or anyone else. This issue works thorough follow-up and collaboration between the end user (laboratory), WHO and the Ministry of Foreign Affairs or Ministry of Health. Most of the time problems are faced in receiving the exemption letter required from Ministry of Foreign Affairs. It was proposed that pre-advice letter from WHO should be copied to the laboratories also, which will help to follow up with concerned quarters.
- Laboratories should keep analysing data for any unexpected increase in workload to prepare the laboratory to deal with the situation, in terms of more need for supplies or staff. At the same time, laboratories should remain vigilant for any emergency situation like cell contamination, sudden breakdown of essential equipment etc. In all such cases the supervisor and laboratory director should be informed immediately for corrective actions and WHO should also be informed to seek their help or advice.
- Data management should be strengthened to monitor the laboratory performance indicators to know any poor performance, and resolve the problem on time or seek WHO advice. To ensure timeliness of reporting, documentation should be reviewed regularly by the laboratory supervisor and cross-checked by the laboratory director. The standard operating procedures should be kept updated and revised as and when required.
- Staff should be provided with incentives, such as by giving them chances to go for training in or out of country or to attend meetings, or giving them appreciation letters or involving them in research projects. If possible, monetary incentives should be given. The director should create an atmosphere of teamwork and sense of family. Staff should be encouraged to enjoy vacations as per regulations; however, backup staff should be prepared to substitute for staff absence. If there is dire need for more staff, the director should communicate with other programme managers or higher authorities to resolve the staff shortage. While dealing with staff, their self-respect and dignity should be considered at all times.
- Continuous or refresher training of staff should remain ae priority. It will motivate the staff and also help to keep them updated on emerging knowledge.
- There should be clear lines of internal communication, delegation of authority and reporting. The job description should be discussed with staff and adjustments made according to needs of the programme and capacity of staff. There should be succession planning: transfer of information and documents to new director.

7. IMPLEMENTATION OF REAL-TIME PCR (RRT-PCR) ASSAY

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

Dr Javier Martin, WHO/EMRO

The introduction of a new algorithm for poliovirus isolation a few years ago led to a significant reduction in the time required for the isolation and identification of poliovirus in clinical samples. The global poliovirus laboratory network has gone a step further by developing an rRT-PCR assay for ITD of poliovirus isolates. An rRT-PCR reaction has also been established to detect VDPV strains, which together with the use of the ITD rRT-PCR will eliminate the need to perform the ITD ELISA test, saving time and resources and moving a step closer to a full molecular method for the detection and typing of poliovirus in clinical samples.

Real-time PCR ITD has been validated completed initial evaluations and pilot testing. The Centers for Disease Control and Prevention (CDC), Atlanta, developed both ITD and VDPV rRT-PCR tests and technical, training and implementation issues were identified and used to refine assays and focus training and implementation plans. The methods were further evaluated by the global specialized laboratories to include all known VDPV strains. The evaluation period led to changes to the real-time assays to improve sensitivity and specificity and to help the laboratories in their efficiency and timeliness goals, in addition to helping them to correctly interpret the collected data.

7.2 Overview of real-time PCR implementation in global polio laboratory network in 2009

Dr Esther de Gourville, WHO/HQ

The rRT-PCR method was development and validation by CDC, Atlanta, and it was evaluated in 9 global specialized laboratories of GPLN. A survey was done in 2008 to know the capacities of the laboratories to perform the rRT-PCR. It came out that 22 laboratories need equipment and training workshops should be conducted in 5 regions. A draft was developed as a supplement to the polio laboratory manual on rRT-PCR ITD and VDPV screening algorithm.

The training material was developed by CDC, and WHO laboratory coordinators were orientated on rRT-PCR methods at CDC in September 2008. The first training workshop was held in Muscat, Oman, 24–28 January 2009, followed by one in Mumbai, India, in March, and one at CDC, Atlanta in July 2009. The participants were from ITD laboratories.

