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Report on the

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

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1. INTRODUCTION

The eleventh intercountry meeting of directors of poliovirus laboratories in the WHO Eastern Mediterranean Region was held in Cairo, Egypt from 29 October to 1 November 2007. Directors of poliovirus laboratories in Egypt, Islamic Republic of Iran, Iraq, Jordan, Kuwait, Morocco, Oman, Pakistan, Saudi Arabia, Sudan, Syrian Arab Republic and Tunisia attended the meeting. Participants also included scientists from the Centers for Disease Control and Prevention (CDC), Atlanta; National Institute of Public Health and the Environment (RIVM), the Netherlands; National Institute for Biological Standards and Control (NIBSC), United Kingdom; and staff from the World Health Organization (WHO) headquarters, Regional Office for the Eastern Mediterranean (EMRO) and Regional Office for Africa (AFRO).

Dr M. H. Wahdan, Special Adviser to Regional Director for Polio, 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 expressed his appreciation the contribution made by the polio laboratory network, emphasizing the vigilance and readiness to detect the circulation of wild polioviruses accurately in a timely manner for quick response in the field to contain the spread of wild poliovirus transmission. He expressed his satisfaction about the successful implementation of new cell culture and intratypic differentiation testing algorithm. He commended the performance of nucleotide sequencing laboratories for providing results with great efficiency and accuracy, and urged the laboratories to maintain high standards of quality assurance to sustain the good laboratory performance indicators.

Dr Eman Al-Maamoun, (Egypt) was elected to Chair 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 TENTH INTERCOUNTRY MEETING OF DIRECTORS OF POLIOVIRUS LABORATORIES IN THE EASTERN MEDITERRANEAN REGION

Dr Humayun Asghar, WHO/EMRO

Dr Asghar reviewed the implementation and main achievements of the recommendations of the tenth intercountry meeting of directors of poliovirus laboratories in the Region. It was clear that all the recommendations addressed to national authorities and those addressed 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

Dr Faten Kamel, WHO/EMRO

The Region continued to make progress towards the goal of polio eradication. The year 2006 witnessed the lowest ever number of cases reported in the history of the Region (107) and by end October 2007, circulation of the wild virus in the Region was restricted to limited areas in Pakistan (17 cases) and Afghanistan (12 cases). In addition, 8 cases were reported from defined areas in Somalia, representing the tail of the epidemic that occurred as a result of importation, with the last case in March 2007.

In the past year, the Region has been taking the lead role in introduction of new tools that are enhancing the impact of eradication strategies. These tools include the use of monovalent oral polio vaccines and new laboratory methods to reduce time needed to confirm the diagnosis.

Several achievements were made in Pakistan and Afghanistan. The vast majority of the population in both countries live in polio-free areas; transmission is focal in two zones (NWFP and adjacent parts of Afghanistan as well as along corridor of population movement extending from southern Afghanistan through Baluchistan to northern Sindh and Karachi). Other achievements include sustained political commitment at national levels, continued improvement in the quality of supplementary immunization activities (especially in high risk areas) and maintaining certification standard surveillance.

The main challenges facing the programme in Afghanistan and Pakistan include difficult access to children in certain areas due to insecurity and conservative communities, increasing percentage of refusals, continued exchange of viruses through the mobile population and the need for more ownership/engagement of political leadership at local level.

The main strategic directions adopted in both countries are concentration of efforts on remaining areas of transmission using appropriate mOPV, improved access to all children (especially those in insecure areas), high level of operational quality of supplementary immunization activities and surveillance with maintaining overall population immunity (through NIDs) and rapid detection of virus and mop-up response (mOPV) in polio-free areas. A lot of efforts were exerted recently to further enhance coordination between the two countries, especially at the border areas and to ensure accessibility to children living in insecure areas in Afghanistan.

Preparedness for poliovirus importation continues to receive special attention as the risk of importation will continue as long as wild poliovirus is circulating anywhere in the world. Guidelines were prepared for developing national plans for preparedness and were updated in line with ACPE recommendations considering that the main pillars for preparedness are: high population immunity; highly sensitive AFP surveillance system to allow early detection and timely response; studying population movement between and within countries; anticipation of possible importation; and putting the national plan into action.

One confirmed case was reported from south Darfur in Sudan with date of onset 10 September, due to imported virus linked to circulation in bordering areas of Chad. The case was timely detected and investigated and followed by adequate response indicating good preparedness.

In Eastern Mediterranean Region, all infected or recently polio-free countries are maintaining the required level of AFP sensitivity and in 2006 the regional rate reached 3.9 per 100 000 children under 15 years of age and 4 to date in 2007. In 2006, the minimum required level of 1 per 100 000 children under 15 was not reached in 3 countries, all with a small number of expected cases.

The second key quality indicator for surveillance is percentage of AFP cases with adequate stool collection. In 2006 this indicator was maintained above the target of 80% at the regional level (89.2%) and in 18 of the 22 countries of the Region. This was further improved in 2007 with a regional figure of 90% and only two countries below the target. The AFP surveillance reviews conducted in 2006 and 2007 confirmed the quality of the surveillance systems and its ability to detect any possible wild poliovirus.

Certification activities are moving forward, with national documentation accepted from 18 countries and provisional reports received from 3 countries with viral circulation. Final national documentation was accepted from 12 countries of those polio-free for 5 years or more and having completed Phase 1 of laboratory containment. Regular updates are submitted by all countries that have submitted national documentation.

The main challenges facing the programme include securing necessary resources both from national funds and external resources, maintaining interest and commitment of national authorities and the public and reaching children living in security-compromised areas with the necessary vaccine.

Regional priorities for polio eradication during the coming year are to:

- Interrupt transmission in the remaining endemic countries;
- Avoid 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;
- Maintain certification-standard surveillance, with focus on performance and indicators at sub-national level and among high risk areas or populations;
- Strengthen coordination activities 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;
- Continue with containment and certification activities;
- Optimize PEI/EPI collaboration; and
- Secure the financial resources required to implement the regional plan for eradication.

3.2 Status of the global polio laboratory network

Dr Esther de Gourville, WHO/HQ

During 2006 and the first half of 2007, progress continued towards the goal of global polio eradication. Transmission of wild poliovirus (WPV) continued in 4 endemic countries (Afghanistan, India, Nigeria and Pakistan); outbreaks due to importations continued in 5 countries (Angola, Chad, Democratic Republic Congo, Myanmar and Niger); and an importation was detected in Sudan in September 2007. Most importations occurred due to WPV1. In 2006, a total of 1997 polio cases were reported, while as of October 2007, 635 polio cases were reported. In 2007, WPV3 circulation was more than WPV1 in India and Nigeria, and WPV1 was detected in a sewage sample from Switzerland.

In February 2007, a consultation of all major polio eradication stakeholders was held at WHO headquarters. The meeting was attended by envoys of the heads of states of the 4 endemic countries and by all major polio eradication partners, and resulted in the development of action plans (and agreement on milestones for intensified polio eradication activities).

Programmatic changes were introduced to address the challenges and included widespread use of type 1 monovalent OPV (mOPV1), implementation of targeted strategies to reach more children thorough supplementary immunization activities, synchronization of cross-border immunization activities, and introduction of new testing algorithm to confirm cases rapidly. Since April 2005, 2.4 billion dose of mOPV1 have been used.

The global polio laboratories network has screened all Sabin-related isolates since 1999. Such isolates were found in 7311 specimens between January 2006 and June 2007. A total of 121 (1.7%) of these were categorized as vaccine-derived polioviruses (VDPVs): 107 were cVDPVs; 12 were isolates from immunodeficient persons; and 2 were aVDPVs. Isolates of iVDPVs were obtained form 7 immunodeficient people detected between January 2006 and June 2007: 3 had serotype 2 (detected in Islamic Republic of Iran, in France in a child of Tunisian origin and in the Syrian Arab Republic); 1 was co-infected with type-1 and type-2 VDPV (in Islamic Republic of Iran); and 3 had type-3 VDPV (in Egypt, Islamic Republic of Iran and Kuwait). Serotype 1 aVDPVs were isolated form a single AFP case in Shanxi, China. Non-AFP sources also provided aVDPVs found in a healthy child in Shanghai, China (serotype 3), and sewage water in Israel. A total of 69 polio cases associated with type-2 cVDPVs were detected in nine northern states of Nigeria in children with acute flaccid paralysis. At least 46 (49%) of the cVDPVs isolates and closely related isolates were from Kano state, which has been a major reservoir for WPV1 and WPV3 circulation. Genetic analysis showed that at least 7 distinct cVDPVs genetic lineages, suggesting independent emergence of multiple cVDPVs transmission chains in 2005 and 2006. The issue concerning Nigeria cVDPV outbreak was that the viruses not flagged by network screening methods of poliovirus for VDPVs. Geographical and temporal clustering of poliovirus type-2 (PV2) Sabin-like viruses led to further investigations at CDC.

