

Report on the

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

Kuwait City, Kuwait
24–26 October 2011

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1. INTRODUCTION

The fifteenth intercountry meeting of directors of poliovirus laboratories in the WHO Eastern Mediterranean Region was held in Kuwait from 24 to 26 October 2011. Directors of poliovirus laboratories in Egypt, Islamic Republic of Iran, Jordan, Kuwait, Morocco, Oman, Pakistan, Sudan, Saudi Arabia, 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; the National Institute for Biological Standards and Control (NIBSC), United Kingdom; and staff from WHO headquarters and Regional Office for the Eastern Mediterranean.

Dr Humayun Asghar, Regional Polio Laboratory Network Coordinator, WHO/EMRO, 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 appreciation for the high quality of work performed by the regional polio laboratory network. He commended the laboratory support extended to countries and the timeliness of results reporting despite recent political turmoil in some countries of the Region. He cautioned about the risk of importation of wild polioviruses into the countries with changing political situations and urged the laboratories to remain vigilant for any importation. He concluded by acknowledging the network laboratories for their active role in evaluating new initiatives.

Dr Qais Al Duwairi, Assistant Under Secretary for Public Health, Kuwait, welcomed the participants on behalf of His Excellency Dr Helal Mosaed Al-Sayer, Minister of Health. He thanked WHO for holding the meeting in Kuwait. He highlighted the importance of sensitive AFP surveillance and role played by the virological surveillance. He expressed satisfaction with the performance of the national poliovirus laboratory in virological analysis of stool samples of AFP cases and contacts in Kuwait.

Dr Siham Al Mufti (Kuwait) was elected to chair the meeting. The programme and list of participants are included as Annexes 1 and 2, respectively.

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

Dr Humayun Asghar, WHO/EMRO

The recommendations of the fourteenth intercountry meeting of directors of poliovirus laboratories in the Region addressed to national authorities and to WHO were implemented. However, it was emphasized that many of these recommendations continue to be valid and their implementation should be pursued by all concerned.

3. OVERVIEW

3.1 Status of the regional polio eradication initiative

Dr Humayun Asghar, WHO/EMRO

Nineteen countries of Region maintained their polio-free status for five or more years. The epidemic that spread in Sudan starting in June 2008 came to an end after one year, with the last case reported from south Sudan in June 2009.

AFP surveillance indicators (non polio AFP rate and percentage of adequate stools) are satisfactory at national and regional levels, but subnational data analysis is showing some gaps. In 2010, AFP surveillance reviews were conducted for 8 programmes: Afghanistan, Egypt, Lebanon, Morocco, Somalia (desk), south Sudan (desk), Tunisia and Yemen. The recommendations of these reviews are being addressed by relevant authorities with actions aimed at improvement of the system. The laboratory network is providing excellent support and biosafety measures are followed up. The isolation of circulating vaccine-derived polioviruses (cVDPVs) from Afghanistan, Somalia and Yemen reflects poor routine EPI coverage in these areas.

Pakistan and Afghanistan are the only endemic countries in the Region. In Afghanistan, circulation is localized in the southern part of the country and remaining areas are without any established circulation.

The majority of the polio cases in Pakistan are from Khyber Pakhtunkhwa/Federally Administered Tribal Areas (KP/FATA) and Sindh, where campaign quality is compromised due to security and management issues. The Government of Pakistan has launched the national emergency action plan with the target of eradicating polio by the end of 2011. Key steps being taken to improve campaign quality include: establishment of the national task force; charging district coordination officers (DCO) with responsibility for supervising activities in their district; updating specific district and Union Council plans with focus on pre-campaign activities to ensure quality implementation; improving mobile population strategies; tightening supervision and monitoring; and not tolerating poor performance.

In the context of milestones of the global strategic plan for polio eradication 2010–2012, the assessment indicates that Pakistan is at risk. For Afghanistan the assessment is that polio eradication is “progressing but with issues of concern” in the south. Surveillance in both countries is on track.

The certification activities are continuing and basic national documentation has been accepted from 20 countries. Afghanistan and Pakistan submitted provisional reports and the Regional Commission for Certification of Polio Eradication (RCC) has shown satisfaction with the quality. Final national documentation has been accepted from 17 countries that have been polio free for 5 years or more and have completed phase 1 of laboratory containment (other than Afghanistan, Pakistan, Somalia, South Sudan, Sudan and Yemen).

Regular annual and abridged annual updates are submitted by all countries who submitted the basic national documentation and final national documentation, respectively.

3.2 Regional progress of the regional polio laboratories network

Dr Humayun Asghar, WHO/EMRO

All Eastern Mediterranean Region poliovirus network laboratories network laboratories are fully accredited and passed the proficiency testing (PT) panels of virus isolation and intratypic differentiation (ITD). The workload of the laboratories is considerably high. During 2010 the network laboratories processed 26 601 specimens from AFP cases and contacts, and the workload continues to be at the same level in 2011. Laboratory performance is being maintained at certification standard. The average time between the receipt of stool samples in the laboratory and reporting the result was 12 days in 2010. Overall, 94% of specimens had culture results within 14 days, 98% had ITD results within 7 days of virus culture positive referral and 97% of final laboratory testing results were provided within 45 days of paralysis onset. The real-time PCR (rRT-PCR) method for rapid characterization of polioviruses was implemented in Morocco's national poliovirus laboratory which brings the total laboratories which can perform ITD methods to 7 out of 12.

The nucleotide sequencing of poliovirus is performed in the Pakistan and Tunisia polio regional reference laboratories (RRLs). Indigenous type 1 wild poliovirus (WPV1) and type 3 wild poliovirus (WPV3) continued to circulate in high risk districts of Pakistan and Afghanistan in 2010. However, as of October 2011, mainly WPV1 is detected in Pakistan and Afghanistan, and only two WPV3 cases have been detected in Pakistan. In 2010, 03 clusters (A3-D2, A3-A1A1 and B4-A1) of WPV1 and one cluster of WPV3 (B1-C5) were circulating in Pakistan; while in Afghanistan 2 clusters (A3-D2 and A3-A1A1) of WPV1 and 2 clusters of WPV3 (B1-C5 and B1-C6A) were detected. In 2011, in Pakistan there are mainly two WPV1 clusters (A3-D2 and A3-A1A1) and 1 cluster of WPV3 (B1-C5), while in Afghanistan only two WPV1 clusters (A3-D2 and A3-A1A1) are circulating. Many small chains of transmission have disappeared but main clusters, especially A3-D2 and A3-A1A1, are widely circulating in both Pakistan and Afghanistan. An importation of WPV1 was detected in Hotan district of Xingjiang province of China which was linked with virus circulating in Ghotki district of Sindh province of Pakistan in 2010.

Between January 2010 and October 2011, circulating vaccine-derived polioviruses (cVDPVs) were isolated from AFP cases and contacts in Afghanistan, Somalia and Yemen. Two iVDPVs (iVDPV2 and iVDPV2) were isolated in Egypt and one iVDPV type 2 was isolated from an immunodeficient child in the Islamic Republic of Iran. One type 2 VDPV was isolated from an AFP case in South Sudan.