Post-workshop implementation plans developed are as follows:

- Phase 1: attaining proficiency using authenticated Sabin strains or sequenced PVs.
- Phase 2: Retrospective screening of Sabin-Like viruses for VDPV detection.
- Prospective ITD and VDPV testing up to a maximum of 100 isolates.
- Evaluation of rRT-PCR proficiency test panel.
- Regional offices to coordinate data management changes and reporting.

Laboratories in the Eastern Mediterranean and South East Asia regions are already implementing the plans. For the African Region, two training workshops will be held in NICD, South Africa in November 2009: one for Anglophone and another for Francophone countries.

7.3 Implementation of rRT-PCR and results of retrospective testing of Sabin isolates

7.3.1 Egypt

Dr Laila El-Bassioni

The evaluation for rRT-PCR was performed in Egypt during 2009. Ten positive Sabin like (SL) isolates were tested 4 times for ITD and VDPV by rRT-PCR. All the results and data were sent to CDC for confirmation and evaluation. The preliminary testing to attain proficiency was accepted by CDC, and VACSERA was allowed by WHO/EMRO to perform the retrospective testing of SL viruses by rRT-PCR: 35 positive isolates SL1, 11 positive isolates SL2 and 40 positive isolates SL3.

The results were: all SL type 1, 2, and 3, were positive for VP1, except four SL type 2 and two SL type 3, which were negative for VP1. All these negative VP1 were flagged as VDPV and referred to CDC for sequencing. The laboratory was given an rRT-PCR proficiency test panel and passed with 100% score.

7.3.2 Islamic Republic of Iran

Dr S. Shahmahmoodi

NPL-Iran started working on rRT-PCR to attain proficiency by working on known SL/NSL/VDPV isolates and monotypic/mixed NIBSC, Sabin standard viruses. The NPL-Iran was equipped with a Corbett Rotorgene 6600 by WHO, this machine is different from the ABI 7500 machine, which is used in most of the laboratories of the network: lacking the adjustment option for ramping time; and difference in heating/cooling system of the machine which is with air. These differences were problematic in setting up the test on Corbett and obtaining satisfactory results. Eventually the problem was solved by decreasing the annealing temperature and increasing the annealing time in addition to breaking the annealing step to 2–3 steps (for different primers). Also for degenerate primers, the stringency was decreased by addition of $MgCl_2$ to the reaction.

The setup and working on 4 runs of 10 specimens to attain proficiency were completed after almost 4 months of hard work and all the results shared with CDC, EMRO and HQ. Two “rRT-PCR ITD” and two “rRT-PCR VDPV” kits were consumed during these steps. Retrospective testing of archived Sabin isolates was completed shortly after attaining proficiency and the results shared with CDC, EMRO and HQ. The 24 specimens with different serotypes were tested. Six SL2 and two SL3 specimens showed discordant results and were sent to CDC for sequencing. Results of all of these specimens were confirmed as SL by CDC. The NPL-Iran passed the rRT-PCR proficiency test panel.

7.3.3 Kuwait

Dr Siham Al Mufti

The virology unit has different PCR instruments such as Roche Light Cycler (1.5, 2.0, and 480 versions), and ABI 7500. Two technicians were trained by the laboratory director and Known isolates were collected for testing on ABI 7500 to attain proficiency.

In April 2009, the first experiment was set-up. The results were not good, especially that of negative controls which gave positive signal. Results were shared with CDC, and they informed that the Buffer B was contaminated and this was also observed by other laboratories. A new Buffer was sent and the test was repeated with the same samples. The results were satisfactory for Sabin types 1, 2, 3, and PanEV (non-degenerate primers) but Sero 123 and PanPV (degenerate primers) did not give good results (Zigzag lines), results were sent to CDC and they suggested some changes. Despite all those changes, repeat testing gave the same results as before. To date the problem has not been resolved.

The laboratory was very much engaged in the H1N1 work and the workload was very high. Two new technologists were appointed and trained on the job and they are now running the test. It is hoped that they may fix the problem and will be able to do all the poliovirus work.