In 2006, 93% of network laboratories were fully accredited by WHO. The network laboratories analysed 137 000 AFP stool specimens in 2006. There was an increase of 106%

in workload between 2003 and 2006, most of the increase in workload occurred in the South East Asia Region. The increase occurred primarily because of efforts aimed at increasing the sensitivity of AFP surveillance in endemic countries and virus importation into several other countries.

Laboratories in all WHO regions met the programme's 28-day target for providing virus isolation results. All, except in the African Region, did not meet the target of providing intratypic differentiation (ITD) results on polioviruses within 14 days of receipt.

To increase the speed of poliovirus confirmation, a new cell culture and ITD algorithm was field tested in Pakistan, India and CDC. Its use halved the time for completing laboratory analysis (from 42 to 21 days) without compromising the sensitivity of poliovirus detection. The algorithm was adopted in June 2006, and by March 2007, the virus isolation component implemented in 42/43 laboratories in endemic regions. ITD component was implemented in 14/18 laboratories in endemic regions. The impact of the efforts to reduce laboratory reporting time is already evident. In 2007, approximately 80% of WPV importation or outbreaks were detected by laboratories of the global poliovirus laboratory network within 21 days of onset of paralysis.

There were some laboratories with special concerns, such as poor performance in proficiency testing (PT), incident of cross contamination of samples (Bangladesh), low cell sensitivity and under-estimation of WPV (Maiduguri), and infrastructural issues (electrical cuts, renovation of laboratory, fire in ERC Mumbai).

3.3 Regional progress in the regional polio laboratories network

Dr Humayun Asghar, WHO/EMRO

The performance of WHO Eastern Mediterranean Region polio laboratory network is sustained at certification standard indicators. All network laboratories were fully accredited, except Kuwait which was provisionally accredited, pending ELISA proficiency panel testing. All laboratories passed the WHO proficiency testing panel for both primary virus culture and intratypic differentiation testing. The workload remains high due to general improvement in AFP surveillance, maintaining a non-polio AFP rate of at least 2 per 100 000 children under 15, and collection of stool samples from contacts. Between 2003 and 2006, there were increases of 61% in AFP sample collection, 392% in contact sample collection and 73% in ITD testing. After the implementation of new test algorithm, there was also an increase in consumption of disposable material and reagents.

A remarkable achievement is implementation of new testing algorithm in all network laboratories, which has shortened the time of reporting of virological investigation results to less than two weeks. The speed of sequencing results for wild polioviruses has also increased, most WPV positive cases have been confirmed within one week of isolation, which in turn has decreased the response time in the field for immunization activities.

All laboratory performance indicators are well above the set targets: cell culture report within 28 days, NPEV rate, ITD with 14 days, and final report within 60 days of onset of

paralysis. After the implementation of new test algorithm, the mean time from onset of paralysis to ITD result is 22 days in 2007, as compared to 29 days in 2006.

In Egypt, AFP surveillance is continuing at good sensitivity, and one more site (Red Sea) has been added to the 33 already-existing sewage collection sites for environmental surveillance. Two more laboratories were given the facilities for ITD testing, and are in the process of establishing the ITD testing. New LABIFA software, version 4.0, was designed in light of the needs of the new algorithm for use in network laboratories in January 2008.

3.4 Regional progress in the African regional polio laboratories network

Dr Francis Kasalo, WHO/AFRO

As of 24 October 2007, 280 WPV cases were confirmed in 5 countries representing a 75% reduction in WPV cases. In addition, cVDPVs type 2 have been reported from Nigeria and Niger. There are two epidemiological zones of WPV circulation in 2007: Nigeria–Niger—Chad (multiple importations from Nigeria into Chad and Niger, and re-appearance of WPV cluster of 2005 in Chad), and Angola–Democratic Republic of Congo (original importation from India into Angola in 2005, subsequently multiple importations into DRC and Namibia in 2006, and recent importation into Angola from India). A significant decline in the number of circulating WPV1 clusters has been observed in Nigeria.

In 2006 the African laboratory network tested a total of 25 918 AFP stool specimens. As of October 2007, the laboratory network had tested 20 214 specimens and the total number in 2007 is expected to rise beyond the 2006 numbers. With the introduction of the new algorithm, there has been an estimated of 20%–25% increase in workload due to the need to have additional cell culture tubes.

In 2006, 14 of 16 African network laboratories were fully accredited; the other two were provisionally accredited (CAR and Maiduguri). In 2007, 13 of 16 laboratories were accredited (Ghana provisionally accredited), and three are pending accreditation. All virus isolation laboratories passed the 2007 PT panel and eight out of nine PCR laboratories passed their PT panel.

The new virus isolation algorithm was successfully implemented in all network laboratories. The overall mean interval for primary isolation has decreased from an average of 23 days to 15 days in 2007. Four out of 9 laboratories with ITD capacity have successfully introduced the ITD algorithm. Overall mean interval for ITD results has improved from an average of 15 days to 8 days. There is a significant increase in workload due to implementation of the new algorithm.

4. VIRUS SURVEILLANCE

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

Dr Mark Pallansch, CDC/USA

As of October 2007, there are only 3 WPV1 and 1 WPV3 clusters circulating in Pakistan and Afghanistan. The WPV1 belonging to B4A cluster was isolated in Australia from an adult Pakistani student.

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

- Declining nucleotide diversity of wild poliovirus isolates in Pakistan and Afghanistan, and Somalia with geographical localization of clusters;
- Re-importation of WPV1 from Chad to Sudan in September 2007.

WPV type 1

A3-A: In 2006, it was circulating in southern Afghanistan, and continued to circulate in Helmand and Kandahar in first quarter of 2007. It was also detected in Karachi (Sindh Pakistan) in January and August of 2007.

A3-D: In 2006, this cluster was circulating in southern Afghanistan (Kandahar and Helmand) and northeastern Afghanistan (Nangarhar and Baghlan). It was also circulating in NWFP Pakistan in Bajour (Federally Administrated Tribal Areas), in Baluchistan (Quetta), and in Sindh (Karachi). In 2007, it was only seen in North-west Frontier Province (NWFP) of Pakistan (Khyber and Nowshera) and Sindh (Kumbar and Thatta).

B4-A: In 2006, this cluster was circulating in NWFP of Pakistan and eastern Afghanistan (Laghman). In 2007, it is circulating in NWFP (Swat and Peshawar) and north-eastern Afghanistan (Nangarhar).

WPV type 3

B1-C: In 2006 and 2007, this cluster is the one circulating continuously in Pakistan and southern Afghanistan.

Horn of Africa and Chad

WPV1 was circulating in Ethiopia and Somalia. The Somalia WPV1 was detected in a refugee camp in Kenya. The Ethiopia outbreak stopped in November 2006, while last WPV case in Somalia was detected in March 2007. All Somali viruses belonged to WEAF-B genotype, I-1C5 cluster. The WPV1 importation in 2004 into Chad continued, the genetic data suggest that there are gaps in surveillance. The WEAF-B genotype, cluster I-1C3 is circulating in Chad and recently its importation has been detected in Sudan, and in a sewage sample in Switzerland.

4.2 Pakistan and Afghanistan: laboratory performance indicators

Mr Sohail Zahoor Zaidi, National Institute of Health, Pakistan

The WHO Regional Reference Laboratory (RRL) Pakistan has been continuously supporting the global polio eradication initiative through its excellent performance and timely reporting, which was particularly impressive given the increased workload in 2006 and 2007.