Environmental surveillance in Egypt continued its valuable work. Through it an importation of WPV1 was detected. After investigation it was diagnosed as linked with 2009 Sudan viruses. Ongoing environmental surveillance was expanded in major cities of four provinces of Pakistan. Large numbers of sewage samples from all collection sites are positive for WPV1; the last WPV3 was isolated from Karachi in October 2010. The nucleotide

sequencing of viruses isolated from sewage samples are linked with the virus isolated from acute flaccid paralysis (AFP) cases.

The network laboratories participated in pilot testing of FTA cards for isolate referral to laboratories, evaluation of selective growth on L20B and high temperature selection for detection of non-Sabin like polio viruses, an iVDPV surveillance project, and seroconversion study in Karachi. Pakistan RRL supported the molecular sequence analysis of China viruses. The biosafety training modules were introduced into the network and biosafety campaign fully implemented in network laboratories. One tissue culture training workshop was conducted for network laboratories in Kuwait. The Regional Office supported the establishment of environmental surveillance in the African Region.

3.3 Status of global polio laboratory network

Dr Ousmane Diop, WHO/HQ

The Global Polio Laboratory Network (GPLN) tested 286 301 faecal samples and 26 188 non AFP samples between January 2010 and June 2011. WPV as detected in 1750 AFP cases and 175 non-AFP samples between January 2010 and 21 September 2011. 22% of all reported WPV cases during the period reviewed were detected in the 4 polio endemic countries of Afghanistan, India, Nigeria and Pakistan. The situation in India is considered to be promising as no WPV has been detected in that country since a WPV1 case was found in West Bengal in January 2011. The reported decline in WPV3 cases in 3 polio endemic countries is also encouraging: no WPV3 was detected in Afghanistan and India in 2011, while the most recent WPV3 positive AFP case from Pakistan had paralysis onset in June 2011 and the most recent WPV3 positive sewage sample in the same country was collected in Sindh province in October 2010. The WPV1 situation in Pakistan is still a major concern to the polio eradication initiative as WPV1 transmission is widespread in the insecure northwestern areas of the country and in Baluchistan, as well as in the highly populated Sindh province from which transmission spills over into other locations within Pakistan. WPV1 transmission continued mainly in southern provinces of Afghanistan in 2011. The number of WPV cases in Nigeria has declined substantially with reported total number of WPV cases of 388 in 2009, 21 in 2010, and 30 up to September 2011. However co-circulation of WPV1 and WPV3 and vaccine derived poliovirus (VDPV) of serotype 2 continues and there are occasional long gaps in genetic information that link related viruses, suggesting some weaknesses in AFP surveillance. The 78% of cases and a single WPV1 in sewage reported globally since January 2011 represented transmission of imported viruses in 23 countries.

Sixteen countries had cases following WPV importation: 7 with new importations and outbreaks (Congo, Kazakhstan, Nepal, Russian Federation, Senegal, Tajikistan and Turkmenistan); 3 with re-established transmission (Angola, Chad and Democratic Republic of Congo); 6 with outbreaks from previous years and new sporadic importations (Liberia, Mali, Mauritania, Niger, Sierra Leone and Uganda). Non-endemic countries with only new WPV importation cases or outbreaks in 2011 included China, Côte d'Ivoire, Gabon and Guinea. Transmission of imported WPV1 has persisted in border areas between Kenya and Uganda since 2009, with long gaps in WPV detection.

Circulating VDPVs (cVDPVs). cVDPV outbreaks were newly detected in Yemen (VDPV2, 4 cases), Mozambique (VDPV1, 2 cases) and Angola (VDPV2, 1 case linked to an older outbreak in Somalia) in 2011. A VDPV type 2 (VDPV2) outbreak that first started in Nigeria in 2006 continues with 27 and 14 related cases detected in 2010 and in 2011. VDPV2 outbreaks in Afghanistan and Somalia that started in 2010 and 2008, respectively, continued into 2011.

VDPVs of ambiguous origin (aVDPVs). Isolates of aVDPV2 from AFP cases were detected in Chad (1), China (2), Democratic Republic Congo (2), India (5), Myanmar (1), Nigeria (1), Peru (1), Somalia (1) and Syrian Arab Republic (1) since January 2010. aVDPV3 was detected in single AFP cases in China (VDPV3) and Tajikistan in 2010. aVDPV2 strains were detected in unrelated AFP contacts in South Sudan (1) and Nigeria (1) in 2011 and a single aVDPV1 was isolated from a healthy child in Turkey. VDPV2 isolates were also reported from non-AFP sources such as single sewage samples collected in Estonia in November 2010 and Finland in July 2011 and in 5 samples collected in Israel since January 2010. In all three countries the VP1 nucleotide sequences of isolates indicates their continued evolution from viruses previously detected in the same locations, albeit from as yet unidentified sources. A single VDPV1 positive sewage sample was collected in Egypt in February 2010.

VDPVs from immunodeficient persons (iVDPVs). Type 2 VDPVs were isolated from immunodeficient persons from China (1) India (1), Egypt (1), Islamic Republic of Iran (1), Iraq (1), Sri Lanka (1), and Turkey (1) since January 2010. VDPV1 isolates were found in immunodeficient persons in Egypt (1) and Tunisia (1) and VDPV3 strains were isolated in China (1) and Algeria (3) also from samples from immunodeficient individuals. A special multi-country study of immunodeficient persons led to the detection of at least four of the reported iVDPV cases.

Some VDPVs reported in 2011 were pending categorization and the outcome of follow-up investigations. These included VDPV2 isolates from Nigeria (1), India (1), China (1) and a VDPV3 from China.

Laboratory accreditation and proficiency tests. Overall, 98.6% of network laboratories were fully accredited in 2010, as were 96.5% of those reviewed up to September 2011. Implementation of a quality assurance programme for sequencing laboratories has started. On-site accreditation evaluations were carried out by expert reviewers in laboratories in India, South Africa, Thailand and Tunisia, all of which attained on-site passing scores of > 90% for their performance. Minor changes to the checklist were recommended by the reviewers to include a requirement for direct sequencing of heterotypic virus mixtures, referral of homotypic mixtures to global specialized laboratories for characterization and archiving of data for isolates tested in parallel with other laboratories. Areas identified for improvement were dye removal cleanup, sequence editing processes, data management and appropriate archiving of test documents and results. Currently 64 laboratories (45% of the network) have ITD capacity, and the majority (54) of them had new rRT-PCR capacity established since mid-2009. Indeed there has been a 100% increase in the total number of

laboratories with any ITD capacity since capacity building commenced in 2006. ITD results are now available more rapidly for programme use, VDPV2 detections have increased and there has been a decrease in the demand and cost for inter-laboratory and international shipment of infectious isolates. ELISA use is now only restricted to 13 laboratories (10 of which also do rRT-PCR), mainly for characterizing PV isolates from sewage.

