

Report on the

**Tenth intercountry meeting of directors of
poliovirus laboratories in the Eastern
Mediterranean Region**

Tunis, Tunisia,
18–20 September 2006

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1. INTRODUCTION

The tenth intercountry meeting of directors of poliovirus laboratories in the WHO Eastern Mediterranean Region was held in Tunis, Tunisia from 18 to 20 September 2006. Directors of poliovirus laboratories in Egypt, Islamic Republic of Iran, Iraq, Jordan, Morocco, Oman, Pakistan, Sudan, Syrian Arab Republic and Tunisia attended the meeting (Directors of poliovirus laboratories from Kuwait and Saudi Arabia were unable to attend). Participants also included scientists from the Centers for Disease Control and Prevention (CDC), United States of America; National Institute of Public Health and the Environment (RIVM), 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 Ibrahim Abdel Rahim, WHO Representative in Tunisia, 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 noted with appreciation the contribution of laboratories to investigate the wild poliovirus outbreaks in Yemen and Somalia. He emphasized the need for early detection of importation into a polio-free country. He also showed his satisfaction towards the development of new testing algorithm aimed at reducing the reporting time of final virological investigation results. He commended the regional reference laboratory for polio eradication in Pakistan for acquiring the technology of genetic sequencing of polioviruses.

Dr Hicham Abdelsallam, Director of Health for Technical Cooperation, Ministry of Public Health, welcomed the participants and highlighted the progress achieved in Tunisia in the fields of routine immunization and the poliomyelitis eradication initiative. He emphasized the need for the certification standard AFP surveillance. He noted that Tunisia had maintained its routine immunization coverage well above 95% and that no wild poliovirus case had been reported since the last case in 1992. He commended the role of Regional Reference Laboratory, Institut Pasteur, for helping in virological analysis of AFP stool samples and in completing the first phase of survey and inventory of laboratory containment of wild polioviruses.

Dr Hinda Triki (Tunisia) was elected Chair. The programme and list of participants are included as Annexes 1 and 2, respectively.

2. IMPLEMENTATION OF RECOMMENDATIONS OF THE NINTH 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 ninth intercountry meeting of directors of poliovirus laboratories in the Region.

No.	Recommendation	Implementation status
1.	Poliovirus laboratories are essential national public health resources. National authorities should support the poliovirus laboratory through the provision of a sufficient budget and personnel. WHO should continue its advocacy with governments to meet the unmet needs.	None of the national poliovirus laboratories received any specific budget allocation from their own government, however, WHO continued to advocate with respective governments in the countries to support polio laboratories.
2.	The EPI should work with the laboratory to assess the incremental surveillance sensitivity obtained by collection of contact stools (and other specimens from excluded cases) in the context of increased workloads in the laboratory and the requirements for additional human resources and logistics support.	No direct discussions were held between laboratory and EPI in most of the countries, however, in few high workload countries, it was agreed to rationalize the collection of contact samples.
3.	Cell culture sensitivity testing should be standardized by using the NIBSC standard Sabin strains and laboratory quality control standards, according to WHO protocol. All tests should be documented and the worksheets shared with NIBSC in the United Kingdom and the WHO Regional Office. All laboratories should perform routine sensitivity testing of cell cultures halfway during the recommended 15 passage of cell lines and a second test just before cells are discarded, to be reassured that cells have maintained sensitivity during their use.	In all the laboratories the cell sensitivity testing was standardized and fully implemented. A few problems were encountered in Morocco laboratory, which were discussed in detail and suggestions were made during in-country visit and by emails to resolve the problem.
4.	Supplies and resource needs should be planned based on the increased workload of laboratories due to increase in AFP rates and any added strategy such as the collection of contact stool samples. In addition, adjustments should be made in case of an unexpected crisis e.g. an outbreak. Efforts should be continued to mobilize resources to cover supplies, personnel and logistic needs and provide budget through specific allocations for laboratory support. WHO should continue to coordinate with national authorities and partners in poliomyelitis eradication to mobilize resources.	The laboratories redistributed work according to available human resources. Most of the time WHO was contacted for logistic support.
5.	In the light of the recent Sudan and Yemen wild poliovirus outbreak experiences, which set an example of efficiency, laboratories are advised that in such situations they should work closely with WHO for prompt response to expedite the transportation, testing and reporting of samples.	There is strong coordination between WHO/EMRO and network laboratories, and a good example was set again by investigating Somalia outbreak.
6.	Day-to-day performance (sensitivity and specificity) of test controls in ELISA-ITD should be monitored continuously. Strict documentation of exact dilutions used, incubation time, as well as registered optical density (OD) values of anti-total reactions, will demonstrate decreasing quality of reagents, should this occur.	It is implemented and worksheets are monitored during accreditation visits.
7.	All polioviruses isolated from clinical specimens should be referred to global specialized laboratories for further characterization to monitor	All isolates are sent for sequencing to global specialized laboratories.

No.	Recommendation	Implementation status
	their genetic diversity for VDPV detection. The referred viruses should be accompanied by referral form.	
8.	Environmental surveillance in Egypt is supplementing the AFP surveillance. The National Public Health Institute, Helsinki (KTL) should continue to characterize all poliovirus isolates of programmatic importance from environmental samples. At the present high level of workload, all poliovirus isolates from the environment surveillance in Egypt should be tested by one molecular method (e.g. probe hybridization). All candidates for wild polioviruses isolates should be flagged and confirmed by intratypic differentiation (ITD) ELISA, reported to the national poliomyelitis eradication programme, and at the same time should be sent to KTL for characterization.	Environmental surveillance is ongoing. VACSERA is successfully continuing testing all poliovirus isolates by probe hybridization method.
9.	WHO annual accreditation helps the laboratory to overcome the technical and managerial problems. A completed accreditation checklist should be sent to concerned laboratories for their records and to facilitate their follow-up.	All laboratories are regularly receiving the annual accreditation checklist.
10.	All poliovirus network laboratories should ensure full immunization of staff against poliomyelitis according to the national immunization policy and document the vaccination of staff in accordance with the national policy. This documentation should be submitted to the regional poliovirus laboratory network coordinator at the WHO Regional Office for the Eastern Mediterranean.	No documentation of vaccination status of laboratories staff was provided, however, during accreditation visits the question is asked. Some of the laboratories have vaccinated their staff, while some of the laboratories are reluctant to vaccinate with OPV and demand IPV.
11.	Poliomyelitis eradication is progressing towards its final stages and there are increasing demands to provide timely virological investigation results of AFP cases. To achieve this target, the national poliovirus laboratories, as appropriate, should be upgraded to perform ITD testing.	Staff of three laboratories of the Region will be given training in ITD testing in 2006, and one more will be given training in 2007.

3. OVERVIEW

3.1 Overview of polio eradication in the Eastern Mediterranean Region

Dr Humayun Asghar, WHO/EMRO

The lowest annual number of cases of poliomyelitis in the Region was in 2003, with 113 cases reported. During 2004 and 2005, progress continued in the three endemic countries; however, explosive epidemics occurred in Somalia, Sudan and Yemen. A total of 187 cases were reported in 2004 and 727 in 2005 (37 from endemic countries). As of mid-September, 78 cases have been reported: 28 in Afghanistan, 19 in Pakistan, 30 in Somalia and one in Yemen.

Pakistan has made great progress towards eradication of poliomyelitis, with clear evidence of decreasing virus diversity and intensity of transmission. In early 2005, poliovirus was detected mainly in southern Punjab, Peshawar Valley and northern Sindh. In the second half of 2005, the virus reappeared in Balochistan and continued in 2006 close to the borders of Afghanistan in a common area of transmission. The general performance of the programme has been good and efforts have been made to address the remaining issues, including reaching the youngest children (especially in conservative communities and in inaccessible areas).

The transmission of poliovirus in Afghanistan continues in the southern region, which has known problems of insecurity. Efforts have been made to ensure the engagement of local community elders and tribal leaders and to use local staff to ensure access to all children with the vaccine.

In Egypt, the last reported case of poliomyelitis was in May 2004. There is clear indication that viral circulation in Egypt has been interrupted, with the last positive environmental sample isolated in January 2005. Political commitment is still evident, with six rounds of national immunization days conducted in 2005 and two in 2006. Monovalent type 1 oral polio vaccine (mOPV1) was used in three rounds in 2005. It is important to maintain high population immunity through routine immunization, and supplementary immunization activities.

The epidemic that occurred in Sudan in May 2004, following the importation of Nigerian poliovirus via Chad, has been contained with the last case reported in June 2005.