In 2006, the RRL received and analysed 6844 stool samples. These comprised 4295 samples from AFP cases and 2549 samples from contacts. A total of 40 wild polioviruses (20 WPV1 and 20 WPV3) were isolated and the NPEV rate was about 23%. From Afghanistan, 1585 stool samples (979 AFP and 606 contacts) were received and processed. The NPEV rate was 24% and 28 wild polioviruses (27 WPV1 and 1 WPV3) were isolated.

In 2007 to date, the RRL received stool samples of 5160 cases from Pakistan and 17 wild viruses (10 P1 and 7 P3) were isolated from 10 districts of Pakistan. The NPEV rate is 22%. For Afghanistan, 1362 stool samples have also been processed and 4 WPV1 and 8 WPV3 have been isolated so far. The NPEV rate is 26%.

The RRL met all criteria for full accreditation by WHO in 2006. The results of the RRL are reliable and accurate as demonstrated by scores of 100% in virus isolation PT panel testing and 97.5% in polymerase chain reaction (PCR). The laboratory has 100% concordance in ITD results with the Centers for Disease Control and Prevention (CDC), Atlanta.

In 100% of the samples, results were reported within 28 days. The mean reporting time for wild viruses after receiving the sample to sequencing results is 12 days.

4.3 Egypt: laboratory performance indicators

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 and Yemen. Between September 2006 and October 2007, a total of 3415 samples (2548 from AFP cases and 867 from contacts) were tested. The performance indicators were sustained at high level: 98% of culture results within 28 days, 97% of ITD results within 14 days, 100% referred for sequencing within 7 days, NPEV rate of 15% and 100% score in virus isolation, enzyme linked immunosorbent assay (ELISA), PCR and nucleic acid probe hybridization (NAPH), PT panel testing.

Environmental surveillance continued with collection of sewage samples from 34 sites in 19 provinces. A Red Sea site was recently added to the list as a new collection site. Almost all sewage samples collected yielded either NPEV or Sabin-like viruses or a combination. The poliovirus isolates and sample concentrate obtained from sewage samples are sent to KTL, Finland, for parallel testing. As of October 2007, 1963 positive environmental isolates and 528 concentrated environmental samples have been sent to KTL. There is high concordance between results of KTL and VACSERA.

4.4 KEMRI: laboratory performance indicators

Mr Peter Borus, KEMRI, Nairobi, Kenya

The polio laboratory is housed in the Kenya Medical Research Institute (KEMRI). The laboratory is an intercountry laboratory serving Kenya, Eritrea, Somalia, south Sudan and Djibouti. It is fully accredited for 2007–2008.

Since April 2007, the laboratory has been implementing the new virus isolation test algorithm. The laboratory tested 1694 stool specimens from January to September 2007. Of these, 1333 were from AFP cases whereas 361 were from contacts. In this period, 636 stool specimens from cases and contacts were received from Somalia, whereas south Sudan sent 275 stool specimens.

Compared to 2006, the laboratory handled more samples month by month in 2007 from Somalia, whereas there were generally more samples in most months in 2007 for south Sudan compared to 2006. Fourteen WPV1 were isolated in the laboratory from 8 AFP cases from Somalia.

The laboratory is meeting all its traditional performance indicators. In-house quality assurance is done through the write-up of SOPs, calibration of equipment and cell sensitivity tests. Cell sensitivity data are documented at least quarterly and results are within the required reference titre.

Results of virological investigation for 100% of samples were given within 28 days. However, the laboratory did not meet the 14-day target for ITD results (score is 45.4%) due to shortage of supplies. All poliovirus isolates are forwarded to RRL (NICD, South Africa) in a timely manner and usually within 2 days of test results. The accuracy of poliovirus detection for the laboratory has been 100%. The overall NPENT rate for the laboratory for the period January to September 2007 was 12.5%. For Somalia, the NPENT rate has been above 10% month-by-month except in July, when it was 4.8%. The rate for south Sudan was 0% in January and February, but was above 10% in all subsequent months.

The main challenge facing the laboratory in 2007 was the difficulty in implementing the ITD test regimen for ELISA. Corrective measures were taken and it is anticipated that this will result in the laboratory fully implementing the ITD ELISA by December 2007 and subsequently the ITD PCR in the first quarter of 2008.

5. LABORATORY QUALITY ASSURANCE

5.1 Accreditation status of EMR polio laboratories

Dr H. Asghar, WHO/EMRO

In 2006, 11 of 12 regional network laboratories were fully accredited. The Kuwait regional reference laboratory was provisionally accredited. As of October 2007, all are fully accredited by WHO, except the national poliovirus laboratories in Jordan, Morocco and

Syrian Arab Republic, which are pending accreditation visits. All national poliovirus laboratories implemented recommendations made during accreditation visits.

There is sustained good quality performance of all laboratories of the network. All network laboratories were able to maintain the timeliness of reporting of virological investigation results. There was uniform implementation of quality assurance programmes.

5.2 Proficiency testing results for virus isolation and typing 2006–2007, and PT panel testing in new algorithm

Dr H. van der Avoort, WHO Temporary Adviser

The regional laboratories have all reached a 100% score in the 2007 proficiency test for isolation and typing. This remarkable achievement illustrated the reliability of the EMR laboratories network.

For 2008 a new type of PT for isolation of polioviruses and enteroviruses in stool samples will be in place, consisting of 10 stool specimens to be analysed according to the isolation part of the new algorithm. This panel will be field tested in 2007; Some EMR laboratories will be included in this field test.

5.3 Report on proficiency testing: PCR and ELISA

Dr Mark Pallansch, CDC/USA, and Dr H. van der Avoort, WHO Temporary Adviser

The PCR PT panel 2007 was distributed among the regional reference laboratories and selected national laboratories in their use of probe hybridization and diagnostic PCR for ITD. All the laboratories sent results electronically of excellent documentation with findings and interpretations.

Three laboratories (Egypt, Islamic Republic of Iran and Oman) scored 100%, while Pakistan, Tunisia and Kuwait scored 95%, 92.5% and 90%, respectively. VACSERA scored 100% in NAPH PT testing. The network laboratories are highly proficient in diagnostic PCR and (where used) probe hybridization

All but one laboratories of the EMR network performing ELISA have passed the PT, with only the Kuwait laboratory having problems. Most of these problems were solved after the visit of a consultant. The new panel for 2007 was distributed after the meeting, with reporting time of 7 days, after arrival at the home laboratory.

5.4 Proposed changes in the polio laboratory checklists

Dr Esther de Gourville, WHO/HQ

In the light of new test algorithm two changes are required: adding two supplements and revising the section on accreditation checklists. One of the two supplements has already been developed and distributed; however, revision is needed to finalize the flowchart and re-distribution. The other supplement is on the adaptation of newly received cells to local conditions.

According to new checklist only 2 titles for laboratories will be used: national (or virus isolation) laboratory (NL) (replacing national polio laboratories), and ITD laboratories (replacing regional reference laboratories nomenclature). The format has been designed to allow evaluating both the traditional (mostly polio-free regions) and new algorithm (used in polio endemic regions). Many sections have been revised and main focus is on revision of target times for reporting using the new algorithm, virus identification section, materials to be stored or referred, variables in the database for reporting of results, supervision and management, internal quality control procedures, equipment maintenance, reagents and supplies, bio-safety and containment, bio-safety practices and procedures and cooperation with Regional Offices.

The scoring system under laboratory operating procedures and work practices has been revised. Six criteria (reporting virus isolation test results, number of tests performed per year, accuracy of poliovirus typing (traditional algorithm), accuracy of identifying poliovirus positive specimens (new algorithm), ≥80% poliovirus isolates referred for ITD, score on isolation/identification proficiency testing, on-site score of laboratory operating procedures and practices, internal quality control (QC) implemented at least quarterly will be used for accrediting NL and six to seven criteria (reporting ITD test results, referral of ≥80% wild polioviruses and suspected VDPV for sequencing, score on ITD proficiency testing.