7.3.4 Oman

Dr Said Al Baqlani

The NPL-Oman started proficiency attaining run for establishment of rRT-PCR in March 2009. Right from the beginning the laboratory faced difficulties in results analysis. The results (graphics and data) were shared with CDC. Suggestions by CDC were implemented: re-passage the NIBSC strains to increase virus titre; increased amount of tissue culture infected fluid from 10 ul to either 30 or 50 ul; treating the samples with chloroform prior to testing; using new Buffer B; and extraction of ribonucleic acid (RNA) for sample preparation (especially for PV3); collecting data at annealing stage and not extension stage.

Results improved after using RNA extraction. However, problems did not resolve completely and samples continued to give low signals for both degenerate and non-degenerate primers. The degenerate set of primers constantly caused problems such as bad plots (zigzag), false positive, false negative and/or inconsistent results. Most of the time the non-degenerate and VDPV primers worked well but inconsistent results were continuously observed with degenerate primers. Oman-NPL is not ready to start rRT-PCR for polioviruses until the problem is fixed.

7.3.5 Pakistan

Mr Shahzad Shaukat

Two separate real-time PCR (rRT-PCR) assays were implemented in the WHO regional reference laboratory for ITD and VDPVs screening since September 17, 2009. Retrospective testing of known as Sabin isolates with rRT-PCR was conducted; they had been previously

reported Sabin-like PVs through conventional PCR and ELISA. A Total of 153 Sabin-like isolates (SL1=31, SL2=75, SL3=23 and Sabin mixtures= 24) were selected from the period from 2006 to March 2008. Truth table was used to calculate the relative sensitivity of CRT-PCR and rRT-PCR and it was found 82% and 100%, respectively for Sabin1 isolates; for Sabin2 and Sabin3 it was 94% (conventional PCR) and 100% (rRT-PCR).

Previously reported SL viruses from 2007 to March 2009 from Pakistan and Afghanistan were screened for VDPVs. Total number of isolates for this period was 1231 (SL1=445, SL2=69 and SL3=17). Only three SL2 samples (two from Pakistan during year 2007 and one from Afghanistan during 2009) were referred for sequencing, as they were found non Sabin-like on VDPV assay. Sequencing data confirmed that these were not VDPVs: the nucleotide divergence was less than 1% from Sabin.

7.4 Estimation of rRT-PCR reagents for ITD laboratories

Dr H. Asghar, WHO/EMRO

The purpose of the estimation scheme was to facilitate and help the ITD laboratories to calculate the annual requirements of reagents i.e. enzymes, which are not only an expensive item but also have a short shelf life. In the calculation formula, for each sample 6 primers (Sabin multiplex, PanEV, PanPV, PV1, PV2 AND PV3) and 12 controls (one negative and one positive for each primer) were considered for ITD and, depending on whether monotypic or mixtures, VDPV primers and controls were added. It was demonstrated to the participants that calculations can vary depending on workload, repeat testing and number of test runs per week. An Excel file with formula was provided to participants for calculation and estimation of the reagents.

8. MISCELLANEOUS TOPICS

8.1 Introduction to pilot testing of two training module on biosafety

Two training modules on biosafety were introduced and pilot tested in the meeting. The purpose was to receive the feedback from the participants on the structure and methods used in these modules, and use it as baseline information before the formal introduction into the global polio laboratory network. The modules were: personal protection equipment; and disinfection, sterilization and waste management. The modules are in the form of videos which are filmed in the real setting of the laboratory; however, some common managerial, supervision and operational mistakes are added intentionally for teaching purposes. After each act, the video goes on pause and this time is used for discussion.

For the purpose of group discussion participants were divided into two groups and each group used one module. The facilitators were asked to run the videos on the computer and lead the group to discuss the mistakes, problems and solutions with the group. It was a very interactive activity and participants enjoyed discussing the problems and solutions.

The personal protection equipment video demonstration contained following parts:

- Part 1 (General biosafety)
- Part 2 (Handling of liquid nitrogen)
- Part 3 (Working in biological safety cabinet (BSC)

The participants noted and commented mistakes: open toed shoes; not wearing goggles wearing while handling liquid nitrogen; kind of gloves worn; safety of equipment; design of laboratory; compliance with good laboratory practices; eating and drinking inside the laboratory; no proper training of new person in laboratory orientation before starting the laboratory work; handling of chemical and chemical safety; emergency measures in case of accident e.g. eye wash solution bottle should be properly labeled; working in the biological safety cabinet (crowded with too many items) things; sequence of steps during processing stool specimen; and biosafety.