In Yemen, low routine immunization coverage and limited sub-national immunization days in 2002 and 2003 resulted in a significant immunity gap that facilitated the occurrence of the epidemic. Weaknesses in surveillance led to late detection of the importation: the virus was introduced in February 2005 and only detected 20 April 2005. A national immunization day was conducted in April before confirmation. In May, a properly planned house-to-house campaign was conducted followed by similar rounds in July, August, September, November, December and January, plus two mop-up rounds. In most rounds, mOPV1 was used. Independent monitoring showed improvement and most gaps seen in May and July were addressed in subsequent rounds. The last case of the epidemic had onset in February 2006.

Very low routine immunization in Somalia, in addition to the war and emergency situation limiting access to many areas, made the country susceptible to the spread of importations. Wild poliomyelitis virus was introduced from Yemen. Despite difficulties in investigation and control, several supplementary immunization activities were conducted early in 2005 as a precautionary measure and these have been instrumental in limiting the epidemic, essentially to Mogadishu and neighbouring districts. The epidemic is on the decline and the spread outside Mogadishu and neighbouring Lower Shabelle remains of a very limited nature. The plan is to continue campaigns until two rounds after the last virus case with focus on the quality of campaigns, refusals and nomads.

The coordination of polio eradication activities between countries in the Horn of Africa is a priority. A cross-border meeting for countries bordering Sudan, an EMRO/AFRO

coordination meeting and a Horn of Africa coordination meeting have taken place. As well, Horn of Africa TAG was held in August 2006. However, more is needed especially at the local level (e.g. direct contact between national staff).

Risk of importation will continue as long as wild poliomyelitis virus is circulating in the world. Guidelines prepared for developing national plans for preparedness were updated in line with the Advisory Committee on Polio Eradication (ACPE) recommendations. Over the last few years, several importations/introduction of virus occurred with no secondary spread (Gaza, Islamic Republic of Iran, Lebanon, Oman, Saudi Arabia and Syrian Arab Republic). However, importations in Somalia, Sudan and Yemen resulted in epidemics as a result of immunity gaps among children aged under 5 years.

The regional strategic plan for poliomyelitis eradication, 2006–2007, was prepared in consultation with nationals, UN agencies and other partners. The plan covers the main elements of intensifying supplemental immunization, enhancing surveillance and maintaining the laboratory network, laboratory containment, certification and strengthening of EPI. It is essential to maintain trained personnel (national and international) and utilize them to support priority health programmes. A PEI/EPI consultation on optimizing collaboration has also been conducted to ensure the experience and benefits of PEI is used to strengthen routine immunization services.

Regional commitment for poliomyelitis eradication is now at its highest level, with national authorities in both polio-endemic and polio-free countries showing great commitment. The Regional Office has continued advocacy for polio eradication since 1988, with visits by the Regional Director to priority countries, dissemination of information and regular updates, and alerting national authorities to developments. Technical support is continuing, using about 100 international and 900 national polio staff in addition to teams of experts and temporary advisers as well as RTAG and country TAGs to advise on strategic directions. Regional priorities for polio eradication are now to:

- Interrupt transmission in the remaining endemic countries;
- Continue supplementary immunization activities with the same intensity;
- Sustain political commitment at all levels;
- Halt transmission in reinfected countries and regain their polio-free status;
- Avoid large immunity gaps in polio-free countries;
- Maintain certification-standard surveillance;
- Coordinate activities between neighbouring countries, especially in the Horn of Africa: synchronization, exchange of information, local level planning and coordination;
- Continue with containment and certification activities;
- Optimize PEI/EPI collaboration and avail the financial resources required to implement the regional plan for eradication.

3.2 Overview of the global polio laboratory network

Dr Esther de Gourville, WHO/HQ

Many indigenous lineages/chains of wild poliovirus serotypes 1 (WPV1) and wild poliovirus serotypes 3 (WPV3) have been eliminated since the start of the initiative. There are two of each, WPV1 (WEAF-B and SOAS) and WPV3 (WEAF-B and SOAS), currently circulating. The NEAF WPV1 genotype was found only in Egypt and was last detected in January 2005. Indigenous wild poliovirus serotype 2 was last detected in western Uttar Pradesh, India, in October 1999.

The polio laboratory network comprises 123 national laboratories, 15 regional reference laboratories and 7 global specialized reference laboratories. In 2005, 94% of network laboratories were fully accredited by WHO. Over 95% of reported cases have 2 stool samples collected and investigated in the laboratory network. There is significant increase in workload in laboratories over the years. The network laboratories tested 120 000 samples in 2005. This represents an increase of 37% in workload over the previously reported 18 months period. Virus isolation results were made available to the programme within 28 days of receipt for more than 95% of samples. For 83% of AFP cases with poliovirus isolates, the ITD tests confirmed the wild or Sabin-like viruses within 60 days of onset of paralysis. There was significant improvement in timeliness in reporting of results of case with earliest onset in outbreaks.

Between 2005 and 15 September 2006, the network laboratories isolated 3200 wild poliovirus (WPV) cases in 19 countries. WPV1 and WPV3 are endemic in four countries (Nigeria, India, Pakistan and Afghanistan). The WPV1 outbreaks/importations occurred in 15 countries (AFR: Angola, Cameroon, Chad, Democratic Republic of Congo, Eritrea, Ethiopia, Mali, Namibia, Niger; EMR: Somalia, Sudan, Yemen; SEAR: Bangladesh, Indonesia, Nepal). The WPV3 importation occurred in Niger. The WPV1 was detected in sewage in Egypt and India. The outbreaks have been controlled in most of the countries, but are continuing in Angola, Bangladesh, Democratic Republic of Congo, Ethiopia, Namibia, Nepal, Niger and Somalia.

All WPVs and polioviruses which give inconclusive results on ITD tests are subjected to genetic sequencing of the VP1 region of the poliovirus genome. From 2003 to 2006, 24 countries have seen importations, out of which 6 importations are linked to India viruses and 18 are linked to Nigeria viruses. Strong inter-laboratory and inter-regional collaboration has been seen in detection and investigation of importations. For example, Pakistan, Oman and CDC laboratories helped in investigation of Yemen outbreak, and Egypt, Kenya, Sudan and the National Institute of Communicable Diseases (NICD), South Africa, worked together to investigate the Somalia outbreak.

Vaccine-derived polioviruses (VDPVs) have been shown to be circulating in the past in Egypt, Hispaniola, Madagascar and Philippines. Type 1 VDPV outbreaks were detected in China in 2004 and in Indonesia in 2005. Type 2 VDPVs were isolated in a single case of AFP in the Lao People's Democratic Republic in 2004 and in Hong Kong and Saudi Arabia in 2005. A type 2 VDPV outbreak is being investigated in Madagascar; type 3 VDPV was

confirmed in 1 AFP case and 8 contacts in Madagascar in 2005. Currently, 30 iVDPVs and one long-term immunodeficient PV2SL excreter have been reported. There were a few more iVDPVs detected in 2005–2006: China (1 co-infected type 2 and 3), Syrian Arab Republic (1 type 2), Islamic Republic of Iran (1 type 2), Spain (type 2, child of Moroccan origin and 3 family contacts), France (type 2, child of Tunisian origin), United States of America (1 type 1 and 3 community contacts).

VDPV from non-AFP sources have also been reported by network laboratories. Type 2 VDPV are the most frequently detected and have been isolated intermittently from sewage water collected in Egypt, Israel and Slovakia. A type 2 VDPV was isolated from a healthy child as part of a stool survey in Japan in 2004. Type 3 VDPV was also isolated in Japan in 2005 from an adult with AFP and from a child in the same household who had been vaccinated against polio.

There are a few laboratories with special concerns in terms of their poor performance. Most of the issues are related to low cell sensitivity, failure to isolate wild polioviruses, and improper implementation of laboratory techniques. A few laboratories suffered from infrastructural problems, such as power cuts in Ibadan, renovation of the laboratory in Greece, and fire in the Mumbai laboratory.

The global polio laboratory network has taken several new initiatives. A field evaluation of new cell culture and ITD algorithm was conducted in India, Pakistan and CDC, aiming at reducing the reporting time of virological investigation results, especially of wild polioviruses. It showed a good potential for 50% reduction in reporting times, increase sensitivity for poliovirus detection, and implications for increasing the number of ITD laboratories. The laboratories supported mOPV clinical trials in Egypt and India with the involvement of ERC, RIVM and CDC.

3.3 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. The network laboratories have supported the polio eradication activities in a timely manner. All network laboratories were fully accredited, except Kuwait which was provisionally accredited. All laboratories passed the WHO proficiency testing panel of unknown viruses for both primary virus culture and intratypic differentiation testing.