5.5 Update on cell sensitivity testing in EMR network laboratories

Dr Javier Martin, WHO/EMRO

Several years of experience have shown that cell sensitivity testing provides the most critical data to reassure that laboratories can isolate polioviruses. There are no major issues in cell sensitivity testing in EMR laboratories. However, minor corrections would improve the quality of testing and facilitate follow-up to correct any possible deficiencies. Laboratories should continue regular testing and report results within 48 hours of virus titre determination.

Trend data should be represented using the appropriate scale to help detection of changes in virus titres. Horizontal lines showing upper and lower titre range limits should be included in graphic representations of data. Relevant changes in procedures or personnel running the test should be indicated in the trend chart. Laboratory directors should work closely with the regional coordinator and colleagues at NIBSC to implement the correct testing and interpretation of results. Detailed training of new personnel is essential to ensure the quality assurance of laboratory procedures necessary for poliovirus isolation and characterization.

5.5.1 Experience with cell sensitivity testing: Syrian Arab Republic

Dr Arraj Amira, NPL, Syria

The data on cell sensitivity testing for the period from January 2006 up to June 2007, and for the period between July 2007 to September 2007 was presented. The titre for both NIBSC and laboratory quality control in 2007 were low. This may be due to less sensitive cells or poor media or problem in procedure performance. The EMR laboratory coordinator

and NIBSC were in continuous discussion and it was finally agreed to resolve during forthcoming accreditation visit.

5.5.2 Experience with cell sensitivity testing: Morocco
Ms Maria El Qazoui, NPL, Morocco

The NIBSC Sabin standards were received at room temperature due to delay in clearance; however, the laboratory inoculated these on RD and L20B cell lines to prepare the laboratory quality control (LQC). Upon testing, titres of virus were low in both NIBSC standards and LQC for all serotypes. The issue was discussed with laboratory coordinator and NIBSC, and it was suggested to check the technique and also replace the cells, because it may be due to less sensitive cells. New cells were received from Kuwait and VACSERA at different times and gave the same results. It was again discussed with laboratory director during the meeting and she suggested another solution to fix the problem.

6. NEW TEST ALGORITHM

6.1 Progress on new test algorithm: proposed changes after field testing

Dr E. de Gourville, WHO/HQ

After field testing, the new test algorithm has been implemented in 3 endemic regions. Achievements include the following:

- Changes and review of SOPs were completed.
- Changes to databases are ongoing in 3 regions.
- The isolation algorithm is in use in 42/43 laboratories in endemic regions (Democratic People's Republic of Korea (DPRK) pending)
- 18/43 laboratories in endemic regions have ITD capacity (14 are using new ITD algorithm, 2 are introduced PCR since June 2007 and 2 not using new ITD algorithm (need PCR capacity))
- 9 additional laboratories will establish ITD capacity by end 2007.
- Various training workshops on cell culture and ITD testing were conducted.

There was significant impact on reporting time of cell culture and ITD results: for the Eastern Mediterranean Region, the average time for cell culture result decreased from 15 days to 13 days; for ITD from 6 days to 3 days; and reporting time from onset to final laboratory result from 27 days to 24 days. In most instances in 2006 and 2007, WPV confirmation results were available within 21 days.

The operational issues were related to needs for interpretation of virus isolation results, adjusting reporting variables, extra PCR and electrophoresis equipment in high workload laboratories, cost of testing isolates from both arms of the algorithm in high volume laboratories and uncertainty about the benefit of doing ITD tests on L+R- and R+L+R- (inconclusive) isolates. Laboratories were reluctant to meet 100% increase in demand and cost of enzymes for PCR. Another issue was slow response to requests for ELISA reagents and P3

ELISA reagents in short supply. Laboratories were also faced with challenges in storing and tracking isolates.

A laboratory survey was conducted after introduction of the new algorithm and laboratories were asked four main questions: 1) What is the frequency of different categories of virus isolation results? 2) What were the outcomes of PCR testing of "virus isolation inconclusive" isolates? 3) What is the frequency of concordant serotyping and ITD results for isolates from different arms of the algorithm? 4) What is the NPEV isolation rate?

According to survey results, a total of 25 538 specimens of 10 468 AFP cases were tested, and 1481 (5.8%) specimens were with isolates for ITD (L20B positive, inconclusive or mixtures). All inconclusive results obtained in the L20B arm were L+R- isolates, inconclusive results were rare (< 1% of more than 25 200 specimens), and no new polio cases were found (one example of polio found from inconclusive isolate from the first stool but the second stool was L20B and polio positive).

ITD tests were done on 1226 specimens using the new algorithm (i.e. L20B positive, inconclusive and mixtures), out of which 29% of specimens had isolates from only 1 arm of the algorithm. Of the 71% of specimens with isolates from both arms of algorithm, 1% specimens had isolates with discordant ITD results when isolates from both arms tested and 99% had concordant serotype and intratype results.

The new algorithm had a negligible impact on overall NPEV rate; however, it is too early to determine impact on NPEV rate for individual laboratories. It is necessary that laboratories work with sensitive cells, and this determines that monitoring of cell sensitivity data is more critical.

6.2 Experience in implementing the cell culture and intratypic differentiation (ITD) testing algorithm

6.2.1 Pakistan

Mr Sohail Zaidi, National Institute of Health

The RRL Pakistan has implemented the new test algorithm from May 2006. A total of 20 635 specimens from 8034 AFP cases were tested. No virus was isolated from 14 709 specimens. A total of 2233 isolates were sent for ITD testing, out of which 901 were positive on both arms (LR and RLR arm), 1100 were positive on LR only, and 1044 were positive on RLR arm. Wild poliovirus was isolated from 175 isolates; 769 are positive for Sabin-like viruses. A total of 205 isolates have mixtures in it.

The new testing algorithm has required an increase of 12 000 culture tubes per month and two-fold monthly increase in cell culture flask, cryovials and fetal calf serum. Cell sensitivity testing was performed 15 times in the past 12 months. There was no cell contamination or problem with cell availability due to the increased demand for virus isolation.

6.2.2 Egypt

Dr L. El-Baissouni, VACSERA

The new test algorithm was implemented in January 2007. A total of 2440 specimens were tested: 1771 specimens of 906 AFP cases and 669 specimens of contacts. Results were available for 2383 specimens: 1945 negative, 438 positive, 339 NPEV positive, 2 WPV positive, 114 Sabin-like PV (16 mixtures).

VACSERA was testing all positive specimens on PCR from both LR and RLR arm; however, they shifted to testing of positive isolates on LR arm after September 2007. This will decrease the workload of PCR and ELISA testing.

There was no change in timing of cell passage, but there is an increase in the quantity of cell culture tubes, flasks (especially for RD cells), serum and media. There is increase in RD cell workload of 25%, and reduction in serotyping by 72%. Due to new algorithm, there was a 7-fold increase in workload in PCR and a 50% increase in ELISA workload. To reduce the workload in PCR, extra PCR and electrophoresis equipment is needed.

6.2.3 Oman

Dr S. Al Baqlani, NPL, Oman

The new test algorithm was implemented in January 2007. A total of 865 specimens were tested: 688 negative, 33 Sabin-like virus positive (9 mixtures), and 144 NPEV positive. All viruses were isolated on both arms, and no inconclusive specimen was reported. There was no change in cell culture cycle; however, there was 10% increase in consumables and media. The laboratory is testing LR positive isolates for ITD. There was two-fold increase in PCR and ELISA testing. Due to the increase in workload, extra personnel or extra working hours are required. Cell sensitivity testing is performed regularly and values are within acceptable range.

6.2.4 Sudan

Mr H. Babikar, NPL

The new test algorithm was implemented in January 2007. A total of 697 specimens of 270 AFP cases and 158 contacts were tested: 599 negative, 2 WPV1, 19 Sabin-like virus positive, and 77 NPEV positive.

All positive cultures were sent to VACSERA within 7 days of isolation. Two inconclusive isolates each on LR and RLR were sent to VACSERA and tested negative and NPEV, respectively. Only 56% results were reported within 14 days after receipt of sample. Most recent WPV1 isolated from south Darfur was reported positive on cell culture after 8 days of receipt in laboratory. There was no change in cell culture cycle.