The video demonstration on disinfection sterilization and waste management contained the following parts:

- Part 1 (Disinfection)
- Part 2 (Autoclaving)
- Part 3 (Waste management)

In this group discussion the participants also noted and commented on the mistakes: problems of supervision; need to develop standard operating procedures for preparation and use of disinfectants; proper technique to disinfect; proper waste disposal, segregating different types of waste appropriately, and disposal of infectious materials inside the cabinet, use of autoclave bags, and correct disposal sequence: materials, gloves, shoe covers, etc; autoclaving and discarding material adequately, autoclaving should be carried out by trained staff using validated methods and correct settings, discard of autoclaved materials correctly; dispose of chemical waste following recommended procedures; use and discard correct personal protective equipment for all procedures

This group discussion was very fruitful, and allowed many conclusions to be drawn. It clearly showed the enthusiasm and willingness of the laboratory directors towards biosafety and at the same time it showed their knowledge and understanding of biosafety and its importance in their work. Many of them admitted that there are gaps in implementation of full bio safety in their setting and proposed comprehensive training of the laboratory staff.

8.2 Establishment of environmental surveillance in Pakistan

The Pakistan technical advisory group recommended starting environmental surveillance in two major cities of Pakistan (Karachi and Lahore).

The main objectives were to:

- Intensify polio surveillance in Karachi and Lahore, Pakistan, to identify possible reservoir communities where wild poliovirus circulation is sustained.

- Target identified reservoir communities in OPV immunization campaigns to interrupt indigenous wild polio virus transmission.
- Compare the nucleotide sequence from the VP1 region of the genome of wild polioviruses isolated from environmental samples and confirmed polio cases to determine transmission links.

For the establishment of environmental surveillance, field visits were conducted to identify the sewage collection sites and training of field staff for collection, storage and transportation of samples. In the mean time, an environmental surveillance laboratory was established in the regional reference laboratory and it was fully equipped with equipments and reagents.

The potential sites in priority towns of Karachi and Lahore were identified with the help of local government, Ministry of Health and WHO staff and necessary SOPs, schedule of sampling and documents were prepared. On-site training for collection of sewage samples was provided to field staff.

The laboratory staff were trained by a consultant and the regional polio laboratory coordinator on sample receipt and processing; data entry, analysis and reporting; and quality assurance. As of October, the laboratory has processed 26 samples and 8 samples were found positive for WPVs: 2 WPV1 in Lahore; 4 WPV3, 1 WPV1 and 1 mixture of WPV1 and WPV3 in Karachi.

8.3 Managing the polio laboratory work at the time of H1N1 pandemic

As of October 2009, Oman NPL have tested 7000 samples of suspected H1N1, with 28% positive cases, and 28 deaths have been documented due to H1N1. Oman NPL is one of the two laboratories which have been authorized to carry out H1N1 testing. The heavy burden of H1N1 testing compromised the smooth running of routine molecular testing of other viral agents. All resources were pooled (human, consumables and equipment) to meet the needs of pandemic. Due to high workload, all molecular biology unit staff were involved in H1N1 testing.

There was no significant challenge in polio laboratory work in virus isolation and ITD testing, except that it affected the time-frame for establishment of polio rRT-PCR. There is need for a separate molecular laboratory for polio, and it is expected to be resolved after new laboratory complex is constructed, very soon.

8.4 Containment

The major objective of laboratory containment is to minimize the post eradication risk of reintroducing wild polioviruses or Sabin strains from the laboratory to the community, at a time when OPV use has stopped. This can be achieved through national destruction and prohibition of polio virus material except in essential facilities in a minimum number of countries and managing the risk of essential facilities through the primary safeguards of containment and secondary safeguards of location.