Evaluation of the biosafety campaign has started and 26 trainers have reported running 119 training workshops that reached approximately 600 trainees. The impact and changes in individual laboratories are to be more formally evaluated through questionnaires and observations during on-site visits linked to the GPLNs accreditation programme.

Experiences with pilot testing of FTA cards showed equivalent performance of ITD tests (> 90% concordance in serotype and intratype results in rRT-PCR assays, respectively) for traditionally referred isolates and those spotted on FTA cards. Shipping costs were reduced by 50% in Nigeria using FTA cards. Total shipping time was generally not reduced and amounted to 5 to 7 days. The total processing time of FTA referred samples was longer than that for traditionally referred isolates, because of extra steps required for elution and RNA extraction of samples. Use of disposable punchers during sample processing of FTA cards decreased the time spent on cleaning reusable punches to reduce the risk of cross contamination.

4. VIRUS SURVEILLANCE

4.1 Laboratory performance

4.1.1 Pakistan and Afghanistan: laboratory performance and molecular characteristics of wild polioviruses

Mr Sohail Zaidi, Pakistan

Between January 2011 to October 2011, the Pakistan RRL processed 37 391 stool specimens of AFP and contact specimens for both Pakistan and Afghanistan. The laboratory met all the laboratory performance indicators: 100% score PT panels of virus isolation and ITD testing methods of rRT-PCR, ELISA and nucleic acid probe hybridization (NAPH). The NPEV rate remained 22% and large number of Sabin like viruses were isolated. The Pakistan RRL is providing vital nucleotide sequencing data on polioviruses which is helping the polio eradication programme to take targeted action in high risk areas and population. The RRL also participated in polio research activities: high temperature sensitive WPV and VDPV isolation from sewage samples and seroconversion study in Karachi.

For Pakistan in 2010, 144 WPVs (116 WPV1 and 28 WPV3) were isolated. As of October 2011, 131 WPV (129 WPV1 and 2 WPV3) were isolated. For Afghanistan in 2010, 25 WPVs (17 WPV1 and 08 WPV3) and as of October 2011, 41 WPV (all WPV1) were isolated.

Summary of molecular characterization of poliovirus in Pakistan and Afghanistan:

P1-Wild: Six clusters (A3-A1A1, A3-A1A2, A3-A1A3, A3D2, B4A1B, and B4A1C) were circulating during 2010 whereas in 2011 only two clusters (A3-A1A1 and A3D2) are active. Recently, A3A1A1 is further subdivided into three clusters: A3A1A1A, A3A1A1B and A3A1A1C. A3D2 is sub divided into five clusters: A3D2A, A3D2B, A3D2C, A3D2D and A3D2E. All eight of these clusters are active in 2011.

A3D2B cluster was widely spread in 2010 and 2011. During 2011, it has been found in all four provinces of Pakistan including Balochistan (Killa Abdullah, Quetta, Noshki, Pishin, Khuzdar, Kalat, Dera Bugti, Kohlu and Lorali), Punjab (Okara and Lodhran), Sindh (Badin, Hyderabad, Jamshoro, Kambar, Sanghar, Tandoallah Yar, TM Khan, Thatta, Umarkot, KHI Baldia, KHI Gadaap, KHI Gulshan Iqbal, KHI Orangi, KHI Sadar, KHI Site) and Khyber Paktoonkhawa (Khyber, Laki Marwat, Mansehara, Torghar, S-Waziristan, N-Waziristan). Viruses from the same cluster were also found from AFP cases in China having close homology with Ghotki-Sindh viruses.

During 2011, A3D2A, A3D2B, A3D2C and A3D2E clusters have been present in KP only. A3A1A1A cluster has been found only in an AFP case of Bannu (KP) with May 2011 onset. Viruses isolated from AFP cases of Mohmand (KP) and Diamer (FANA) with onset January and May 2011 respectively fall in A3A1A1B cluster. A3A1A1C cluster remains active in both Pakistan (Killa Abdullah and Pishin) and Afghanistan (Farah, Hilmand and Kandahar). This cluster is active in southern Afghanistan and Quetta-Killa Abdullah Block. The virus of this cluster is also found in environmental samples collected from Quetta during 2011.

P3-Wild: During 2010, two clusters (B1C5A and B1C5B) were circulating in Pakistan while in Afghanistan B1C5A and B1C6A were active. In 2011 only B1C5A has been detected in Pakistan (KP) whereas no wild type 3 poliovirus has been found in Afghanistan so far. None of the environmental samples collected during 2011 has been found positive for wild type 3 poliovirus.

4.1.2 Egypt

Dr Iman Al Maamoun, VACSERA/Egypt

The laboratory at VACSERA, continued to support the polio eradication initiative by testing stool specimens and virus isolates for Egypt, Iraq, Lebanon, Syrian Arab Republic, Sudan and Yemen. Between October 2010 and October 2011, VACSERA tested 3210 samples (2615 from AFP cases and 595 from contacts) from Egypt and other countries. The quality of performance indicators was sustained at certification standard. In 2011, 100% score was obtained in PT panels of virus isolation, rRT-PCR, ELISA and NAPH test.

Environmental surveillance is continuing and is providing vital information. In 2010, one WPV1 importation was detected in December 2010 in a sewage sample collected from Aswan which was related with 2009 Sudan viruses. One aVDPV was also isolated from a Helwan sewage sample in February 2010.

The iVDPV type 1 and 2 were isolated from cases in Behira in May and June 2010 and 2011 respectively. One VDPV2 was isolated from a case in Iraq whose sample was collected in December 2010, which was characterized as iVDPV2 based on clinical diagnosis. Six isolates (5 poliovirus type 2 and one type) from Yemen cases and contacts were found discordant on ITD testing (positive as SL2 but negative in VP1) and referred to CDC. Four of these were confirmed as cVDPV2.

The biosafety campaign was successfully implemented and 21 laboratory staff were trained. VACSERA continued to support the polio laboratories through training: one scientist was recruited to Ibadan laboratory in Nigeria to train laboratory staff in sewage water testing methods and two virologists from Iraq were trained in VACSERA for polio diagnostic methods.

4.1.3 South Sudan and Somalia

Dr Peter Borus, Kenya

The intercountry polio laboratory based at the Kenya Medical Research Institute (KEMRI), Nairobi, supports poliovirus surveillance in Somalia and South Sudan, in addition to other countries of the African Region. The laboratory carries out poliovirus isolation and ITD. The latter capacity was developed over a period of approximately one year with the support of WHO AFRO, the regional Reference laboratory at NICD, South Africa, and CDC. The laboratory was officially allowed to report ITD results to the programmes from 1 October 2011. Wild poliovirus isolates as well as VDVPs are still referred for sequencing at NICD.

The general performance of the laboratory in 2011 has been good, with all sections of cell culture, virus isolation and ITD showing stable performance. There was pressure on the laboratory to meet cell culture needs and timeliness of reporting indicators as a result of increased workload from South Sudan, but this was mitigated through prudent change of work schedule. The Regional Office, through the South Sudan office also assisted with an additional US\$ 5000 to meet supplies and other recurrent expenditure resulting from this increase.