6.2.5 Jordan

Dr M. Karsaneh, NPL

The new test algorithm was implemented in January 2007. A total of 119 specimens of 31 AFP cases and 88 contacts were tested: 103 negative, and 13 NPEV positive. All positive cultures were observed on both LR and RLR arms. Twelve positive cultures were sent to Tunis RRL, which confirmed all as NPEVs; however, 5 cultures positive on LR arm were confirmed as NPEV. Since most NPEV cannot grow on L20B, it was assumed that L20B cells were contaminated with RD cells. The NPL was advised to change the L20B cell line. There is no change in cell culture propagation cycle; however, the quantity of cell culture tubes, media and other disposable material and reagents has increased.

6.3 Group discussion: new testing algorithm

Group 1: New cell culture testing algorithm

This group comprised participants from Egypt, Iraq, Jordan, Morocco, Pakistan, Saudi Arabia, Syrian Arab Republic and KEMRI. The following salient points were highlighted by the group, which recommended action to address them where indicated.

- The new algorithm is fully implemented without major problems.
- Cell culture maintenance and quality is an issue; some of the laboratories reported short survival of cells, i.e. 4–5 days. The following actions were suggested.
  - Laboratories should check water quality, pH with standardized buffers for calibration, temperature of incubators, reagents quality etc.
  - The flasks and tube should be seeded with appropriate amount of cell inoculums i.e. concentration should be adjusted for longer survival of cells.
  - Frequent passaging of cells should be avoided by using sets of flasks per week, especially for high workload laboratories.
  - Cells should not be put in the incubator directly on the metal tray.
- With regard to mycoplasma contamination, mycoplasma testing is not advised routinely. To prevent contamination, the following actions were suggested.
  - Ensure good laboratory practice.
  - Always discard cultures after 15 passages.
  - Always use cells that have been tested as mycoplasma-free on supply.
- It was noted that NPEV rate is declining in some countries with the implementation of new algorithm. The following actions were suggested.
  - Observe the trend of NPEV isolation by season and analyse country data after data are available for 12–18 months.
  - All laboratories are using similar worksheets, which are too squeezed. Laboratories can make changes by having 2 or 3 cases per sheet.
  - Pooling of tubes should be done when they reach 3+ cytopathic effect (CPE) at the same time for each arm, because 3+ CPE takes 1–2 days to show CPE.
- Surveillance colleagues do not understand the reporting format for preliminary reports. The following actions were suggested.

  - There is a need to sensitize surveillance staff through meetings with EPI/surveillance staff.
  - There is concern of losing the confidence of surveillance staff if a positive culture sent for ITD turns out to be negative on PCR.
- With regard to quality of documentation, it was noticed that laboratories are not properly recording cell count, cell passage history, temperature monitoring and actions taken, and revival of cells from liquid nitrogen. The following actions were suggested.
  - Documentation should be used as a way of having evidence of work done. It ensures traceability on who did what, when and trouble shooting, whenever problems occurred. Concerns were shown on quality of documentation, as an observation of accreditation visits.
  - The cell culture log book should have continuous recording of all procedures. The PT panel should be documented like any other sample in receiving/logbook.
  - Power back-up should be ensured, but in case of electricity interruption this needs to be documented.

Group 2: New ITD testing algorithm

This group comprised participants from Egypt, Islamic Republic of Iran, Kuwait, Oman, Pakistan and Tunisia. A number of issues were raised and recommended actions were made.

- With regard to responsibilities of NPL and ITD laboratories, the NPL should refer all the positive culture tubes from the both arms, i.e. LR and RLR arm. The ITD laboratory should perform PCR on LR arm only. If LR arm is negative on PCR, perform PCR for RLR arm. If referred isolate becomes negative on PCR, then passage on RD cells and repeat PCR. Perform sequencing on LR arm isolate.
- With regard to reporting of results of testing on L20B cells and PCR, the following actions were suggested.
  - The samples positive on L20B cell but negative on RD cells should be reported as negative.
  - A Sabin type isolate detected by PCR but not picked up by micro neutralization test should be reported Sabin-like.
- With regard to documenting L20B positive results, the following actions were suggested:
  - Unique laboratory identification should be allotted to each referred sample.
  - All L20B positive samples should be reported and documented within 7 days for ITD.
- All ITD discordant test results should be sent immediately to sequencing laboratory.
- Regarding problems with ELISA and requesting kits of PCR and ELISA, the following actions were suggested.
  - Whenever any problem arises with ELISA, immediately contact RIVM through email, fax or telephone and also copy to the regional and global laboratory coordinators.
  - Prior to adding the virus, process it with chloroform, freeze and thaw at least twice, and vortex for 5 minutes to thoroughly mix antibodies prior to dilution. In case of virus mixtures, properly mix sample before adding.

- For PCR, when requesting primers send the request to CDC (USA), with copies to Mark Mandelbaum and Dave Kilpatrick and the regional and global laboratory coordinators.

7. VACCINE-DERIVED POLIOVIRUSES

7.1 VDPVs overview

Dr M. Pallansch, CDC, USA

VDPVs can cause paralytic polio in humans and have the potential for sustained circulation of poliovirus. Poliovirus isolates can be divided 3 categories, based upon extent of VP1 nucleotide divergence from corresponding Sabin strain: wild, >15%; OPV-like, <1%; VDPVs, 1%–15%; VDPVs are further categorized as: 1) circulating VDPVs (cVDPVs), which emerge in areas with inadequate OPV coverage; 2) immunodeficient-associated VDPVs (iVDPV0), which are isolated from persons with primary immunodeficiencies shown to have prolonged VDPV infections after exposure to OPV; and 3) ambiguous VDPVs (aVDPVs), which are either clinical isolates from persons with no known immunodeficiency or environmental isolates whose ultimate source has not been identified as VDPVs.

These VDPVs are detected by global poliovirus surveillance. Before 2006, 36 iVDPVs reported and 1 long term PV2 Sabin-like (SL) excreting immunodeficient person were reported. In 2006 and to 31 May 2007, more were added to this list from several countries. Many cVDPV outbreaks were reported from January 2006 to October 2007: type 2 cVDPV in Nigeria; type 1 cVDPV in Myanmar; type cVDPV in Cambodia.

New issues were raised by recent cVDPV outbreaks like Type 2 cVDPV viruses were not "flagged" by the network's current screening policy for VPDVs (SL in both PCR and ELISA). Several viruses were also detected with < 1 % VP1 sequence divergence from Sabin, with some mutations in common with "true" VDPVs, suggesting parental links. There is probable need to revise the VDPV definition.

The primary risk factor for VDPV emergence remains inadequate immunization with OPV. Inadequate surveillance can allow undetected circulation of cVDPVs, in some cases resulting in extensive outbreaks. Until widespread implementation of new laboratory tests, epidemiological suspicion of potential clusters of AFP cases with vaccine-related viruses (SL; particularly type 2 and 3) is important.

7.2 VDPVs detected in countries of the Region

7.2.1 Islamic Republic of Iran

Dr S. Mahmoudi, NPL

Since 1995, 5 VDPV cases were detected in Iran, all classified as iVDPVs. All these were AFP cases except the first, which was an OPV vaccinee contact. All were with immunodeficiency. Discordant results were obtained on ITD testing.

The first VDPV case was detected in 1995 in a child of 17 months who was an OPV vaccinee contact and who received IPV because it was known that she was suffering from antibody deficiency. Three (3) stool specimens were collected and yielded P2-DR in ELISA and P2-SL in NAPH. Sequencing showed 2.2% divergence from Sabin-2, and P2–P1 recombination at position 5355 which is located in 3A region of the genome. This patient died 8 days after paralysis onset.

The second VDPV case was in a 7 month old male, reported from Isfahan in August 2005. Immunological investigation for this patient showed severe combined immunodeficiency (SCID). The last OPV dose was administered one month before. Five specimens were collected from the patient (the first 2 were 5–6 days after paralysis onset, and then 3 follow-up specimens). All specimens were positive for PV2 DR in ELISA and SL in PCR. Six contact specimens of this case were negative. Sequencing result showed 1.1%–1.5% divergence from Sabin-2, P2–P1 recombination at position 5358 (3A). Mixed bases at multiple positions were detected and this case was categorized as iVDPV. The patient died almost 6 months after paralysis onset due to renal failure and multiple infections.