There was a generalized increase in workload due to improvement in AFP surveillance and two major outbreaks in Somalia and Yemen. Another factor for the increase in workload was the collection of stool samples from contacts of AFP cases. All laboratory performance indicators were well above the set targets, except transportation of samples within 3 days, which was only 77%. Timeliness of reporting the virological investigation results, from onset paralysis to final results, significantly improved from 32 in 2005 to 28 days in 2006. Another remarkable achievement was the implementation of new testing algorithm aimed at shortening the time of reporting results. From April 2006, it was implemented in the Pakistan polio

laboratory, and results showed that final results could be obtained in just under 2 weeks after the stool samples were received in the laboratory. This testing algorithm will be introduced in the whole network by mid-2007.

In Egypt, AFP surveillance is supplemented with environmental surveillance to increase sensitivity for detection of wild poliovirus. The last wild poliovirus type 1 was isolated from Fayoum and Sohag in January 2005. There was an evident decrease in genetic diversity of polioviruses in Pakistan and Afghanistan. Importations of wild poliovirus into Sudan led to widespread outbreak and later led to importation into Saudi Arabia, Somalia and Yemen. The last wild type 1 polioviruses in Sudan and Yemen were isolated in June 2005 and February 2006, respectively. In Somalia the importation occurred in July 2005, and as of May 2006 the outbreak was continuing, but it is now on decline and sporadic cases are reported. In 2005, three vaccine-derived polioviruses were reported from the Islamic Republic of Iran, Saudi Arabia and Syrian Arab Republic.

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

3.4 Progress in the African regional polio laboratories network

Dr Francis Kasalo, WHO/AFRO

The WHO/AFR Regional Polio Laboratory Network consists of 3 regional reference laboratories (RRLs) located in Central African Republic, Ghana and South Africa and 13 national polio laboratories (NPLs) found in Algeria, Cameroon, Cote d'Ivoire, Ethiopia, Nigeria (Ibadan and Maiduguri), Kenya, Madagascar, Democratic Republic Congo, Senegal, Uganda, Zambia and Zimbabwe.

Over the past two years, the African laboratory network has made significant achievements, namely:

- Coping with an 18% increase in stool specimen workload in 2005
- Providing timely results to the polio eradication programme
- Full accreditation of all the 16 laboratories in 2005 and upgrading of two national laboratories to perform ITD (Ibadan and IP Senegal laboratories) testing and the regional genomic sequencing facilities and dendrogram generating capacity at the RRL in South Africa
- All laboratory indicators are improving and maintained.

The African network continues to perform at very high standards, despite the fact that it is faced with problems such as:

- Increasing workload in an environment of static or reduced workforce (loss of staff to other competing programmes)
- Frequent electricity disruptions leading to loss of reagents/isolates and threatened laboratory performance quality

- Unreliable air travel in African countries continues which effects shipping timeliness (in some instances isolates had to be re-routed via Europe).

The network performed commendably in 2005 and first half of 2006, after a number of lessons were learnt.

- To improve ITD turnaround time there is need to redistribute ITD work to existing laboratories and to expand ITD capacity strategically.
- There is need for additional onsite support to strategic laboratories in order to ensure sustained high quality performance
- There is need for closer collaboration between the laboratory and programme staff at country level in order to address local problems such as data management gaps and enhance monitoring.
- High quality laboratory performance should be sustained and enhanced by providing frequent onsite technical support to priority laboratories.
- Training and implementation of the new testing algorithm is needed by end 2007.

4. VIRUS SURVEILLANCE AND MOLECULAR EPIDEMIOLOGY IN ENDEMIC/REINFECTED COUNTRIES

4.1 Characteristics of viruses in countries of the Eastern Mediterranean Region

Dr Humayun Asghar

As of September 2006, there are only two countries (Pakistan and Afghanistan) endemic for WPVs, while three countries were re-infected due to importations between 2004 and 2006. The genetic sequencing data show that most of the lineages are localized and most of chains of transmission are already eliminated or on the verge of elimination in endemic countries.

Most significant achievement was the eradication of wild poliovirus from Egypt. There was rapid decline in genetic diversity of wild polioviruses from 12 WPV1 clusters in 2001 to just 2 clusters in 2004 and 2005. The last WPV1 isolates were isolated in January 2005 in sewage samples from Fayoum and Sohag, belonging to clusters E and H of the genetic sequencing dendogram, respectively. No WPVs have been isolated from AFP surveillance or environmental surveillance since June 2004 and January 2005, respectively.

The sequence data suggest good surveillance in most of the countries. The data helped to detect gaps in surveillance and target supplementary immunization activities in reservoir areas in endemic countries.

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, however cluster A-3 was widespread in southern Afghanistan;

- Spread of wild poliovirus type 1 from northern Nigeria to Chad to Sudan to Yemen and Somalia.

The sequence data indicate very high proficiency in performance in several regional polio network laboratories dealing with wild poliovirus endemic and re-infected countries. Despite the heavy workload due to improvement in AFP surveillance and outbreaks in Yemen and Somalia, the proficiency was maintained in providing the accurate results in timely manners. The Pakistan laboratory acquired the capability of genetic sequencing of polioviruses, which helped the polio eradication programme to target the activities very fast. All WPVs are referred to CDC Atlanta and VP1 sequences of polioviruses at CDC from the referred stool specimens matched those of the isolates in Atlanta originally obtained by the national laboratories. The recent sequence data show:

- Existence of a persistent reservoir for wild poliovirus type 1 in southern Afghanistan, southern Punjab, Peshawar valley and adjacent Federally Administrated Tribal Areas;
- Continued circulation of wild poliovirus type 3 in Quetta, Multan, Sukkur, Jaffarabad and southern Afghanistan;
- Importations of wild poliovirus type 1 from Sudan into Yemen and Somalia with a subsequent explosive spread;
- Importation/outbreak in Somalia is on decline and many of the chains of transmission have already disappeared;
- Multiple lineage imported into Yemen which led to an explosive outbreak of wild poliovirus type 1.

In Sudan, the last WPV1 was isolated from a contact in August 2005. In Yemen, the last WPV1 was isolated in February 2006. Since then no wild poliovirus has been isolated from these countries in the presence of sensitive AFP surveillance.

4.2 Pakistan and Afghanistan: virus surveillance

Mr Sohail Zahoor Zaidi, National Institute of Health, Pakistan

The WHO regional reference laboratory in Pakistan has been continuously supporting the polio eradication initiative by its excellent performance and timeliness of reporting which was particularly impressive given the increased workload that laboratory handled in 2005 and 2006.

Over the years there has been an increase in the number of stool samples analysed in the RRL. This is due to improved AFP surveillance in Pakistan and Afghanistan and also due to the increase in contact sampling. During 2005, the Pakistan RRL received and analysed 6044 stool samples collected from Pakistan, out of which 3926 were of AFP cases and 2118 were contacts. The 28 wild polioviruses (27 WPV1 and one WPV3) were isolated from 18 districts of Pakistan and NPEV rate was about 24%. From Afghanistan stool sample of 1170 cases (819 AFP and 351 contacts) were received and processed. The NPEV rate was 19% and only nine wild polioviruses (5 WPV1 and 4 WPV3) were isolated.

As of September 2006, WHO RRL received stool samples of 4464 AFP cases from Pakistan and 19 wild viruses (13 WPV1 and 6 WPV3) have been isolated from 12 districts of Pakistan. The NPEV rate is 22%. The 1081 cases from Afghanistan have also been processed and 26 wild polioviruses (25 WPV1 and one WPV3) have been isolated so far. The NPEV rate is 21%.

All polioviruses isolated are subjected to genetic sequencing and results are shared with CDC. The WHO RRL met all criteria for full accreditation by WHO in 2005. The results of the RRL are reliable and accurate as demonstrated by scores of 100% in all proficiency tests virus isolation and identification and ITD and 100% concordance in ITD results between the RRL and CDC.

4.3 Yemen: virus surveillance

Dr Sulieman, National Poliovirus Laboratory, Oman

The Oman NPL started testing the Yemen AFP cases and contact stool samples in 1998. Initially there were problems in labelling of samples and/or improper packaging and/or leakage, but now the transportation has improved. No cold chain problem has been observed during this period.

The number of samples received from Yemen has increased over the years. A sharp increase occurred during the outbreak of poliovirus in 2005. During 2005, a total of 521 wild polioviruses were isolated from AFP cases, and only 1 wild poliovirus was isolated in 2006. The NPEV rate is always above 10%.

The Yemen outbreak started in Hodeidah and spread rapidly all over the country. The rapid diagnosis and well coordinated communication between the laboratory and Yemen polio eradication programme played a major role in targeting the immunization activities. The polioviruses were sequenced in record shortest time. Since the Oman NPL was suddenly dealing with a large number of stool samples, and also isolated a large number of polioviruses, laboratory supplies were depleted very quickly. However, the NPL received good support from national health authorities and WHO to replenish the laboratory supplies.