On performance indicators for 2011, the laboratory processed 3131 stool samples as of 21 October 2011, with South Sudan and Somalia accounting for approximately 68% of this workload. Samples from South Sudan were 1424 whereas 695 stool samples were received from Somalia. In terms of timeliness in generating laboratory results within 14 days, the laboratory had a score of 87.7% over this period, with timeliness for South Sudan and Somalia samples being slightly over 90%. All isolates to be referred to the RRL met the 7 days timeliness limit. Accuracy of poliovirus detection was 100%, and the non-polio enterovirus isolation rate for this period was 11.3%. The laboratory scored 100% proficiency test for real time poliovirus PCR as well as for the VDVP assay.

Sixty poliovirus isolates were obtained from stool samples from Somalia. Out of these VDVPs were intratyped from 5 cases and 9 contacts. Fifty two poliovirus isolates were obtained from samples from South Sudan, out of which VDVPs were intratyped from one

case and two contacts. No wild poliovirus was isolated from samples originating from cases or contacts in the two countries.

4.2 VDPV detection and molecular epidemiology

4.2.1 Outbreak in Afghanistan

Dr Humayun Asghar, WHO/EMRO

A total of six cVDPV2 cases have been reported: 5 in 2010 and one in January 2011. All these cases are reported from Nade Ali district of Helmand. These cVDPVs share 9 non-Sabin nucleotides which showed a clear evidence of genetically related VDPVs that are circulating in Helmand and indicated persistent circulation.

Almost 30/50% of the target population remains inaccessible in most of the rounds in 2010. Supplementary immunization activities coverage ranged between 50% and 60%. Routine vaccination coverage among AFP cases is 14%. Six round of tOPV used from July 2010 to June 2011 and no VDPV2 have been isolated since January 2011.

In the district, more than 50% of the target children were missed at least in the last 9 campaigns during 2008 and 2009. In February 2010 the entire district was not covered due to military operations. In the other campaigns in 2010 (March to June), 33% to 50% children were not accessed. A number of clusters and villages are still being completely missed, including the villages from where the VDPV2 cases are reported.

It is also important to mention that most of the zero dose AFP cases reported during 2009/2010 are from Nade Ali/Marja and its bordering districts including Maywand and Lashkargah (Bust) indicating presence of pockets of population which were not accessed by vaccination teams for more than 1/2 years.

In 2009, 3 VDPV2 were found and data may suggest that there is circulation but no obvious epidemiological evidence was found at that time. The epidemiological data clearly indicate that Nade Ali and surrounding areas are at risk of spread of cVDPVs.

4.2.2 Outbreak in Somalia and Yemen

Dr Steve Oberste, CDC

Somalia has a history of VDPV2 circulation dating back to at least 2008. Five cases were identified in Somalia in 2011; the most recent case had a date of onset of 24 March. The cases were clustered in the Banadir area, near Mogadishu. They were genetically related to VDPVs that circulated in the same area in 2009, indicating persistent circulation for at least two years.

In Yemen, eight VDPV2 cases have been identified in 2011, clustered in the Sa'dah area, with latest onset in June 2011. Laboratory investigation is continuing, to better define

the genetic relationships among the viruses. There are at least two different genetic clusters, implying two separate emergences.

Such circulation identifies gaps in both immunization and surveillance, both of which require follow-up by the polio eradication programme.

4.2.3 VDPVs in the Islamic Republic of Iran

Since 1995, eight confirmed iVDPVs (5 iVDPV2, one iVDPV3, and one mixture of iVDPV1 and iVDPV2) have been detected in 7 Iranian AFP cases with different humoral immune deficiency disorders. There was also another immune deficient AFP case whose specimens were positive for PV2-discordant; the sequencing confirmation of this case is pending to date. Among these AFP cases, 5 patients died due to different infections, and 3 are still alive, two of which have been shedding virus for almost 5 months.

In a pilot study that searched for long term excretors of poliovirus in humoral immune deficient patients, which was performed in the national polio laboratory from June 2009 to October 2010, 84 specimens from 43 patients with different types of humoral immune deficiency were checked for poliovirus excretion. Only one male patient with X-linked agammaglobulinaemia was positive for PV3 Sabin like (SL). Sequencing showed 9 nucleotide substitutions in VP1 region of the genome and all 3 critical positions in PV3 genome were similar to the wild strain. Follow-up specimens were requested but the patient died before specimen collection; no paralysis occurred. This study has been expanded and screening of immune deficient individuals for poliovirus excretion will be continued in the Islamic Republic of Iran.

4.2.4 VDPVs in Egypt

Dr Laila El Bassioni, VACSERA/Egypt

Historically, from 1988 to 1993, 30 cases of WPV2 were reported from 8 governorates in Egypt. In 1993 these viruses were referred to CDC Atlanta, and nucleotide sequencing suggested these were cVDPV2 originated from one source. Since 2005, 7 VDPVs have been detected in Egypt: through AFP surveillance, 1 iVDPV case (iVDPV3 from Kafr el Sheikh in July 2007, patient died); through the iVDPV project, 1 iVDPV1 from Behira in May 2011 and one iVDPV2 from Cairo in June 2011; and through environmental surveillance, 3 aVDPV2 from Behira in April 2005, December 2007 and April 2008, and 1 aVDPV1 from Helwan in February 2010.

Close contact samples of iVDPV patients were collected sequentially one month apart until 3 consecutive samples were negative for VDPV isolation. For all environmental sample collection sites which were positive for VDPV, the frequency of sample collection, and in some cases the number of sites, has been increased and is continued until 3 consecutive samples are negative for VDPV isolation.

With the implementation of rRT-PCR in 2009, the screening of poliovirus isolates for VDPVs has become more accurate and efficient.

4.2.5 Update on the long-term poliovirus excreter in the United Kingdom

Dr Javier Martin, Temporary Adviser

The rate of vaccine-associated paralytic poliomyelitis among healthy vaccinees is very low and the vaccine is considered very safe. The situation is different in persons with defects in their immune system. Immunodeficient individuals, particularly those with antibody deficiencies, have been shown to have a much higher risk of VAPP (an estimated 3000-fold excess). While immune competent individuals excrete poliovirus for short periods after vaccination with OPV, which rarely exceeds several weeks, immunodeficient patients can excrete poliovirus for several months and even years. To date, about 45 such cases have been reported around the world. Among them, an individual in the United Kingdom is known to have been excreting Sabin 2-derived poliovirus for an estimated 25 years and is still excreting at present despite several attempts to interrupt poliovirus excretion. Molecular analyses of sequential isolates from this individual including an isolate from June 2011 have revealed that the viruses have incorporated a large number of amino acid mutations in antigenic and receptor binding sites which might lead to changes in biological properties. The viruses are very virulent in animal models and fail to interact with a large proportion of type 2 monoclonal antibodies available at NIBSC. Inactivated vaccines have shown to induce a reduced immune response against these iVDPV strains in rats which might mean that the strains have potential to circulate in populations with low immunity against type 2 poliovirus. The prevalence of poliovirus excretion among immunodeficient individuals should be further assessed to estimate the possible impact for the post-eradication era.