Nineteen 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, United Arab Emirates and Yemen), have reported completion of the Phase 1 of laboratory survey and inventory activities. National plans of action has been developed by Afghanistan and submitted for the approval of the Ministry of Public 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 laboratory survey and inventory of laboratories for containment of wild poliovirus and potentially infectious material.

All countries that have completed Phase 1 of containment activities were required to submit the quality assurance report. Documentation of the quality of Phase 1 containment activities was submitted by 18 countries (Bahrain, Djibouti, Egypt, Islamic Republic of Iran, Iraq, Jordan, Kuwait, Lebanon, Libyan Arab Jamahiriya, Morocco, Oman, Qatar, Saudi Arabia, Sudan, Syrian Arab Republic, Tunisia, United Arab Emirates and Yemen). The original or revised report has not been submitted by 4 countries (Djibouti, Egypt, Lebanon and Syrian Arab Republic).

Currently, the Global Action Plan III (GAP-III) draft is available and official adoption will await review by the World Health Assambly.

9. CONCLUSIONS

The Eastern Mediterranean Region has made good progress towards the polio eradication goal. Of 22 countries of the Region, 19 continue to maintain polio-free status, 2 others (Afghanistan and Pakistan) are still considered endemic, and there was continuation of an outbreak due to local circulation of an imported virus in Sudan. In 2008, a major setback occurred to polio eradication efforts, when 117 cases were reported from four provinces of Pakistan and 31 cases were reported from Afghanistan. The main case load in Pakistan was due to WPV1 in Punjab, followed by WPV3 in North-West Frontier Province (NWFP) and Federally Administered Tribal Area (FATA). There was evidence suggesting geographic restriction and decreasing genetic diversity among isolates from Afghanistan and Pakistan, despite the upsurge in the number of confirmed wild polio cases in both countries. In northern Sudan, two cases signalled two separate importations of virus from Chad with no secondary cases; however, in south Sudan an outbreak started in June 2008 and resulted in 24 WPV1 cases up to end of 2008. In Egypt, in April 2008, one a VDPV type 1 was isolated from sewage in Behira governorate, and two WPV1 were detected in two environmental samples from the greater Cairo area in September and December 2008. The WPVs were genetically linked to viruses that recently circulated in Ethiopia and south Sudan (the September isolate) and India (the December isolate). As of October 2009, 72 confirmed WPV cases have been reported in Pakistan, 24 in Afghanistan, 40 in south Sudan and 5 in northern Sudan. Indigenous circulation of WPVs is ongoing in well identified areas of Pakistan and Afghanistan: NWFP/FATA (Swat and Bajour); Quetta Block (Quetta–Pishin–Killa Abdalla) linked with southern Afghanistan; Karachi with recurrent importations and secondary spread;

southern Punjab (continuation of 2008) outbreak; and in southern Afghanistan. In south Sudan, the most recent wild poliovirus case had paralysis onset on 27 June 2009.

In 2008, network laboratories analysed 26 226 faecal specimens (20 903 from AFP cases and 5323 from contacts), which represented approximately a 12% increase in workload compared to 2007. As of October 2009, 20 543 specimens have been analysed in network laboratories. Between January 2008 and October 2009, no vaccine-derived polioviruses (VDPV) were isolated from acute flaccid paralysis (AFP) cases. However, in the Islamic Republic of Iran, one type 2 VDPV was isolated from follow up stool sample collected in 2008 from an AFP case that had onset in 2007

The performance of the regional laboratory network is being sustained at certification standard and AFP surveillance activities are efficiently supported. All network laboratories passed the WHO proficiency tests for both poliovirus isolation, and intratypic differentiation (ITD) testing laboratories also passed the proficiency tests. All laboratories are fully accredited, except Kuwait, which is provisionally accredited. The average reporting time from sample receipt in laboratory to final ITD results decreased from 18 days in 2008 to 16 days in 2009.