The third VDPV case was in a male AFP patient, 10 months old from Lorestan, suffering from SCID. Date of paralysis onset was on 21 October 2006, and the last OPV dose was almost 3 months earlier on 7 July 2006. Stool specimens were positive for PV2 DR ELISA and PCR was SL. Eight contact specimens were negative for virus isolation. Sequencing results showed 1.7–2% divergence from Sabin-2, recombinant or extensively mutated (more analysis is needed), numerous mixed bases and several lineages. The case was categorized as iVDPV. The patient died less than 3 months after paralysis onset (6 January 2007).

The fourth case was in a 15 month old male AFP patient from Tehran suffering from X-linked agammaglobulinemia. Paralysis onset was on 17 December 2006, and the last OPV dose was given on 27 March 2006. Six specimens were taken from this patient, the first and third were PV3 DR on ELISA and SL on PCR. The patient was treated using intravenous immunoglobulin and all 3 follow-up specimens were negative. Five contact specimens were negative for any virus. Sequencing results showed ~2% divergence from Sabin-3 and recombination with Sabin-1 in 3Dpol, a few mixed bases, and only one amino acid change despite numerous nucleotide changes. The patient is alive to date but is completely paralysed, and is hospitalized periodically due to respiratory dysfunction.

The fifth VDPV case was in a 5 month old female from Tehran, who was suffering from SCID. She had paralysis onset on 7 March 2007, and received the last OPV dose on 21 November 2006. Two specimens were collected 3–5 days after paralysis onset, which were both positive for Polio1–Polio2 mixture. ELISA results showed NSL1 and PV2 DR, while PCR was SL for both types. Eight contact specimens were negative for any virus. Sequencing results showed a mixture of PV1/PV2-iVDPV. Numerous mixed bases were detected, and 3Dpol did not have ordinary Sabin sequences. Plaque purification is needed for more analysis, and the percentage of VP1 divergence has not yet been determined. The patient died as a result of sepsis one month after paralysis onset.

7.2.2 Syrian Arab Republic

Dr A. Arraj, NPL, Dr H. Asghar, WHO/EMRO

Between 2001 and 2006, five iVDPV cases were reported from the Syrian Arab Republic. The first case occurred in a 9 month old child from Hama-Muhardah, with date of onset of paralysis on 19 February 2001. Both PCR and ELISA gave results as PV2SL, but upon sequencing it was characterized as P2 iVDPV, with 1.3% (12/903) and ~1.0% (9/903) divergence from Sabin 2.

The second case was in a 29 month old child, from Deir Al-Zowr, with date of onset of paralysis on 20 December 2001. Both PCR and ELISA gave results as PV1SL and PV2SL, but upon sequencing it was characterized as P2 iVDPV, with 1.5% (14/903) divergence from Sabin 2.

The third case occurred in a 3 month old child from Edlep, with date onset of paralysis on 18 December 2001. It was PV2SL on both PCR and ELISA. Upon sequencing it was characterized as PV2 iVDPV, with 1.5% (14/903) divergence from Sabin 2. All these three cases have S2-derived sequences in 2C region and S1-derived sequences in 3Dpol.

The fourth patient was 6 months old, from Jaramana in rural Damascus, with onset of paralysis on 19 September 2005. The PCR and ELISA results showed co-infection with SL2 and SL3. Upon sequencing it was characterized as P2 iVDPV with 1.3% (12/903) divergence from Sabin 2 and <1.0% (8/903) from Sabin 3.

The fifth case occurred in a 7 month old patient from Damascus-Mahrdah with onset of paralysis on 5 July 2006. Both PCR an ELISA results showed as PV2SL. Sequencing results characterized it as P2 iVDPV, with 2.2% (20/903) divergence in complete VP1 with numerous mixed bases positions.

7.2.3 Kuwait

Dr S. Al-Mufti, Kuwait. Dr H. Avoort, WHO/EMRO

A case of poliomyelitis associated with VDPV occurred in an AFP patient born in April 2006 in Kuwait to an Egyptian family. The first dose of OPV was given at birth. She left to Egypt in June 2006, where second dose of vaccine was given. The third dose was given on 27 August 2006. She was admitted to hospital on 28 August 2006 with diarrhoea, vomiting and acute flaccid paralysis but with no fever. Poliovirus type 3 was isolated from stool samples collected on 28, 29 and 30 August 2006. All isolates were DR by ELISA and Sabin-like by PCR. On sequencing all isolates were Sabin-like. The child was diagnosed as SCID. The stool sample collected on 13 January 2007 yielded DR isolate by ELISA and Sabin-like by PCR. Sequencing showed more than 1% (12 mutations) divergence in VP1 region. Follow-up samples were collected; the last sample was collected on 2 October 2007, and all samples were negative for virus isolation.

7.2.4 Egypt

Dr E. Al Maamoun, VACSERA; Dr M. Pallansch, CDC, USA

From 1988 to 1993, 30 cases of poliomyelitis associated with cVDPV2 were reported from 8 governorates (Giza, Qalubia, Menofia, Cairo, Fayoum, Beni Suef, Sharkia and Minya) in Egypt. Analysis suggests that all cases of infection originated from one source. Low OPV coverage was a contributing factor in origin and spread. VDPV2 circulation ceased with rising OPV coverage, and the last VDPVs were detected in Egypt in this group in 1993.

In 2007, one iVPV3 was detected in an AFP case, 4 months of age, from Kafr El-Sheikh, with date of onset of paralysis 2 April 2007. The child was suffering from repeated chest infection and was treated with corticosteroids. The ITD results gave discordant results (DR on ELISA and SL3 on PCR). The patient later died. The sample was sent to CDC, USA, for sequencing. The sequencing showed 10 nucleotide differences from Sabin 3, seven of these mutations were mixed bases. This virus does not appear to be recombinant, based on 3Dpol real-time PCR assay results. One aVDPV type 2 was detected in a sewage sample collected in 2005.

8. OTHER ISSUES

8.1 Development, evaluation and performance characterization of real-time PCR reagents for poliovirus identification and ITD and VDPV

Dr M. Pallansch, CDC, USA

Real-time PCR assay has been developed to completely replace previous PCR assay for identification and ITD of poliovirus isolates.

Real-time PCR can detect the serotype and intratype of polioviruses including VDPVs. It has high sensitivity and specificity, and less risk of contamination (PCR product carryover) because there is no opening of tubes. It automatically plots the amplification product verses time. The automated report is generated which can be exported to database.

Real-time PCR was evaluated against viruses with known results and there was 100% concordance between the results. It was also evaluated on different real-time PCR platforms and satisfactory results were obtained. Parallel testing in CDC diagnostic laboratory has begun, however, field evaluation is needed.

Multiplex Sabin VDPV assays target antigenic sites in VP1 and also target the 3D region for likely recombination. This assay could replace ELISA for screening of SL viruses following PCR ITD. Real-time PCR has been developed to identify isolates for possible non-capsid recombination. For this test, evaluation was completed with more than 100 poliovirus isolates. Parallel testing with ELISA has shown higher sensitivity for cVDPV detection. There is need for field evaluation.

8.2 Recombination and genetics drift in the VP1 of polioviruses isolated in Tunisia

Dr H. Triki, Tunisia

Genetic characterization of polioviruses remains highly important even in countries where wild poliovirus circulation was interrupted. Recent recommendations required enhanced surveillance of vaccine-related isolates with the aim of detecting all isolates with increased potential for transmissibility in humans (cVDPVs). Recombination is a frequent event in vaccine strains and was found in most of the VDPVs that caused the recent outbreak associated to drifted vaccine strains.

A study looked at the genetic characteristics of 113 vaccine-related polioviruses isolated in Tunisia from 1991 to 2006. Vaccine-related isolates were assessed for genetic recombination by a double PCR/RFLP, in the VP1/2A junction and in the 3D region of the genome, as well as sequence analysis of amplicons when RFLP patterns are non specific to Sabin viruses. All recombinant viruses detected were assessed for genetic drift in the VP1.