5. LABORATORY QUALITY ASSURANCE

5.1 Accreditation status of regional polio network laboratories

Dr Humayun Asghar, WHO/EMRO

In 2010, 11 of 12 network laboratories were fully accredited. The Kuwait regional reference laboratory was provisionally accredited in 2010 and in 2011 was fully accredited. As of October 2011, all network laboratories are fully accredited by WHO, except the national poliovirus laboratory in Saudi Arabia which is pending accreditation visits in 2011. On-site visits were waived for Egypt, Islamic Republic of Iran, Iraq, Oman, Pakistan and Tunisia. All national poliovirus laboratories implemented recommendations made during accreditation visits.

It is concluded from the accreditation visits that the laboratory staff are well-trained, hardworking and dedicated. All laboratories are implementing quality assurance programmes satisfactorily and maintaining certification standard quality performance indicators, especially, timely reporting of stool testing results to the national polio eradication programme. The regional laboratories have shown high efficiency in implementing new polio diagnostic methods and are also supporting polio research activities. A few laboratories did

not report problems unless the problem went out of control or was detected by routine regional monitoring.

5.2 Quality assurance by proficiency testing in a changing diagnostic world: regional data

Dr H. van der Avoort, WHO Temporary Adviser

The proficiency test performance of the regional polio laboratory network in 2011 has been excellent all 12 laboratories passed the annual proficiency test for isolation of polioviruses and enteroviruses according to the new algorithm; ten laboratories reached a 100% score. Non-optimal scores were due to a reporting error (1 laboratory) while one laboratory did not follow all the steps of the new algorithm two laboratories performed the PT for ITD by ELISA each with a 100% score.

Regional laboratories have participated for 20 years in the annual PT quality assurance scheme of the polio laboratory network: in the early years (1991–2000) when the regional laboratory network was built up, PT scores for isolation and typing varied from year to year with mean annual score of 88%; in the past 10 years period performance improved significantly with mean annual score of 97%. All these results were achieved despite the more stringent scoring system that was in use during the past 10 years. Only occasionally one or two laboratories could not fulfil the annual requirements directly, but after rapid implementation of actions formulated in a plan of action to overcome weaknesses observed, these laboratories always passed a second PT often with 100% scores.

PT scores for ELISA were always far above the passing score of 90% in 5 of the 6 laboratories using this test for ITD of polioviruses, with optimal scores on almost all occasions. One laboratory with a low sample volume had over the years repeated problems with obtaining valid P3 ELISA test results. ITD results from this laboratory have always been confirmed at the specialized reference laboratory in the Netherlands. At present the ELISA is not used anymore for ITD purposes in the laboratory network, only the two laboratory analysing environmental surveillance samples Egypt and Pakistan use the test to separate mixtures of wild and Sabin viruses of the same serotype.

In the near future new methods for direct detection of polioviruses in stool samples might become available for general use in the network, but introduction will occur only after thorough and intensive field testing in specialized and selected national laboratories, a practice of proven success that has been used in the last 20 years to guarantee rapid quality laboratory data for action in the field.

5.2.1 Summary of proficiency test performance-PCR methods

Dr Steve Oberste, CDC

The ITD PT panels are distributed to test proficiency of global polio laboratories network (GPLN) polio ITD laboratories in their use of probe hybridization, diagnostic PCR and rRT-PCR for ITD and VDPV detection. The existing ITD PT panel format was

maintained and combined to create one panel for conventional and real-time PCR ITD, VDPV PT panels have now been sent to all laboratory using routine real-time PCR methods. At the same time PT panel field tests the reliability and durability of reagents developed at CDC and the WHO-sponsored training in the use of molecular methods. The regional polio laboratory network continues to perform the molecular assays for ITD and VDPV detection with high proficiency. In 2011, all regional ITD laboratories scored 100% on the ITD PT panels. Regional polio network laboratories are highly proficient in diagnostic PCR and they have excellent turnaround time. Their PT results correlate with routine results.

Improvements in VDPV rRT-PCR assays are in progress to include primers/probes that target Sabin-specific sites in VP1 and 3D (for each of the 3 serotypes). It is known that VP1 sites are those that are known to revert in VDPVs. The 3D site is not specific for VDPVs because of high frequency of recombination, even in non-circulating Sabin strains due to high load of HEV-C viruses in high risk areas. Omission of 3D primers/probes results in more robust VP1 detection by increasing VP1 signal. Sensitivity in detecting normal Sabin viruses increased (total tested ~450, all detected as Sabin) while still not detecting VDPVs (80+ VDPVs, all were NSL). New kits will contain VP1 primers/probe only. Results in real-time diagnostic PCR are occasionally difficult to interpret due to low virus titre especially in mixtures. Mixtures are relatively common, especially in endemic areas where vaccination coverage is improving. Two-stage rRT-PCR increases sensitivity for minor components of virus mixtures. This is especially advantageous for high volume laboratories which are most likely to have serotype mixtures.

The advantage of improved assay is that it increases sensitivity of assays by over 10-fold and tissue culture results with two-stage assay are as sensitive as using extracted RNA with standard assay. There is no need to extract RNA, thus saving time/cost. It will also reduce additional sequencing. There will be no changes in kit reagents, so two-stage real-time assay can be implemented immediately. The GPLN recommended implementation in early 2012, as current kit stocks are exhausted.

5.2.2 Improving the quality control methods in Kuwait RRL

Dr Siham A. Al Mufti, Kuwait

During the last accreditation in 2010 the reviewer made recommendations to improve laboratory performance. These were mainly related to laboratory set-up and routine work. A clean area was separated from the infectious area and cell culture work is done in a completely separate area. The biosafety cabinets (BSC) were serviced and the filters of the BSC were changed by a certified company. The office areas have been brought outside the laboratory. A new autoclave was ordered but did not arrive to date. The consumable items/reagents are kept in a separate store and inventory is well maintained. The number of samples tested in the laboratory has improved and the laboratory has tested than 300 stool samples during the past 12 months with 87% timely reporting within 14 days. A new ITD method (rRT-PCR) was successfully implemented and they were offered the new rRT-PCR PT panel. They secured 100% in PT panels of ITD (rRT-PCR method). All isolates are sent to

RIVM for confirmation and sequencing and they have 100% concordance of their results with RIVM.