Two separate real time PCR (rRT-PCR) assays have been developed by the Centers for Disease Control and Prevention, Atlanta (CDC) for intratypic differentiation and screening for VDPVs. These assays were validated at CDC and evaluated at 6 global specialized laboratories, 2 high workload regional reference laboratories located in South Africa and Pakistan that serve polio endemic countries and a regional reference laboratory in Australia that serves the non-endemic Western Pacific Region. The first rRT-PCR training workshop in the global polio laboratory network was held in the Eastern Mediterranean Region in January 2009 in Muscat, Oman. As of October 2009, rRT-PCR has been fully implemented in three laboratories (Egypt, Islamic Republic of Iran and Pakistan) and work required for implementation is ongoing in four other laboratories (Kuwait, Morocco, Oman, and Tunisia). The main challenge in implementing the rRT-PCR was the increase in requirement for laboratory reagents. The laboratory database (LABIFA 4.1) was revised to include variables or options for recording rRT-PCR results.

Network laboratories in Iraq and Pakistan continue to experience frequent electricity failure and there is also a general problem of insecurity in Iraq. Despite these challenges these two laboratories are maintaining high performance standards with accurate and timely reporting of laboratory results.

A significant achievement was the establishment in 2009 of a fully functional environmental surveillance laboratory in Pakistan where sewage samples are being collected and analysed from the provinces of Sindh and Punjab.

The laboratory infrastructure developed for polio eradication in the Region is proving to be beneficial for supporting the pandemic (H1N1) 2009 laboratory diagnostic work. In this regard, it has been documented that there is sharing of laboratory personnel and/or equipment in four laboratories (Iraq, Kuwait, Oman and Pakistan) for detection of influenza viruses.

The meeting concluded that the regional laboratory network continues to perform at a high standard and that it is successfully meeting the needs of the polio eradication initiative, while playing an expanding role in the evolving H1N1 influenza pandemic.

10. RECOMMENDATIONS

1. The directors of all network laboratories should strengthen management and supervisory practices to improve and sustain laboratory performance. Laboratory directors are encouraged to explore opportunities for participation in local training courses in management and supervision.

2. Ongoing work required for full implementation of rRT-PCR assays for ITD and VDPV screening should be completed in laboratories in Kuwait, Morocco, Oman and Tunisia by the end of the first quarter of 2010.

3. The sample of choice for rRT-PCR assays should be the cell culture supernatant of the isolate. RNA extraction and repeat testing should be done only for those isolates that initially give invalid rRT-PCR test results. Laboratories that are in the process of establishing the rRT-PCR method (Kuwait, Morocco, Oman, Tunisia) and those that are experiencing difficulties should share rRT-PCR data (graphics and .eds files) with CDC and the global and regional polio laboratory network coordinators, for assistance with troubleshooting.

4. LABIFA version 4.1 software should be installed in all ITD laboratories by the end of 2009 to update databases to include new variables and codes for reporting rRT-PCR results for ITD and VDPV screening.

5. Two training modules on biosafety were discussed and pilot tested at the meeting in separate discussion groups. Positive feedback was given by the participants on the format and training approach. WHO should organize a regional training of trainers workshop on biosafety before the planned biosafety campaign is launched in the Region.

6. Network laboratories should develop plans to implement a biorisk campaign in 2010 using training materials developed by WHO for use in the global polio laboratory network. The plan should include designation of a focal point for biosafety in each laboratory and making provision for space and time for all personnel to complete the training programme, with documentation of participation.

7. WHO should advocate with national governments for continued support of polio network laboratories. Expansion of activities to support surveillance or diagnosis of H1N1 influenza or other vaccine-preventable diseases should be pursued in ways that do not jeopardize services or divert resources that are specified for polio eradication.