4.4 Somalia and south Sudan: virus surveillance

Mr Peter Borous, Kenya Medical Research Institute, Kenya

The polio laboratory at the Kenya Medical Research Institute (KEMRI) provides virological diagnostic support to AFP surveillance in the WHO polio eradication initiative. The laboratory is classified as a national intercountry laboratory and serves Kenya, Eritrea, Somalia, south Sudan and Djibouti. Kenya and Eritrea are countries within the WHO African Region, while Somalia, Sudan and Djibouti are in the Eastern Mediterranean Region.

The laboratory has supported surveillance in Somalia and Sudan since the beginning of the decade. Some challenges in 2005 arising from failure to satisfactorily analyse a proficiency test panel resulted in the suspension of the testing of stools from these countries in September 2005. However, these issues were resolved and the laboratory started to once again

process stools from countries of the Eastern Mediterranean Region in April 2006. The laboratory has maintained excellent performance indicators in 2006.

The laboratory had processed a total of 695 stool samples from all countries served as of 15 September 2006. From April 2006, 152 samples were received from Somalia from 78 AFP cases, while 123 were received from 64 AFP cases in south Sudan.

The Somalia samples came from 20 regions with Mudug, Togdher, Banadir and Galgadud having the most samples in diminishing order. Over 30 samples were received each in April, May and July, while 22 and 18 were received in June and August, respectively.

Samples from south Sudan rose steadily from 3 in April to 19 in August. All south Sudan samples came from 5 regions, with Bahr-el-Ghazal having the most (65) followed by Upper Nile/Jongli (32) and East Equatoria (14). Six stool samples were received each from Upper Nile state and West Equatoria.

The overall non-polio enterovirus rate for the laboratory between April and August 2006 was consistently above 10%. This rate was highest in May and June (21.6% and 21.1% respectively), and lowest in April.

The NPEV rate for Somalia rose from 8.8% in April to 27.8% and 27.3% respectively in May and June. In July and August, the rate was 22.2% and 25% respectively. The south Sudan NPEV rate was generally higher than the Somalia rate. The rate for April, May, June, July and August was 37.3%, 38.8%, 18.2%, 33.3% and 29.4% respectively.

Thirty-eight type 1 polioviruses were isolated from AFP stools from 22 cases. Of these 26 isolates from 14 cases were confirmed as wild by ITD. Three type 3 poliovirus isolates were isolated in stools from 2 AFP cases, and all were Sabin-like viruses. No poliovirus was isolated from 110 stools received from AFP cases from south Sudan.

In Somalia and south Sudan, 164 and 40 samples were received from stools of contacts respectively. The overall NPEV rate for the contact stools was 25.5%, with a rate of 26.7% and 13.3% for Somalia and south Sudan samples respectively.

Four (4) WPV1 were isolated from stools of contacts of 3 AFP cases. Of these contacts, 2 cases had WPV1 infection. Virus surveillance is going on well in Somalia and south Sudan and there is need to maintain and consolidate laboratory standards as well as the close collaboration between the laboratory and the programmes.

5. VIRUS SURVEILLANCE IN POLIO-FREE COUNTRIES

5.1 Egypt: virus surveillance

Dr Iman Al Maamoun, VACSERA, Egypt

There was an increase in workload due to improvements in AFP surveillance activities in Egypt and other countries like Iraq, for which VACSERA was parallel testing the stool samples. Additional increases in workload also occurred due to outbreak in Somalia, Sudan and Yemen.

In 2005 the laboratory received 2152 stool samples from 866 AFP cases and 269 contacts from Egypt. All laboratory performance indicators were maintained at certification standard: reporting of results within 28 days was 96%, ITD results within 14 days was 94%, and NPEV rate was 22.5%.

As of September 2006, a total of 1791 stool samples from 687 AFP and 357 contacts from Egypt have been processed. All laboratory performance indicators were maintained at certification standard: results reporting within 28 days were 89%; ITD results within 14 days were 89%; NPEV rate was 19.5%. Delay in reporting occurred due to increased workload and late receipt of laboratory supplies.

The laboratory passed the proficiency tests with a score of 100%, and passed the accreditation in 2005 by obtaining 99% for on-site review. No wild poliovirus has been isolated from any stool samples from AFP case or contacts collected in Egypt for the past 28 months, and from sewage samples for the past 20 months. A large number of Sabin viruses have been isolated from stool samples and sewage samples.

VACSERA dealt with AFP stool samples from other countries: Iraq (1294), Sudan (295), Somalia (1130), Yemen (354), Lebanon (49), Saudi Arabia (25), Syrian Arab Republic (12), and Djibouti (19). From re-infected countries (Sudan, Yemen and Somalia), 609 wild polioviruses were isolated and characterized.

All wild polioviruses were referred to CDC for sequencing. There is very high concordance between the results of VACSERA and CDC. The laboratory assisted in a randomized double blind trial to study the comparative evaluation of immunogenicity of monovalent type 1 oral poliovirus vaccine (mOPV1) versus trivalent oral poliovaccine (tOPV), and help in storage and transportation of study samples to RIVM.

VACSERA has started implementing the new testing algorithms of cell culture and ITD from the beginning of August 2006. To date, good results have been produced, with a maximum of 12 days from date of receipt at laboratory to date of notification, except in 3 samples with special circumstances.

In environmental surveillance, 42 sewage samples are collected each month from 33 locations in 18 Egyptian provinces. The last WPV1 was isolated in January 2005 from

Fayoum and Sohag. The positive samples from Fayoum belonged to cluster E, and those from Sohag belonged to cluster H.

The laboratory is working at high sensitivity, as either NPEV and/or Sabin viruses are isolated from almost all the sewage samples. The concentrated environmental samples and positive isolates are sent to KTL, Finland, to compare the results. As of September 2006, 1142 positive environmental isolates and 569 concentrated environmental samples have been sent to KTL. There is high concordance between results of KTL and VACSERA.

5.2 Sudan: virus surveillance

Dr Hatim Babikar, National Poliovirus Laboratory

The last WPV1 was isolated from a contact sample from Sennar state in August 2005. The AFP surveillance is working in close collaboration with laboratory. In 2005, stool samples were collected from 381 AFP cases and 194 contacts. From these stool samples, 21 WPV1 and 17 Sabin-like viruses were isolated. The NPEV rate was 15%.

In 2006, there was improvement in AFP surveillance and as a result more stool samples were collected. As of September 2006, stool samples were collected from 381 AFP cases and 80 contacts. From these stool samples, only 9 Sabin-like viruses were isolated. The NPEV rate was 20%.

The laboratory is working with highly sensitive cells and maintaining the laboratory indicators at certification standards. The timeliness of reporting virological investigation results improved from an average of 19 days in 2005 to 16 days in 2006.

5.3 Islamic Republic of Iran: virus surveillance

Dr Hemedeh Tabatabaia, National Poliovirus Laboratory

In 2005, a total of 876 stool specimens from AFP cases and their contacts were tested. No wild viruses were detected, and 21 Sabin-like viruses were isolated. One poliovirus type 2 iVDPV was isolated. There were 3 specimens that contained polio mixtures (Polio 1+3, Polio 2+3 and Polio 1+2+3).

As of September 2006, the 688 stool specimens from AFP cases and contacts were processed in the laboratory. No wild virus or VDPV were isolated. Fourteen Sabin-like viruses were isolated from these samples. The NPEVs rate is 5.7%. All NPEV are typed and found to be ECHO 6 (10%), ECHO 11 (7.5%), ECHO 20 (5%), ECHO 25 (10%) and ECHO 33 (5%).

6. QUALITY ASSURANCE

6.1 Accreditation status of poliovirus regional network laboratories

Dr H. Asghar, WHO/EMRO

In 2005, 11 of 12 regional network laboratories were fully accredited by WHO. The Kuwait regional reference laboratory was provisionally accredited. The Iraq national poliovirus laboratory was accredited based on its performance during the past 12 months. The data for this period were analysed and on-site review was waived. As of June 2006, the national poliovirus laboratories of Islamic Republic of Iran, Oman, Saudi Arabia, Sudan and Tunisia have been fully accredited by WHO. The remaining laboratories (Egypt, Iraq, Jordan, Kuwait, Morocco, Pakistan, Syrian Arab Republic) are pending accreditation visits by reviewers. 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 virology results. There was uniform implementation of quality assurance programmes.

6.2 Report on proficiency testing: virus isolation, identification and intratypic differentiation and intratypic differentiation by ELISA evaluation

Dr H. van der Avoort, WHO Temporary Adviser

The excellent proficiency of the 12 regional network laboratories was demonstrated once more with the results obtained in the 2005 proficiency test for isolation and typing of polioviruses. All laboratories passed the test, all but one with an optimal score of 100%.