5.3 Poliovirus detection sensitivity: influence of number of specimens analysed per case

Dr Steve Oberste, CDC Atlanta

CDC has conducted a study of AFP surveillance data in India to determine the contribution of the second stool sample to detection of wild poliovirus in AFP cases. The second stool specimen was found to be less sensitive than the first sample. It is unclear why this is the case, but could potentially be due in part to the later time of second stool collection and viral excretion patterns, or perhaps factors related to the field investigation and how the second stool is being collected. Despite lower sensitivity, the second stool contributed an additional 7.3% sensitivity overall, for a combined sensitivity of 98.7%. The second stool made a larger contribution if specimens were collected later, particularly after the first week, if stools were inadequate, or if the child was older. This is indicated by the decline in specimen and combined sensitivity, as well as the increase in added sensitivity for each of these factors. In addition, the second stool identified 382, or 7.4%, of polio confirmed cases that would not otherwise have been detected from 2000 to 2010. The percentage of cases that were identified by only the second stool increased by the same factors as those identified in the sensitivity analyses: as stool collection time increased, for inadequate stools, and as age of the child increased. As a result of these findings, where maximum sensitivity is required, we should continue to collect two stools. This recommendation becomes even more important when samples are collected after the first week after onset of paralysis, or when the sensitivity of the first stool is low, and therefore, the contribution of the second stool will be greater. As we approach eradication, identifying every case is potentially relevant, and therefore maximizing sensitivity is imperative. In the post-elimination era, meaning eradication of polio within India, but not globally, high levels of intense surveillance may be difficult to maintain. At this point, if the first stool specimen continues to be collected in a timely manner, we may consider dropping the second stool specimen. This falls in line with PAHO's recommendation to collect one stool after certification of eradication from the Americas.

5.4 A new protocol for cell authentication using real-time PCR

Dr Javier Martin, WHO Temporary Adviser

During the past few years, a small number of WHO polio network laboratories have reported an unusually high proportion of isolation of non-polio enteroviruses in L20B cells from AFP stool samples. Such findings may be genuine (for virus that grow in the mouse L-cells) or may signal cross contamination of cell lines, or problems either in the preparation of cell cultures or reading of CPE. One of the possible explanations could be the contamination of L20B cells, from mouse origin, with other laboratory cell lines more permissive for infection by enteroviruses. NIBSC was asked to look into possible methods for authentication of cell lines used in diagnostic laboratories. The aim was also to develop molecular tests to detect possible contamination of L20B cells with cells of different animal origin. We chose

the bar-coding technique, used at the Division of Cell Biology in NIBSC, which is based on the molecular analysis of a region at the 5' end of the cytochrome c oxidase subunit 1 gene in the mitochondrial genome. This 648bp region is flanked by conserved regions, enabling a single set of PCR primers to be used for (nearly) all species. The PCR product is sequenced and fed into a database. The intra-species variability is low (>1–2%) and the inter-species variability high (several % for closely related species): of 2238 animal species in 11 phyla, 98% of closely related species had >2% sequence difference. Thus, nucleotide analysis in this region allows for an accurate identification of the origin of any particular cell line. We amplified and analysed cytochrome C oxidase subunit 1 gene sequences of some of the most common cell lines used in the laboratory. There was high similarity between sequences of cell lines from human origin (Hep-2c, RD and CACO2), whereas DNA sequences from Vero (vervet monkey) and L20B cells (mouse) were very different.

We then devised a real-time PCR assay to be able to detect contamination of L20B cell lines of different animal origin. Based on nucleotide sequences at the cytochrome c oxidase subunit 1 gene, we developed a very sensitive assay to detect contamination of L20B cells with cells of human origin. This was done by setting up two separate real-time PCR reactions using primers specific for human or mouse sequences. In this way, we could detect the contamination of between 1 to 10 RD cells in a background of 10^6 L20B cells, even when the deliberately mixed cells had been passaged a few times. A new detailed SOP that can be used in all relevant polio network laboratories is being prepared at NIBSC.

5.5 Results from evaluating authenticity of identity of cell lines stocked in global specialized laboratories

Dr Javier Martin, WHO Temporary Adviser

We developed a very sensitive assay to detect contamination of L20B cells with cells of human origin. This was done by setting up two separate real-time PCR reactions using primers specific for human or mouse sequences. The analysis of the results was based both on the positive/negative DNA amplification using different primer combinations and on the melting curve analysis of double stranded amplified products at the end of the real-time PCR reaction. This analysis is very sensitive to nucleotide sequence differences and can be used to distinguish PCR products from different origin. We then analysed master cell banks from WHO global polio specialized laboratories that distribute cell lines to national laboratories. RD cells from Global Specialized Laboratories are positive in human rRT-PCR reaction and negative in mouse rRT-PCR reaction. DNA samples from RD and L20B cells were received from Global Specialized Laboratories both dried in tubes and FTA cards. DNA was quantified and 125 ng were used per reaction. Human and mouse separate rRT-PCR reactions were performed with all samples. As expected, L20B cells were positive in the mouse rRT-PCR reaction and negative in the human rRT-PCR reaction while RD cells were positive in the human rRT-PCR reaction and negative in the mouse rRT-PCR reaction. Ct values for the rRT-PCR reactions with DNA from cells from different Global Specialized Laboratories differed which suggests that there might be differences in the DNA quality of samples extracted using different methods. Ct values for rRT-PCR reactions with DNA from cells extracted from samples in tubes and FTA cards also differed which also suggests differences in DNA quality

of samples. There was a low positive signal (equivalent to < 1 cell) in DNA samples from L20B cells extracted from FTA cards from 3/4 laboratories. This suggests the presence of a very low level of contamination that it is hoped will be eliminated by using disposable punchers when processing samples in FTA cards. The project will continue by analysing samples from regional laboratories that distribute cell lines to national laboratories.

6. FTA CARDS PERFORMANCE

6.1 Experience of shipping of FTA cards and processing in the laboratory: presentation and practical demonstration

Mr Hatim Babiker, Sudan, Dr Eman Al Maamoun, Egypt

In some countries national regulations for shipment of infectious material are meticulous and make it difficult to ship infectious materials to ITD/sequencing laboratories. Whatman FTA Cards are evaluated as a means to inactivate virus and facilitate shipping. A trial was conducted in August 2008 at KTL to check the preservation of sample integrity for safe referral of virus nucleic acid for rRT-PCR and evaluation of consensus protocol for use of FTA cards – experiences at CDC and with other laboratories in June 2009 (Cameroon, Senegal and South Africa). Field evaluation in the Eastern Mediterranean Region was done for shipping from the Sudan national polio laboratory and testing of FTA cards for ITD at VACSERA in 2011. The Sudan laboratory received protocol and FTA card in 2010 and sent isolates and FTA cards in April 2011. No difficulty was observed in sample preparation and also shipment through courier services. Each shipment cost between US\$90 and US\$100. The post (sample) arrived door-to-door without customs clearance. VACSERA received the protocol (Version 1.2) in October 2010 and a biopsy punch and other material were received in February 2011. A total of 11 FTA cards were received and kept at 20°C. The FTA cards were processed according to protocol. The FTA cards were labelled with the Egypt laboratory number and 8 punches with 3mm biopsy punch were used for elution. The ITD was performed by rRT-PCR. There was 100% concordance between FTA card and isolate ITD testing results. It is proposed to test more FTA cards to establish the authenticity of the method. More studies may be made to check the possibility of sending the isolates from ITD laboratories to sequencing laboratories and environmental isolates and concentrates to sequencing laboratories. The audiovisual training material has been produced and it was demonstrated during the meeting.