Annex 1

PROGRAMME

Monday, 26 October 2009

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 H. Ali El Zein, WR Jordan
	Election of the Chairman and Rapporteur	
	Session 1: Overview	
09:30–09:45	Implementation status of the recommendation of the 12th Intercountry Meeting of Directors of Poliovirus Laboratories, and regional progress of EMR polio laboratory network	Dr H. Asghar, WHO/EMRO
09:45–10:00	Overview of polio eradication in EMR	Dr F. Kamel, WHO/EMRO
10:00–10:15	Status of global polio laboratory network	Dr E. de Gourville, HO/HQ
10:15–10:30	Regional progress in AFR polio laboratory network	Dr F. Kasolo, WHO/AFRO
10:30–11:00	Discussion	
	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
12:10–12:30	Molecular characteristics of wild polioviruses in Pakistan, Afghanistan and Sudan	Mr S. Zaidi, Pakistan Dr H. Asghar, WHO/EMRO
12:30–13:00	Discussion	
	Session 3: Laboratory quality assurance	
14:00–14:10	Accreditation status of EMR polio laboratories	Dr H. Asghar, WHO/EMRO
14:10–14:50	Report on proficiency testing (PT) of virus isolation in cell culture and ELISA	Dr H. Avoort, WHO/EMRO
14:50–15:00	Report on proficiency testing of PCR	Dr H. Asghar, WHO/EMRO
15:00–15:30	Discussion	
16:00–16:20	Update on cell sensitivity testing in EMR laboratories	Dr J. Martin, WHO/EMRO
16:20–16:40	Training module on interpreting genetic sequence data	Dr H. Avoort, WHO/EMRO
16:40–17:00	Issues of quality assurance in Kuwait laboratory and their follow-up	Dr S. Al-Mufti

Tuesday, 27 October 2009

Session 4: Management and supervision

08:30–08:50	Management and supervision in the network laboratories	Dr E. de Gourville, WHO/HQ
08:50–09:00	Introduction to the group discussion: highlighting the management and supervision issues faced in the network laboratories in sustaining the good quality laboratory performance	Dr H. Asghar, WHO/EMRO
09:00–11:00	Egypt, Islamic Republic of Iran, Iraq, Pakistan, Sudan Jordan, Kuwait, Morocco, Oman, Syrian Arab Republic	Moderators: Dr J. Martin, WHO/EMRO Dr H. Avoort, WHO/EMRO Dr F. Kasolo, WHO/AFRO Moderators: Dr E.de Gourville, WHO/HQ Dr H. Asghar, WHO/EMRO
11:30–12:30	Group presentation and discussion	

Session 5: Implementation of rRT-PCR assay

12:30–12:45	Overview of real-time PCR assay for ITD and VDPV screening	Dr J. Martin, WHO/EMRO
12:45–13:00	Overview of real-time PCR implementation in global polio laboratory network in 2009	Dr E. de Gourville, WHO/HQ
14:00–15:00	Implementation of rRT-PCR and results of retrospective testing of Sabin isolates	
	Egypt	Dr L. El-Bassioni
	Islamic Republic of Iran	Dr S. Shahmahmoodi
	Kuwait	Dr S. Al-Mufti
	Oman	Dr Said Al Baqlani
	Pakistan	Mr S. Shawkat
15:00–15:30	Discussion	
16:00–16:20	Estimation of rRT-PCR (ITD and VDPV) reagents for ITD laboratories	Dr H. Asghar, WHO/EMRO
16:20–17:00	Discussion	

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Session 6: Miscellaneous topics

08:30–10:30	Introduction to pilot testing of two training module on biosafety	Dr E. de Gourville, WHO/HQ

	Video demonstrations Group 1 Personal Protection Equipment (PPE) Sudan Group 2 Disinfection, sterilization and waste management Kuwait	Moderators: Dr H. Avoort, WHO/EMRO, Dr F. Kasolo, WHO/AFRO, Dr E. de Gourville, WHO/HQ, Dr J. Martin, WHO/EMRO, Dr H. Asghar, WHO/EMRO
10:30–11:00	Discussion	
11:30–11:45	Establishment of environmental surveillance in Pakistan	Dr H. Asghar, WHO/EMRO
11:45–12:00	Managing the polio laboratory work at the time of H1N1 pandemic	Dr Said Al Baqlani, Oman
12:00–12:15	Status of survey and inventory required in Phase 1 of laboratory containment of wild polioviruses	Dr H. Asghar, WHO/EMRO
12:15–13:00	Discussion	
14:00–15:00	Discussion on conclusion and recommendations	

Annex 2

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