Two laboratories, Kuwait and Tunisia, were able to detect the adenovirus present in one of the samples. Both laboratories have diagnostic duties next to their function as national polio laboratory. Detection of adenoviruses in stool samples is not a requirement for network laboratories. The sample was part of the PT panel to test the adherence of the laboratories to the instructions given in the laboratory manual for samples showing a CPE on L20B cells, by viruses that can not readily be typed as polioviruses. The 2006 PT was distributed to all laboratories at the end of the meeting.

Results for the proficiency testing for intratypic differentiation of polioviruses by RIVM ELISA were also good: 5 of the 6 laboratories passed the test with a mean score of 99%. One laboratory, Kuwait, had consistent problems with the P3 test that could not be resolved through study of worksheets, advice via email and provision of new reagents. A consultant will be sent in the near future to assist in solving the problem.

6.2 Update on cell sensitivity testing in regional network laboratories

Dr Javier Martin, WHO/EMRO

Screening of polioviruses by two methods for intratypic differentiation (one antigenic, one molecular) is the WHO-recommended approach for detecting wild polioviruses and VDPV.

The discordant result (NSL, DR or NR) in the RIVM ELISA for polioviruses that by molecular methods (PCR and/or sequencing) are clearly proven to be of OPV origin, is a common feature for almost all VDPV (two possible exceptions have been claimed, still to be confirmed by RIVM). However the aberrant reactivity in the ELISA is not confined to VDPV alone: antigenic changes, with impact on ELISA reactivity, occur also in isolates that differ less than 1% in genetic content from the Sabin parent, and that by definition are not VDPV.

The molecular basis for the changes in ELISA reactivity was studied by sequencing all known neutralizing antigenic sites of a series of isolates as well as by testing the reactivity of these viruses with neutralizing monoclonal antibodies. Data for Polio 1 suggest that changes in antigenic site 3 are associated with aberrant behaviour in ELISA. However this is not the only factor, as non-neutralizing antigenic sites do play a role.

Similar studies are under way for type 3 polioviruses, where there is a need for improving the sensitivity and specificity of the ELISA, as reagents difficult to produce because of the low degree of differences between the Sabin strain and the P3 wild prototype strain (just 13 nucleotides) and the low immunogenicity of the antigenic sites that constitute the difference between wilds and vaccine P3 viruses.

7. RAPID TESTING ALGORITHM

7.1 A new approach for increasing the speed of poliovirus detection and reporting

Dr E. de Gourville, WHO/HQ

The global target was set at 60 days for reporting of virological investigation results. All the regions dealing with endemic countries or those with importations/outbreaks have achieved this target. In its October 2005 meeting, the Advisory Committee on Polio Eradication recommended to reduce significantly the period from onset to implementation of the first supplementary immunization activity with mOPV. A 30-day target was set, which has significant implications for the laboratory network. Timeliness is affected by both field and laboratory performance. In the field, sample transportation is through shipping companies, which is a major factor affecting the timeliness of results.

In the field, many efforts are made to reduce the time, like flagging the cases as “hot”, improving the transportation time, and reducing the shipping steps by increasing ITD and sequencing laboratories. This has resulted in limited success. In the laboratory, consideration was given to change the current testing algorithm and introduction of new technologies. In the laboratory, there are two steps where time can be reduced: the cell culture step and ITD test step.

While attempting to reduce the time it was noted that in the test algorithm currently used, the time can be reduced by reducing the cell culture observation time and omitting the neutralization step. The following considerations were made in developing the cell culture step of new test algorithm:

- Standard reagents and quality assurance programme are available
- Maintain or improve poliovirus detection sensitivity by inoculation in two cell lines (RD and L20B)
- Experience from several years of testing in LabNet
 - 95% of polioviruses show CPE within 3–4 days of inoculation
 - L20B has higher specificity than RD for poliovirus isolation
 - polioviruses grow to higher titre in RD than L20B
 - passage of isolates in RD before ITD reduces problems in ITD tests
 - ~ 100% agreement in serotype results between PCR and neutralization test
 - PCR provides simultaneous serotype second intratype in shorter time than NT followed by ITD
 - need to retain capacity for NPEV isolation in some laboratories
 - some NPEVs grow in L20B (rare).

The following considerations were given in developing the ITD step of new test algorithm:

- Highest priority is reporting of WPV
- Continued need for screening for VDPVs: 2 ITD methods to be retained
- Using CDC-PCR (6 primer sets):
 - no need to separate viruses in mixtures prior to testing
 - gives serotype, intratype for poliovirus mixtures
 - WPV in homotypic (NSL/SL) mixtures may be missed
 - some technical problems when L20B isolate used. CDC recommends passage of L20B isolates into RD prior to PCR
- Using RIVM-ELISA
 - monotypes (separated viruses) needed
 - high titre virus needed, preferably grown in RD cell line.

In the new test algorithm, the cell culture step was shortened, changes were made in the passage of positive cultures, the neutralization step was omitted, and it was made obligatory to pass L20B into RD before ITD. The positive culture was subjected to PCR for simultaneous serotyping and ITD, and immediate reporting of WPV and referral for sequencing. The monotypic isolates should be tested by ELISA, and mixtures should be separated by neutralization followed by ELISA on separated viruses.

The new test algorithm was field evaluated in three locations: Pakistan, India and CDC Atlanta. While comparing the results of new test algorithm with the traditional algorithm, there is 95% comparability in cell culture, specimen and case base analysis of results. There is 50% reduction in reporting time. The mean time for final poliovirus results was 7–12 days by

the new algorithm as compared to 10–29 days by the traditional algorithm. There were implications of increase in workload in cell culture (18%), ELISA (72%) and PCR (100%).

7.2 Group discussion: new testing algorithm

The new test algorithm differs from the traditional algorithm in two parts, i.e. cell culture steps and ITD testing. For that reason it was essential to discuss these steps with laboratory directors in detail for better understanding before implementation by mid-2007. The laboratories were divided into two groups: NPLs doing cell culture only and NPLs and RRLs doing cell culture and ITD testing. Most of the discussions were centred on the implications of the new algorithm. It was very encouraging that all the laboratories accepted the changes and showed willingness to adapt.

Group 1: Changes in virus isolation and identification

Egypt, Iraq, Jordan, Morocco, Saudi Arabia, Sudan, Syrian Arab Republic, Tunisia, Pakistan (Moderators: Drs E. De Gourville, Humayun Asghar, Francis Kasal)

The following were the salient findings of the discussion:

- The standard operation procedures (SOPs) should be updated according to proposed changes in the new algorithm. The worksheets of specimen inoculation and observation should be modified accordingly to accommodate the different steps of cell culture passages into different cell lines.
- The database should be updated for entry of new variables, checks, analysis and reporting
- The cell culture preparation cycles should also be adjusted to the needs of the new algorithm; a minimum of two cycles per week may be needed.
- The NPLs should refer all L20B+/RD+, L20B+/RD-, RD+/L20B+/RD+ and RD+/L20B+/RD, to the ITD testing laboratory, and report the programme to alert that the sample is potentially positive for poliovirus.
- Any ambiguous samples which is L20B+ and RD-, RD+/L20B+/RD- should be referred immediately to the ITD testing laboratory.
- None of the isolates should be subjected to serotyping before referring the isolate to ITD testing laboratory.
- Close coordination with the ITD laboratory will be necessary to follow-up the results.
- The EPI in the country should be kept informed of all the stages of testing and should be informed of the results as soon as received. There will be need to change the wording of the preliminary report from NPLs to the EPI, like “No virus isolated”, NPEV, L20B positive-referred for serotyping and ITD.
- Training will be needed for laboratory staff at country level in implementing new algorithm
- There will be an increase in workload in terms of more need for reagents and disposable laboratory supplies.

Group 2: Changes in ITD

Egypt, Islamic Republic of Iran, Kuwait, Oman, Pakistan, Tunisia (Moderators: Drs H. Van der Avoort, Javier Martin)

Most of the issues related to operational aspects were the same as those in the first group discussion.

- The standard operation procedures (SOPs) should be updated according to proposed changes in ITD step of new algorithm. The worksheets of specimen referral and ITD testing should be modified accordingly.
- The database should be updated for entry of new variables, checks, analysis and reporting.
- All ITD laboratories should replace the probe hybridization test by PCR for molecular method. Since the laboratories will be using six primer sets for PCR, there will be an increase in workload and consumption of reagents.
- There should be a strong mechanism to track the referred samples for reporting and follow-up. The reporting of positive isolates should be transmitted to referring laboratory rapidly. The WPV should be reported on highest priority.
- In case of queries regarding positive isolates, the WHO regional laboratory coordinator and EPI should be involved for facilitating the transportation.
- Training will be needed for laboratory staff at country level in implementing the new algorithm.
- There will be an increase in workload in terms of more need for reagents and disposable laboratory supplies.