6.2 Shipping from national laboratory to global specialized laboratory: Ibadan Nigeria to CDC

Dr Ousmane Diop, WHO/HQ

To solve shipping problems frequently faced by polio laboratories in several countries, due to national or IATA regulations for infectious substances, the GPLN has developed, validated, pilot and field-tested shipment of polioviruses rendered non-infectious after spotting on FTA cards. This new procedure facilitates referral of polioviruses and also: allows substantial reduction of shipment costs compared to referral of fresh isolates using card boxes and frozen-icepacks or dry ice, and reduces the risk of breaches of containment of wild or

vaccine-derived polioviruses. For the African Region, five countries have participated in the pilot and field-testing following recommendations of the GPLN's small working group in 2010 (South Africa and CDC Atlanta as receiving laboratories and Cameroon, Nigeria, Central African Republic and Democratic Republic of Congo as sending laboratories).

In summary key points from these studies are as follows:

- There is an excellent correlation between ITD tests performed on fresh isolates and on RNA elute from the FTA cards.
- There is need to closely monitor titer of the isolates, storage and shipping conditions of spotted FTA cards to determine if there is a correlation between sequencing success and treatment of the cards prior to processing. Indeed sequencing of a few FTA cards was problematic.
- Based on the costing of shipments from the polio laboratory in Ibadan, Nigeria, the cost of sending spotted-FTA cards is three times less compared to shipment of infectious isolates (US\$60 versus US\$180).
- Based on Central African Republic Experience, substantial gain in time can be obtained where shipping of infectious biological materials can take several weeks.

Future directions for the GPLN following analysis of the outcomes from the African and Eastern Mediterranean experiences are as follows:

- To conduct a comprehensive inventory of laboratories experiencing or that can experience problems in shipping infectious isolates (stringent regulations, few courier available, delays due to long clearance procedures, etc.).
- To provide FTA cards to more laboratories but with a clear reminder that shipment of fresh isolates remains the gold standard and must be used whenever possible.
- CDC to finalize and distribute protocols and instructions for FTA processing and downstream applications (rRT-PCR, sequencing and transfection using eluted RNA).

7. ENVIRONMENTAL SURVEILLANCE FOR POLIOVIRUSES: PROGRESS IN PAKISTAN

Ms Farzana Malik, Pakistan

The objective of the implementation of supplementary environmental surveillance is to intensify polio surveillance in the major and most populous cities of Pakistan to identify possible reservoir communities where wild poliovirus circulation is sustained. This will enable comparison of the nucleotide sequence from the VP1 region of the genome of wild polioviruses isolated from environmental samples and confirmed polio cases to determine transmission links. It will also allow the programme to monitor for the presence of VDPV in environmental samples collected in Karachi and Lahore.

A new environmental surveillance laboratory was established in Pakistan RRL. It has a designated laboratory and staff. Environmental surveillance was formally initiated in August 2009 and initially six sewage sample collection sites were identified and sampled from

Karachi and 3 from Lahore per month. It was further expanded and to date 17 sewage samples are collected monthly: 6 in Karachi, 3 in Lahore, 3 in Multan, 2 in Peshawar, 2 in Quetta, and 1 in Rawalpindi total of 357 samples were collected to date; out of them 186 were wild, 115 were Sabin and 54 were NPEV positive. The WPV1 is found in all sewage sample collection sites, except one site in Karachi Baldia (Kumhar Wara), which has been closed for sampling. Last WPV3 was detected in week 41 of 2010. The WPV3 detected mostly in Karachi and only once in Quetta in week 41 of 2010. WPV3 was detected in combination with WPV1 which shows the co-circulation of both serotypes.

Isolated wild viruses are subjected to molecular sequencing and dendrogram showing the relationship of polioviruses isolated from sewage samples with viruses isolated from AFP cases. Orphan viruses were isolated through environmental surveillance from all districts over the years. Genetic clusters isolated through environmental surveillance in 2011 shows the persistent circulation of A3D2B in Rawalpindi, Multan, Lahore, Karachi, while 5 different clusters are isolated from Peshawar that shows a multiple importation and lineages circulation. A3A1AC is isolated from Quetta in addition to A3D2 that has links to Afghanistan. WPVs of similar lineages were found in the environment and AFP cases, in a few cases viruses were detected from a sewage sample (Lahore) but there was no evidence from AFP surveillance. Molecular epidemiology of environmental samples clearly identified local endemic reservoirs.

Some samples showed non-specific cytopathic effect CPE on L20B and RD cell lines which were confirmed by CDC as untypeable non-enteroviruses including bovine enteroviruses.

8. RESEARCH TOPICS

8.1 iVDPV surveillance project

Dr Humayun Asghar, WHO/EMRO

The primary objective of this project is to integrate iVDPV surveillance into the national AFP surveillance system in order to allow the successful identification, genetic characterization and monitoring of vaccine-derived poliovirus excretion among persons diagnosed with primary immune (B-cell or combined B/T-cell) deficiency disorders (PIDD). The secondary objective is to establish a pilot national iVDPV surveillance project that can serve as a model for other countries to implement as a means of minimizing the global risk of iVDPVs in the post-OPV cessation era and building capacity to monitor and respond to iVDPVs. The project was proposed to be piloted in Egypt for which a feasibility meeting was held in July 2009. It was concluded that this is doable and participating institutions confirmed their willingness, including the Ministry of Health. It was also estimated that the burden of such a project on the current AFP surveillance system, including laboratory analysis, would not be massive. It was agreed to implement this project in three pilot governorates initially.

The draft project proposal was finalized in March 2010 and key issues like case definition/recognition/detection and improved laboratory capacity were addressed. A project planning meeting was held in July 2010 to agree on project structure and timeline and second resource needs.

The screening is in progress and 25 cases have been investigated and their stool samples have been tested at VACSERA. Two iVDPVs cases have been detected (one each of iVDPV1 and iVDPV2). The project extended to another year and expansion of project scope and training needs are being discussed.

Discussion points were as follows:

- Continue collection of stool samples of any immunodeficient from which Sabin like virus has been isolated because he may be enroute to long term excretion (with PID) and sequence SL viruses until 3 consecutive samples are negative.
- If patient is PID then he should be followed up, if a stool sample is negative.
- If patient dies then contact sampling should be conducted of close contacts. In principle it should be followed up but consideration should be given to ethical issues.