19% (n=22) of vaccine-related isolates were Sabin/Sabin intertypic recombinants; no recombinant with non-Sabin sequence in the 3D was identified. This may be associated with the relatively low endemicity of non polio enteroviruses in Tunisia, especially from the HEV-C group. Mutational differences in the VP1 sequences of recombinant viruses ranged from 0.0 to 0.7%, indicating a limited replication period.

This study provides evidence that in countries like Tunisia, with continuous high vaccine coverage and low endemicity of non-polio enteroviruses from the C group, transmission of vaccine-related polioviruses is time-limited.

8.3 Progress on establishing the ITD

8.3.1 Morocco

The Morocco laboratory was included among the new ITD laboratories. In response, one scientist from the NPL attended the ITD training workshop held in Uganda in November 2006. WHO headquarters was provided the list of reagents for establishing the ITD testing. There was a long delay in provision of supplies; however, most of the supplies have been received. Two items are still pending, which are supposed to arrive soon. The pilot ITD testing will be completed in the first quarter of 2008.

8.3.2 Syrian Arab Republic

The Syrian NPL was listed among the new ITD laboratories. The head of laboratory attended the training workshop in Uganda in November 2006. Later, she left the laboratory. She could not establish the ITD testing due to delay in supplies of equipment and reagents. All the teaching material is available in the laboratory. Supplies have now been received. The WHO regional polio network coordinator will conduct ITD training for staff and it is expected to start pilot testing in first quarter of 2008.

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

Dr H. Asghar, WHO/EMRO

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 the laboratory survey and inventory Phase 1 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. A national containment coordinator was nominated by Pakistan and a national plan of action was developed, but no concrete progress has been documented. To date, 19 775 laboratories have been surveyed and only 8 laboratories have been identified to be storing WPV material.

All countries that have completed the Phase 1 of containment activities were required to submit the quality assurance report. Documentation of the quality of Phase 1 of containment activities was submitted by 14 countries (Bahrain, Djibouti, Islamic Republic of Iran, Jordan, Libyan Arab Jamahiriya, Morocco, Oman, Qatar, Saudi Arabia, Sudan, Syrian Arab Republic, Tunisia and Untied Arab of Emirates). It was not submitted by 4 countries (Egypt, Kuwait, Lebanon and Palestine). Egypt and Palestine recently completed Phase 1 of containment.

8.5 Potential problems and their solutions in LABIFA data management

Dr H. Safwat, WHO/EMRO

EMR polio laboratories update their databases weekly and forward a copy to the Regional Office to be reflected in the weekly Polio Fax. EMRO is in direct and continuous contact with the laboratories asking for clarifications, sending feedback and resolving problems due to data entry errors. Most laboratories use the check reports in the lab system databases. This reflects on the quality of data, which is always improving. Errors due to duplicate entries or missed entries have diminished. Further validations of dates are needed to improve the quality of data used in calculations of timeliness. EMR polio laboratories are encouraged to communicate regularly with the surveillance teams in countries they serve and RRLs are urged to be in direct and regular contact with NPLs they support.

A new LABIFA system is being designed for the alternative test algorithm. This would be an opportunity for improvements regarding better checking during data entry and using Excel pivot tables for analysis. It is recommended that all EMR polio laboratories implement the new system by 1 January 2008, that LABIFA version 3 continue until end 2007 and that laboratories using the test version during 2007 continue doing so until the switchover to version 4.

9. CONCLUSIONS

Very encouraging progress has been made in the Eastern Mediterranean Region towards achieving polio eradication. Twenty of the 22 countries of the Region stopped endemic transmission of poliovirus; only Afghanistan and Pakistan remain endemic, with a decrease in

intensity and extent of transmission. The outbreak due to importation in Yemen and Somalia seems to be controlled with the last cases in February 2006 and March 2007, respectively. In September 2007, an importation due to wild poliovirus type 1 occurred in Sudan.

The regional polio laboratory network performance is being sustained at certification standards. All network laboratories passed the WHO proficiency panel tests for both poliovirus isolation and intratypic differentiation testing. All laboratories are fully accredited, except the Kuwait laboratory which is provisionally accredited. The network supported AFP surveillance through rapid and accurate detection of wild polioviruses in a timely manner and facilitated the timely response in the field to interrupt transmission.

Between January 2006 and October 2007, network laboratories tested approximately 32 000 stool samples from AFP cases, and detected wild polioviruses in 5 countries. Analysis of nucleotide sequences data showed that endemic type 1 and type 3 viruses were circulating in Afghanistan and Pakistan in early 2007. Imported wild poliovirus type 1 was detected in Yemen and Somalia through March 2007. Recently an imported type 1 virus genetically linked to Chad viruses was detected in an AFP case in Sudan.

Between January 2006 and October 2007, vaccine derived polioviruses (VDPV) were isolated from acute flaccid paralysis (AFP) cases. The VDPVs were found in immunodeficient children in the Islamic Republic of Iran (one each of type 2 and 3 VDPVs, and one mixture of type 1 and 2), Syrian Arab Republic (one type 2), Kuwait (one type 3 in a resident Egyptian child) and Egypt (one type 3). A type 2 VDPV was detected in a Tunisian child in France. There was no evidence of sustained transmission of any of the detected VDPVs.

For rapid confirmation of wild polioviruses and VDPVs, the global polio laboratory network developed a new algorithm of testing to decrease laboratory reporting time without compromising poliovirus detection sensitivity. The new algorithm has been fully implemented in all EMR polio network laboratories. The average reporting time from onset of paralysis to ITD results has decreased from 29 days in 2006 to 22 days in 2007, while the average reporting time from sample received in laboratory to ITD has decreased from 16 days in 2006 to 13 days in 2007. The main challenge in implementing the new algorithm has been the increase in workload in terms of logistics and ITD reagents.

Highly sensitive cells have paramount importance in isolation of viruses. Cell sensitivity testing provides the most critical data to reassure that laboratories are able to isolate poliovirus in optimal conditions. Historical data review and trend analysis of cell sensitivity data are used to identify problems and corrective measures are taken immediately. A few laboratories were found facing problems of setting up the proper cell sensitivity test and interpretation of results. Emphasis is made on the regular cell sensitivity testing and correcting measures to ensure that the laboratories are working with sensitive cells.

There has been a sustained increase in workload over the years in the Region due to improved AFP surveillance and importations of wild polioviruses into polio-free countries. Between 2003 and 2006, the total number of tested samples was 11 023, 13 192, 18 279 and

17 795, respectively. This increase in sampling from AFP cases along with the increase in workload due to implementation of the new algorithm has led to more demand for resources to meet higher logistic support and operational costs. Despite workload increases, and difficult conditions in certain countries like Iraq, laboratories have continued to maintain high standards of performance, meeting reporting timelines and quality assurance requirements.

10. RECOMMENDATIONS

1. WHO and network laboratories should continue to advocate with national governments and partner agencies to continue their support of the poliovirus laboratory network, especially in countries where laboratories are facing problems with their infrastructure. It is essential that national authorities share the rising costs, given the increasing workload and the need to sustain high levels of laboratory performance and increase the speed of poliovirus confirmation.
   Responsible: WHO/EMRO and Directors of Polio Laboratories

2. In line with the recommendations of the 13th informal consultation of the WHO global poliovirus laboratory network in June 2007, all laboratories should revise their standard operating procedures (SOPs) in the light of the supplement to the WHO Polio Laboratory Manual, *An alternative test algorithm for poliovirus isolation and characterization*, distributed in September 2007.

   2.1.The category of "inconclusive" should be removed as a choice for reporting L+R- and R+L+R- culture results; these should be reported as "negative"

   2.2 The results of virus isolation should be tracked separately for each "arm" of the test algorithm and for each specimen.