7.3 Expected increase in workload due to implementation of the new testing algorithm

Mr A. Nasim, WHO Pakistan

During field testing of new algorithm, it was noted that there was an increase in workload, which consequently led to an increase in consumption of the laboratory supplies. In order to assess the expected increase in workload, calculations were made on the basis of a 2–3 month period from 1 May to 31 July 2006.

A total of 3901 stool samples of 2382 cases were analysed, out of which 1074 specimens were positive for viruses. During the same period a total of 30 cases having 38 cell culture isolates showed growth on L20B cell line. After passing into the RD cell line they were negative, so the L20B cell line was used again for the passage and PCR was performed on these isolates. PCR results revealed that 18 isolates were NPEVs while 20 were negative.

There was a decrease of 91% in micro-neutralization assay, while a 75% increase was observed in ELISA and a 115% increase in PCR. There was also an increase in consumption of reagents like fetal calf serum, MEM, disposable laboratory supplies and molecular biology reagents as six primer sets were used in PCR. Keeping in view the increase in workload, more ELISA and PCR kits are needed on a regular basis.

8. CHALLENGES IN LABORATORY INVESTIGATIONS

8.1 Evaluation of reasons for Sabin-like strains showing NSL reactions in ELISA and implications

Dr H. van der Avoort, WHO/EMRO

Screening of polioviruses by two methods for intratypic differentiation (one antigenic, one molecular) is the WHO recommended approach for detecting wild polioviruses and VDPV.

The discordant result (NSL, DR or NR) in the RIVM ELISA for polioviruses that by molecular methods (PCR and/or sequencing) are clearly proven to be of OPV origin, is a common feature for almost all VDPV (two possible exceptions have been claimed, still to be confirmed by RIVM). However, the aberrant reactivity in the ELISA is not confined to VDPV alone: antigenic changes, with impact on ELISA reactivity, also occur in isolates that differ less than 1% in genetic content from the Sabin parent, and that by definition are not VDPV.

The molecular basis for the changes in ELISA reactivity was studied by sequencing all known neutralizing antigenic sites of a series of isolates as well as by testing the reactivity of these viruses with neutralizing monoclonal antibodies. Data for polio 1 suggest that changes in antigenic site 3 are associated with aberrant behaviour in ELISA. However, this is not the only factor, as non-neutralizing antigenic sites do play a role.

Similar studies are under way for type 3 polioviruses, where there is a need for improving the sensitivity and specificity of the ELISA, as reagents are difficult to produce because of the low degree of differences between the Sabin strain and the P3 wild prototype strain (just 13 nucleotides) and the low immunogenicity of the antigenic sites that constitute the difference between wild and vaccine P3 viruses.

8.2 Antigenic characterization of VDPV

Dr Javier Martin, WHO/EMRO

Intratypic differentiation (ITD) of poliovirus isolates is essential for polio surveillance since it allows discriminating between wild and vaccine poliovirus strains which in the absence of an effective ITD test would add an enormous workload to the process of poliovirus characterization. ITD tests have been successfully used for a number of years and exploit the significant differences, molecular (sequence) or antigenic, found between both types of poliovirus strains. However, a “new” type of poliovirus, vaccine-derived polioviruses (VDPV), has been found to be of increasing importance having been associated with paralytic polio cases and long-term virus excretion in immunodeficient individuals. VDPV, defined as those isolates related to a vaccine strain showing evidence of extensive transmission/excretion in humans (>1% VP1 sequence drift from the corresponding Sabin vaccine strain), share some properties with the vaccine strains.

Neither of the molecular ITD tests in use in the polio network seems suitable to detect VDPV strains. Although the ITD ELISA test, the most extended antigenic test in the network,

effectively distinguishes VDPV isolates there have been a few exceptions which suggest that some VDPV may escape detection with the current ITD testing. It is proposed that monoclonal antibodies of known specificity are used in the ELISA ITD so as to increase its sensitivity. A number of studies were undertaken to investigate the evolution of the antigenic properties of vaccine viruses following long-term replication in humans in order to identify suitable antibody candidates. The availability of an effective test to detect VDPV is of great significance as we approach the latest stages of polio eradication because we need assurance that there is no poliovirus of any kind circulating in the population in order to be able to devise suitable and safe policies to interrupt polio vaccination after global polio eradication.

8.3 Mechanisms and costs for implementing the new cell culture and ITD algorithm

Dr E. De Gourville, WHO/HQ

The advantages of implementing the new test algorithm are many-fold, such as the, increase in sensitivity of poliovirus detection, reduction in reporting time and capacity building in polio laboratories. The goal is that 75% of AFP samples in all polio-endemic WHO regions should be tested in laboratories with cell culture, PCR and ELISA capacity by end 2007. The implementation will be in three phases, depending on the capacity of PCR and ELISA test facilities. By the end of 2007, there will be many NPLs with ITD testing facilities in WHO regions: in the Eastern Mediterranean Region 4 NPLs will be added to the list of laboratories having capacity to perform ITD test. Introduction of the new algorithm has implications for WHO and national polio laboratories and ITD laboratories.

The following are implications for WHO:

- Train laboratory personnel in new procedures
- Provide standard protocol to laboratories (by end October 2006)
- Establish new reporting targets and accreditation criteria: likely to be 15 days for cell culture and 7 days for ITD (laboratories to meet new targets by December 2007)
- Mobilize resources: supplies, training of personnel, equipping of more ITD laboratories
- Make changes to laboratory databases for new reporting requirements
- The following are implications for national laboratories
- Need to update SOPs, change worksheets and database
- Changes to cell culture preparation cycles
- Changes in what material is referred to ITD laboratories: L20B+RD+; L20B+RD-; RD+L20B+RD+; RD+L20B+RD-
- No serotyping of isolates before referral to ITD laboratories
- Shorter reporting time (15 days).

The following are implications for ITD laboratories:

- Need to update SOPs, change worksheets and database
- Replace probe hybridization by PCR for molecular method
- Shorter reporting time (7 days)
- Implication for programme/surveillance personnel
- Faster reporting of wild polioviruses

- Changes in wording of preliminary reports from national laboratories:
- No virus isolated”; NPEV; “L20B positive virus – referred for serotyping and ITD”
- No change to wording of reports received from ITD laboratories.

The challenges in implementation are the availability of resources. At present, a cost of approximately US\$ 2 million has been anticipated. At country level, reliable electricity supply in some locations, commitment of laboratory staff and the need for on-site assignment of consultants for follow-up work with new ITD laboratories are a few of the immediate challenges in implementing the new test algorithm.

8.4 Managing the sensitive cell culture demand for increased workload: lessons and suggestions

Dr Laila El Bassiouni, Egypt

The VACSERA laboratory is dealing with a high workload due to improvements in AFP surveillance of Egypt polio eradication and also dealing with the recent outbreaks in Somalia Sudan and Yemen. The laboratory also acts as NPL for Iraq since 2003. There is also high demand for cell culture, as more cells are used in environmental surveillance.

Monitoring the cell sensitivity and keeping the cell lines free from mycoplasma contamination are key factors for isolation of viruses. This can be achieved through quality control of media and its ingredients e.g. fetal bovine sera, MEM, water, and testing for mycoplasma contamination.

Sensitivity testing of cells is done after receipt of a new batch of sera with different lot number and sources of MEM. This helps to monitor the effect on cell sensitivity. In VACSERA, sensitivity testing is performed at the beginning, middle and end of passages of cells. Due to the high workload, the cell lines are passaged frequently and are used within 2 months and the number of passages does not exceed more than 13. Cell sensitivity testing results are within allowed limits and are shared regularly with NIBSC and WHO/EMRO. Mycoplasma testing was done twice last year and it was found that cells were free from mycoplasma.

8.5 Meeting the polio eradication programme needs to provide rapid ITD results: country experience in coordination and communication

Mr Assif Naim, Pakistan

There has been demand for speedy results from the programme for a long time. One factor responsible for the strong is the long time taken in reporting the results, therefore leading to late implementation of supplementary immunization activities.

Possible means to meet the field or programme demand were to label the cases “hot” and expedite their processing in the laboratory. Moreover, any of such cases show rapid growth on L20B cell line then PCR is performed on a priority basis and results are conveyed to programme.

Implementation of the new algorithm is expected to boost the time-line in reporting of results. Use of the hot case label contributed to isolation of 4% wild polioviruses in 2005, and to 9% in 2006. Proper use of the “hot” label for cases can be improved using the new algorithm. The mean reporting time for the hot cases was also reduced, from 18 to 12 days. Mean time for the sequencing results also decreased from 18 days to only 6 days in Pakistan RRL.

During the past 2 months, the mean reporting time for the wild poliovirus isolates has been 5 days only as a result of implementation of new algorithm. This ensures speedy and accurate results for the programme.

8.6 Process, cost, and constraints of stool specimen transportation

Dr F. Kasalo, AFRO

The African regional polio laboratory network consists of 3 regional reference laboratories (RRLs) 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 of Congo, Senegal, Uganda, Zambia and Zimbabwe.

The African laboratory network receives stool specimen from surveillance officers in the various Member States. Subsequently all poliovirus isolates are forward for ITD testing to laboratories within the network with such facilities.

Several methods of stool specimen and isolate shipment are employed in the African Region. These include overland transportation, (hand carrying on buses, courier), and air transportation, such as through unaccompanied baggage, courier, use of alternative air shipment, e.g. humanitarian flights between countries, hand carrying on airplanes.

The total cost of transportation of 256 shipments carrying 2385 isolates and stool specimens between different countries was US\$ 102 500. The cost of a single shipment was in the range of US\$ 25 455, depending on the countries of origin.

The following are constraints of stool specimen transportation in African Region:

- Strict IATA shipment regulations
- Inadequate or infrequent air travel, especially in West Africa
- Varying courier companies making it difficult to enter in a regional shipment contract
- Varying country regulations/laws on importation and export of infectious verses diagnostic materials
- Increasing and varying shipment costs (including packaging costs).

9. OTHER ISSUES

9.1 Report on VDPV in the Islamic Republic of Iran

Dr Hamidah Tabatabaei, Islamic Republic of Iran

Since 1994, two VDPVs have been isolated from 2 AFP cases, one during 1994 and the other in 2005. Both were poliovirus type 2 iVDPV.

The first iVDPV case was a female, 17-month-old patient from Tehran province. She was suffering from antibody deficiency and had received IPV but was in contact with an OPV vaccinee. The time of exposure is not known. Three specimens were received from this patient. ELISA showed a double-reactive reaction, while the probe hybridization test result was Sabin-like. Sequencing revealed 2.2% divergence in VP1 region from Sabin2, and also recombination with polio1 in 3A region. The patient died 7 days after paralysis onset.

The second iVDPV case was a male patient from Isfahan province. His parents were originally from Afghanistan. He was 7 months old at the time of paralysis onset and was suffering from severe combined immunodeficiency. There was a 35-day interval between his last (fourth) dose of OPV and paralysis onset. Five specimens were received from this patient, in all of which was detected polio 2, with double-reactive result in ELISA and Sabin-like in PCR. Sequencing showed 1.1%–1.5% divergence in VP1 from Sabin2, and also recombination with polio 1 in 3A region. The patient died due to multiple infections, pneumonia and renal failure 3 months after paralysis onset.

9.2 Further progress on direct detection of enteroviruses by PCR in stool specimen

Dr Hinda Triki, Tunisia

Direct detection of enterovirus genomes by PCR in clinical samples is widely used at present in diagnostic laboratories. Amplification in the 5' untranslated region of the genome (5'UTR) is the most sensitive approach but does not allow serotype identification; this is one of the main reasons this technique is not yet included in the WHO standard protocol for poliovirus surveillance. EV detection by PCR amplification of the 5'UTR was introduced in the RRL, Tunisia in 2000. It was first used for diagnosis of meningitis and conjunctivitis cases on cerebrospinal fluid or conjunctival swabs. In 2003, a study on its possible use for polio surveillance was initiated. A retrospective study conducted in 81 stool samples previously tested EV positives in cell culture (CC) confirmed the high sensitivity of the method and its ability to detect viruses with altered infectivity. A prospective study included 572 stool samples which were tested in parallel, upon reception, by PCR and isolation on cell culture. Only 1% of samples were PCR(-)/CC+, probably due to the presence of PCR inhibitors in the stool sample. In contrast, 28 (4.8%) samples were PCR(+)/CC-, 50% of them suffered long transportation time to the laboratory since collection. PCR+/CC-samples are re-tested, especially in case of a decrease in the NPEV isolation rate, to check for any sensitivity problem in cell culture techniques. EV detection by PCR amplification of the 5'UTR was also used to identify isolates showing unusual CPE, non-polio isolates growing on L20B and non-polio isolates that can not be typed by the A-G RIVM pools.

Even if the results reported to the programme remain based on the standard cell culture technique, the use of PCR amplification of the 5'UTR is very helpful for polio laboratories, especially those conducting other diagnostic and/or research activities.

9.3 Status of survey and inventory of phase 1 of laboratory containment of wild polioviruses

Dr H. Asghar, WHO EMRO

Out of 20 polio-free countries, excluding two re-infected countries (Somalia and Yemen), 16 (Bahrain, Djibouti, Iraq, Islamic Republic of Iran, Jordan, Kuwait, Lebanon, Libyan Arab Jamahiriya, Morocco, Oman, Qatar, Saudi Arabia, Sudan, Syrian Arab Republic, Tunisia and United Arab Emirates) have reported completion of the laboratory survey and inventory phase I activities, and two countries (Egypt and Palestine) are in the process of completion. Sudan was reinfected in 2004, after completion of laboratory survey and inventory phase I activities in 2003, and so performed further containment activities in February 2006. National containment coordinators have not been nominated in Afghanistan and Pakistan. WHO and UNICEF will jointly take up the assignment to complete phase I containment activities.

As of September 2004, 22 210 laboratories have been surveyed and only four have been identified storing wild poliovirus material. Most of these belong to the Eastern Mediterranean regional network of national polio laboratories. One laboratory in Razi Institute, Islamic Republic of Iran, is storing quality control neurovirulence strains.

As of September 2006, 14 countries (Bahrain, Djibouti, Iraq, Islamic Republic of Iran, Jordan, Libyan Arab Jamahiriya, Morocco, Oman, Qatar, Saudi Arabia, Sudan, Syrian Arab Republic, Tunisia and United Arab Emirates) have submitted draft reports to WHO for comments. These reports have been reviewed by the reviewer, and findings were discussed with countries. The reports will be submitted to Regional Certification Commission (RCC) through the National Certification Committees (NCC).

9.4 Proposed changes in LABIFA to adjust the new testing algorithm

Dr H. Asghar, WHO/EMRO

In the light of the proposed new testing algorithm, changes were proposed in the LABIFA. It was suggested that no major changes should be made in the LABIFA so as to avoid complications and an increase in data entry work.

Changes were suggested to address the inoculation in two different cell line and passage, referral of L20B isolate, ITD part, and reporting. Most of the variables already exist and need to be re-named for entry and analysis purpose. The most important change is the entry of different dates for different stages of algorithm. The reporting will also be updated in the main menu to include the results of different time intervals and analysis related to cases, specimen, etc.

10. CONCLUSIONS

A great deal of 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, including Egypt, which was declared polio-free in 2006: only Afghanistan and Pakistan remain endemic. Sudan, Yemen and Somalia were re-infected due to importations of wild poliovirus, but the outbreaks in Sudan and Yemen were curtailed and it is declining in Somalia. A network of 12 laboratories contributes to the eradication initiative through providing support for AFP surveillance facilitating detection of wild polioviruses. Information from the network is vital for targeting of public health interventions to interrupt transmission.

Network laboratories tested approximately 40 000 faecal samples from AFP cases between January 2005 and September 2006 and detected wild polioviruses in 5 countries during that period. Additional data from the analysis of viral VP1 nucleotide sequences was used to trace transmission pathways and revealed that endemic type 1 and type 3 viruses were circulating in Afghanistan and Pakistan, whereas imported type 1 viruses genetically linked to Nigerian viruses were found in AFP cases in Sudan, Somalia and Yemen. Outbreaks in Sudan and Yemen have been controlled and transmission in Somalia is on the decline. Wild polioviruses indigenous to Egypt were last isolated from sewage samples collected in January 2005.

Network laboratories continue to provide feedback on the detection of programmatically important vaccine derived polioviruses (VDPV). Between January 2005 and September 2006, type 2 VDPV were found in immunodeficient children in the Islamic Republic of Iran, Syrian Arab Republic, France (from a child of Tunisian origin) and Spain (from a child of Moroccan origin). Other type 2 VDPV were also isolated in 2005 from a single AFP case in Saudi Arabia and from a sewage samples collected in Egypt. There was no evidence of sustained transmission of any of the detected VDPV.