- If isolates are obtained from both the "L20B arm" (LR) and the "RD arm" (RLR) of the algorithm, the virus isolation laboratory should refer both isolates to the ITD laboratory.
- ITD testing should be performed only for the isolate from the L20B arm (LR isolate).
- ITD testing should be performed on the isolate from the RD arm (RLR isolate) only if the result from the L20B arm (i.e. LR isolate) is non-enterovirus (NEV) or non-polio enterovirus (NPEV).
- If only one virus arm is positive (and the other arm negative), the laboratory performing the isolation should always forward the available positive arm isolate for ITD testing.
  Responsible: Directors of Polio Laboratories

3. The laboratories should work towards the timeliness target for testing and reporting of results using the new algorithm, which should be 14 days for virus isolation and 7 days for ITD tests. The laboratories will be accredited by the new checklist and criteria after adoption in January 2008.
   Responsible: Directors of Polio Laboratories and WHO/EMRO Regional Polio Laboratory Coordinator

4. Laboratories should continue regular cell sensitivity testing as per the schedule shown in the Polio Laboratory Manual. Results should be documented and reported to the Regional Polio Laboratory Coordinator within 48 hours of completion of the test. Reported trend data should include all relevant information and list changes in the procedure or material used. Trend data should be presented using appropriate scale to detect changes in titre values. Follow-up should be conducted and corrective measures implemented, if necessary.
Responsible: Directors of Polio Laboratories and WHO/EMRO Regional Polio Laboratory Coordinator

5. LABIFA version 4.0 should be installed in all network laboratories as soon as possible after receiving the software. Laboratories are encouraged to try out the system before the end of the year in order to sort out any problems and/or questions.
Responsible: Directors of Polio Laboratories and WHO/EMRO Data Manager

6. Good laboratory practices should be observed in compliance with occupational safety in handling of biological materials and chemicals. All measures should be taken to avoid fire accidents through institutional involvement of all concerned. The laboratories should develop plans to deal with any emergency situation.
Responsible: Directors of Polio Laboratories

7. New molecular based procedures under development for ITD testing and VDPV detection should be evaluated in pilot study sites in the Region.
Responsible: Directors of Polio Laboratories WHO EMRO/HQ and Global Specialized Laboratories

8. Until full implementation of new methods to reliably detect VDPVs, retrospective genetic characterization of all type 2 and type 3 Sabin related viruses from January 2006 onward by sequencing or real-time molecular screening should be completed as soon as possible in collaboration with the Global Specialized Laboratories.
Responsible: WHO EMRO/HQ and Global Specialized Laboratories

9. All laboratories requests for molecular and antigenic testing reagents, cell sensitivity standards, and serotyping antisera should be addressed properly to the concerned personnel in CDC, RIVM and NIBSC. All such correspondence should be copied to Global and Regional Polio Laboratories Network Coordinators.
Responsible: Directors of Polio Laboratories

Annex 1

PROGRAMME

Monday, 29 October 2007

08:00–08:30	Registration	
08:30–09:30	Opening session	
	Welcome and opening remarks	Dr M. H. Wahdan
	Message from Dr Hussein A. Gezairy, WHO Regional Director for the Eastern Mediterranean Region	
	Election of the Chairman and Reporter	
	Implementation status of the recommendation of the tenth Intercountry Meeting of Directors of Poliovirus Laboratories	Dr H. Asghar
Session 1: Overview		
09:30–09:45	Overview of polio eradication in EMR	Dr F. Kamel
09:45–10:00	Status of global polio laboratory network	Dr E. de Gourville
10:00–10:15	Regional progress of EMR polio laboratories network	Dr H. Asghar
10:15–10:30	Regional progress in AFR polio laboratories network	Dr F. Kasolo
10:30–11:00	Discussions	
Session 2: Virus surveillance		
11:30–11:45	Characteristics of wild polioviruses in Afghanistan, Pakistan, Somalia and Sudan	Dr M. Pallansch
	Laboratory performance indicators:	
11:45–12:00	Pakistan and Afghanistan	Mr S. Zaidi
12:00–12:15	Egypt	Dr E. Al Maamoun
12:15–12:30	Somalia and south Sudan	Mr P. Borus
12:30–13:00	Discussion	
Session 3: Laboratory quality assurance		
14:00–14:10	Accreditation status of EMR polio laboratories	Dr H. Asghar
14:10–14:30	Proficiency testing (PT) results for isolation and typing 2006–2007, and PT panel testing in new algorithm	Dr H. Avoort
14:30–14:50	Report on proficiency testing-PCR and ELISA	Dr M. Pallansch, Dr H. Avoort
14:50–15:10	Proposed changes in the polio laboratory checklists	Dr E. de Gourville

15:30–15:50	Update on cell sensitivity testing in EMR laboratories	Dr J. Martin
15:50–16:30	Experience with cell sensitivity testing	
	Syrian Arab Republic	Dr A. Arraj
	Morocco	Mrs Maria El Qazoui
16:30–17:00	Discussion	

Tuesday, 30 October 2007

Session 4: New test algorithm

08:30–09:00	Progress on new test algorithm: proposed changes after field testing	Dr E. de Gourville
09:00–09:45	Experience in implementing the cell culture and intratypic differentiation (ITD) testing algorithm	
	Pakistan	Mr S. Zaidi
	Egypt	Dr L. El-Baissouni
	Oman	Dr S. Al Baqlani
09:45–10:15	Experience in implementing the cell culture testing algorithm	
	Sudan	Mr H. Babikar
	Jordan	Dr M. Karsaneh
10:15–10:45	Discussion	
11:00–13:00	Group discussion: New testing algorithm	Moderators:
	Group 1: New cell culture testing algorithm: Discuss issues related to SOPs, worksheets, frequency of cell culture propagation, documentation, problems and trouble shooting etc.	Dr E. de Gourville Dr F. Kasolo Dr J. Martin
	Egypt, Iraq, Jordan, Morocco, Saudi Arabia, Sudan, Syria, Pakistan	
	Group 2: New ITD testing algorithm:	Moderators:
	Discuss issues related to SOPs, worksheets, testing of referred isolates, documentation, problems and trouble shooting etc.	Dr H. Avoort Dr M. Pallansch Dr H. Asghar
	Egypt, Iran, Kuwait, Oman, Pakistan, Tunisia	
14:00–14:30	Report on group discussion	

Session 5: VDPVs

14:30–14:50	VDPVs overview	Dr M. Pallansch
14:50–15:20	VDPVs detected in countries of the Region	
	Islamic Republic of Iran	Dr S. Mahmoudi
	Syrian Arab Republic	Dr A. Arraj Dr H. Asghar

	Kuwait	Dr S. Al-Mufti
	Egypt	Dr H. Avoort
		Dr E. Al Maamoun
		Dr M. Pallansch
15:20–15:45	Discussion	
Session 6: Other issues		
16:00–16:15	Development, evaluation and performance characteristics of Real time PCR reagents for poliovirus identification and ITD and VDPV detection	Dr M. Pallansch
16:15–16:30	Recombination and genetic drift in the VP1 of polioviruses isolated in Tunisia	Dr H. Triki
16:30–17:00	Discussion	

Wednesday 31 October 2007

09:00–09:40	Progress on establishing the ITD	
	Morocco	Mrs Maria El azoui
	Syrian Arab Republic	Dr A. Arraj
09:40–10:00	Status of Survey and Inventory of Phase 1 of laboratory containment of wild polioviruses	Dr H. Asghar
10:00–10:20	Potential problems and their solutions in LABIFA data management	Dr H. Safwat
10:20–11:00	Discussion	
11:30–12:30	Open discussion on remaining issues	
12:30–13:00	Discussion on conclusions and recommendations for the technical sessions	

Thursday, 1 November 2007

Laboratory Information for Action, version-4 (LABIFA4) Practical session

08:30–08:40	Introduction to LABIFA4 in accordance with new testing algorithm	Dr A. Middlekoop Dr H. Safwat
08:40–10:30	Data management: new variables, codes, checks. New vs old database	Dr A. Middlekoop Dr H. Safwat
11:00–13:00	Data management: new indicators, analysis, reports and changes in the fax	Dr A. Middlekoop Dr H. Safwat
14:00–15:30	Data management, remaining issues: Specific needs of laboratories and addition to be made in LABIFA4	Dr A. Middlekoop Dr H. Safwat
15:30	Closing	

Annex 2

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