Monovalent oral poliovaccine (mOPV) is increasingly being used in the Region during supplementary immunization activities, especially in areas with current or recent transmission of a single wild polio virus serotype. mOPV has been used in supplementary immunization activities in Egypt, Yemen, Somalia, Pakistan and Afghanistan. There are ongoing efforts to improve the quality of supplementary immunization actions and OPV immunization coverage, although lack of security reduces access to populations in parts of Afghanistan, Pakistan and Somalia. Polio-free countries of the Region remain at significant risk of wild poliovirus importation from remaining endemic countries. The development and implementation of national plans to respond to virus importations are critical to protect previous investments. High quality and sensitive AFP surveillance continues to be important for early detection of infection and transmission. Recent improvements in AFP surveillance have led to increases (of between 100% and 500%) in detected AFP cases in some high-population countries and this has had a significant impact on laboratory workload.

The laboratory network workload in the Region has been increasing over the years and the total number of tested samples was 9743, 10 911, 12 986, and 18 059 in consecutive years

between 2002 and 2005. This presents managerial challenges within laboratories and necessitates the securing of resources to meet demands for higher logistic support and operational costs. Despite workload increases, laboratories have continued to maintain high standards of performance, meeting reporting timelines and quality assurance requirements.

The regional reference laboratory in Pakistan has been one of only three sites in the global laboratory network that implemented a collaborative field evaluation of a new test algorithm that has been shown to provide sensitive poliovirus confirmation in a shorter time. With the new approach, reporting times could be reduced to 12 to 15 days in laboratories with on-site capacity for virus isolation in cell culture and intratypic differentiation of poliovirus strains using PCR and ELISA tests. This is a significant reduction in reporting times compared with the average time of 32 days that was achieved in the Eastern Mediterranean Region in 2005.

11. RECOMMENDATIONS

1. WHO should continue to advocate with national governments and partner agencies to continue their support of the laboratory network. It will be critical to meet rising costs, given the increasing workload and the need to sustain high levels of laboratory performance and increase the speed of poliovirus confirmation.
2. Rapid detection and reporting of wild and vaccine-derived polioviruses continues to be a high priority in order to facilitate early public health interventions. Laboratories should therefore report all wild polioviruses and viruses with discordant results to national authorities and WHO global and regional polio laboratory coordinators within 24 hours of virus detection.
3. Cell sensitivity testing should be continued in network laboratories. Results should be documented and reported to the regional laboratory coordinator within 48 hours of completion of the test. There should be collaboration between the laboratories and the coordinator to develop a follow up plan, including re-testing of samples, as appropriate, if there is evidence of reduced cell sensitivity for poliovirus isolation. Cell sensitivity trend data should be summarized every quarter by the regional laboratory coordinator and shared with EMRO and NIBSC.
4. The new test algorithm for poliovirus isolation and ITD should be implemented in all laboratories of the Eastern Mediterranean Region. WHO will develop a standard protocol for the algorithm which will be distributed to all network laboratories by October 2006.
 - a. National laboratories should amend their standard operating procedures (SOPs), worksheets and cell culture and virus isolation practices in accordance with the new algorithm. The SOPs should be shared with the regional laboratory coordinator who will provide feedback and signal that all relevant operational issues have been appropriately addressed before start up. The cell culture

component of the algorithm should be implemented in all laboratories by mid-2007, and should meet a reporting target of 14 days for virus isolation results by December 2007.

- b. All ITD laboratories in the Region should amend SOPs and worksheets, and ITD practices and should implement the ITD component of the new test algorithm for testing of isolates from AFP cases by December 2006. Laboratories should meet an ITD reporting target of 7 days by December 2006.
 - c. WHO should mobilize resources to increase the number of ITD laboratories in the Region.
 - d. The LABIFA database software should be updated to include the new variables related to the new algorithm and to automate the analysis and generate reports, by December 2006.
5. All network laboratories should work with EPI staff to discuss the expected increase in workloads due to the updated AFP surveillance. Laboratories should prepare a list of annual laboratory supplies expected to be required.
 6. WHO should collaborate with laboratories, as appropriate, to train personnel so that they can be formally certified for the shipping of infectious materials.

Annex 1**PROGRAMME****Monday, 18 September 2006**

- 08:00–08:30 Registration
- 08:30–09:30 Opening session
 Message from H.E. Dr Mohammad Ridha Kechrid, Minister of Public Health, Tunisia
 Message from Dr Hussein A. Gezairy, WHO Regional Director for the Eastern Mediterranean Region
 Election of the Chairman and Rapporteur
 Implementation status of the recommendation of the 9th Inter-country Meeting of Directors of Poliovirus Laboratories / Dr H. Asghar, WHO/EMRO
- 09:30–09:45 Overview of polio eradication in the Eastern Mediterranean Region / Dr H. Asghar, WHO/EMRO
- 09:45–10:00 Overview of the global polio laboratory network / Dr E. de Gourville, WHO/HQ
- 10:00–10:15 Regional progress in the EMR polio laboratory network / Dr H. Asghar
- 10:15–11:00 Regional progress in the AFR polio laboratory network / Dr F. Kasalo, WHO/AFRO
- 11:00–11:30 Discussions on the overviews
- 11:30–11:50 Characteristics of viruses in countries of the Region/ Dr H. Asghar, WHO/EMRO
- 11:50–12:10 Pakistan and Afghanistan, virus surveillance / Mr S. Zaidi, Pakistan
- 12:10–12:30 Yemen, virus surveillance / Dr S. Al-Busaidy, Oman
- 12:30–12:45 Somalia and south Sudan virus / Mr P. Borus, KEMRI
- 12:45–14:00 Discussion
- 14:00–14:20 Egypt RRL, virus surveillance / Dr E. Al-Mamoun, Egypt
- 14:20–14:40 Sudan, virus surveillance / Dr H. Babikar, WHO Sudan
- 14:40–15:30 Iran, virus surveillance / Dr H. Tabatabaei, Iran
- 15:30–15:50 Accreditation status of EMR polio laboratories / Dr H. Asghar, WHO/EMRO
- 15:50–16:10 Evaluation of proficiency test for isolation and typing 2004/5/6 / Dr H. Avoort, WHO/EMRO
- 16:10–16:30 Update on cell sensitivity testing in regional laboratories / Dr J. Martin, WHO/EMRO
- 16:30–17:00 Discussion

Tuesday, 19 September 2006

- 08:30–09:00 A new approach for increasing the speed of poliovirus detection and reporting / Dr E. de Gourville, WHO/HQ

- 09:00–11:00 Group discussion: New testing algorithm
Group 1. Changes in virus isolation and identification: Egypt, Iraq, Jordan, Morocco, Sudan, Syria, Tunisia, Pakistan
Group 2. Changes in ITD: Egypt, Iran, Oman, Pakistan, Tunisia
- 11:30–12:30 Comments by the groups and moderators on the group discussion
- 12:30–14:00 Expected increase in workload due to implementation of new testing algorithm / Mr A. Naeem, WHO Pakistan
- 14:00–14:20 Evaluation of reasons for Sabin like strains showing NSL reactions in ELISA and implications / Dr H. Avoort, WHO/EMRO
- 14:20–14:40 Antigenic characterization of VDPVs / Dr J. Martin, WHO/EMRO
- 14:40–15:00 Mechanisms and costs for implementing new cell culture and ITD algorithm / Dr E.de Gourville, WHO/HQ
- 15:00–16:00 Discussion
- 16:00–16:20 Managing the sensitive cell culture demand for increased workload: lessons and suggestions / Dr L. Al-Baissouni, Egypt
- 16:20–16:40 Meeting the polio eradication programme needs to provide rapid ITD results: countries experience on coordination and communication / Mr A. Naeem, WHO/Pakistan
- 16:40–17:00 Process, cost, and constraints of stool specimen transportation / Dr F. Kasalo, WHO/AFRO

Wednesday, 20 September 2006

- 17:00–17:30 Discussion
- 09:00–09:20 Report on VDPVs in Iran/ Dr H. Tabatabai, Iran
- 09:20–09:40 The use of EV detection by PCR in the 5'UTR for enterovirus detection in stool specimens / Dr H. Triki, Tunisia
- 09:40–10:00 Status of survey and inventory of Phase 1 of laboratory containment of wild polioviruses / Dr H. Asghar WHO/EMRO
- 10:00–10:20 Proposed changes in LABIFA to adjust the new testing algorithm variables / Dr H. Asghar, WHO/EMRO
- 10:20–11:30 Discussion
- 11:30–12:30 Open discussion on remaining issues
- 12:30–13:00 Closing session
Discussion on conclusions and recommendations

Annex 2

LIST OF PARTICIPANTS

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Ms Nancy George, Secretary, WHO/EMRO