8.2 Evaluation of high temperature incubation for enriching for wild poliovirus and VDPVs in cell cultures

Dr Humayun Asghar, WHO/EMRO

Selection of poliovirus by growth in L20B cells followed by passage into RD (A) or African green monkey cells and incubation at 40° C were evaluated as methods to enrich for WPV and VDPVs in sewage concentrates with known NSL positive results using traditional cell culture approaches. A total of 10 samples, both concentrate and isolates, containing monotype and mixtures of WPVs were used. In initial experiments, the inoculated concentrates of samples known to be positive for WPV were correctly flagged by showing cytopathic effect at 40° C. Characterization of isolates by rRT-PCR ITD showed that although there was high concordance of results (> 80%) with those obtained using the traditional cell culture method for samples containing monotypes of NSL, not all isolates in samples containing PV mixtures (whether NSL mixtures) could be recovered when using the high temperature protocol. When compared to traditional protocol for serotyping and ITD, high temperature incubation and rRT-PCR ITD. The isolates required RNA extraction of sewage isolates to increase frequency of valid rRT-PCR ITD results. It showed 73% to 87% sensitivity for obtaining final NSL positive result if isolates incubated at 40 °C before doing rRT-PCR ITD. High temperature and rRT-PCR ITD protocol for testing sewage can achieve higher efficiency; reduced cost and faster results in high WPV incident situation through ~ 50% reduction in number of cell cultures used and ~ 65% reduction in volume of MM used; elimination of serotyping step by neutralization testing; reduction of “hands on” personnel time; better PV containment from reduced manipulation of cultures; replacement of 2 ITD tests (ELISA and HYB) with more rapid rRT-PCR ITD; and reduced total test time by ~ 40% from 18 to 11 days. Prospective parallel testing phase using 2 protocols has started.

8.3 Group discussion: biosafety campaign

The GPLN developed training materials and launched a biosafety campaign in 2010 with the goal of improving poliovirus containment, reducing incidents accidents and decreasing the risk of poliovirus infections in laboratory workers. Standardized audiovisual training materials are now available in 4 language versions (Chinese, English, French and Russian) and are being used as a cost effective way to reach a wide target audience. One training workshop has been run in the regional polio laboratory network. A “training of trainers” approach is being used. The impact and changes in individual laboratories are to be more formally evaluated through questionnaires and observations during on-site visits linked to the GPLN’s accreditation programme. The participants of group discussions were asked to comment on their experience. The following is summary of their comments.

- All countries have implemented the training modules in their national poliovirus laboratories and in some cases, expanded to non-polio laboratories.
- There is need for an Arabic language version.
- Biosafety is responsibility of the director of the laboratory; however, everyone has a role to play in implementing biosafety.
- Need modules specific for cleaning staff and other non-technical staff.
- Need module on how to perform a risk assessment.
- There are problems in obtaining new Personnel Protection Equipment (PPE) and with access restriction.
- There is a need for continuous training focusing on specific topics (e.g. waste disposal, etc.) and periodic refresher training.

9. CONCLUSIONS

The meeting concluded that the regional poliovirus laboratory network is maintaining high performance of certification standards of laboratory indicators. Network laboratories are providing virological analysis of stool samples of AFP cases and contacts to the national polio eradication programmes with accuracy and in a timely manner. As well new poliovirus diagnostic methods are successfully being implemented in the network laboratories.

10. RECOMMENDATIONS

All regional laboratories performing intratypic differentiation methods should implement the revised rRT-PCR technique as soon as reagents and protocols are available in the laboratory. The responsible scientist should carefully set up the new thermocycling parameters for the rRT-PCR reaction according to instructions provided from CDC and analyse, interpret and record the rRT-PCR results of all samples and controls assigning a final result for each isolate.

1. Laboratories willing to implement ITD and VDPV rRT-PCR and/or nucleotide sequencing techniques should prepare a formal proposal that includes justification for this request and a plan of action for evaluation by WHO EMRO.

2. As for wild polioviruses, laboratories should continue to recognize the importance of detecting, identifying and correctly reporting VDPV isolates. Efforts should be made to advocate with both private and public sector laboratories and hospitals including medical practitioners that might have access to:
 - Registries of primary immunodeficient individuals so the possibility of conducting stool surveys can be evaluated.
 - All other possible sources of polioviruses, which should be discussed with the national public health authorities and WHO EMRO.
3. Network laboratories shipping virus isolates to ITD and nucleotide sequencing laboratories using FTA cards should continue sending the virus sample dried onto a FTA card in addition to the original virus isolate tissue culture supernatant. All laboratories sending or receiving virus isolates on FTA cards should follow the detailed standard operating procedures for preparation and processing of FTA cards. Supporting video materials are also available for training. FTA materials and reagents should be ordered as part of the laboratory annual supplies.
4. Environmental surveillance activities should continue in Pakistan and Egypt and more support should be provided if the number of collection sites is expanded in these countries. Expansion of environmental surveillance to other countries should be explored guided by polio eradication programme needs.
5. The laboratory performing environmental surveillance in Pakistan should continue to explore the use of selective viral growth in L20B cells at high temperatures to isolate non Sabin-like poliovirus isolates, up to May 2012. The laboratory should use both the new and the traditional algorithm for virus isolation from environmental samples in parallel testing and share the results with WHO EMRO.
6. Heads of the polio laboratories are responsible for the full implementation of a biosafety programme for laboratory practices. The polio laboratory biosafety programme should be an integral part of the institutional and national biosafety policies. In addition the programme should include:
 - Assessment of training needs.
 - Establishment of specific requirements for laboratory staff and for non-technical staff entering the polio laboratory.
 - Clear instructions for control access and waste management.

WHO should assist the countries/laboratories in implementation and advocacy for the biosafety programme requirement with the government.

7. Regional laboratories supplying cells to other network laboratories should send DNA samples extracted from L20B and RD working cell banks for cell authentication analysis at NIBSC. Both DNA samples air-dried in Eppendorf tubes and DNA solutions dried onto FTA cards should be sent to NIBSC following instructions provided by NIBSC.

Annex 1**PROGRAMME****Monday, 24 October 2011**

08:00–08:30	Registration	
08:30–09:30	Opening session	
	Welcome and Opening Remarks	
	Message from H.E. the Minister of Health, Kuwait	Dr Qais Al Duwairi, Assistant Under Secretary for Public Health, Kuwait
	Message from Dr Hussein A. Gezairy, WHO Regional Director for the Eastern Mediterranean Region	Dr H. Asghar, WHO/EMRO
	Election of Chairman and Rapporteur	
<i>Session 1: Overview</i>		
09:30–10:00	Implementation status of the recommendation of the 14th intercountry meeting of directors of poliovirus laboratories, and regional progress of EMR polio laboratories network	Dr H. Asghar, WHO/EMRO
10:00–10:20	Status of the regional polio eradication initiative	Dr H. Asghar, WHO/EMRO
10:20–10:50	Status of global polio laboratory network	Dr O. Diop, WHO/HQ
10:50–11:15	Discussion	
<i>Session 2: Virus surveillance</i>		
11:30–12:20	Laboratory performance Pakistan and Afghanistan Egypt South Sudan and Somalia	Mr S. Zaidi, Pakistan Dr E. Al Maamoun, Egypt Dr P. Borus, Somalia Mr S. Zaidi, Pakistan
12:20–12:40	Molecular characteristics of wild polioviruses in Pakistan, Afghanistan	
12:40–13:00	Discussion	
<i>Session 3: VDPV Detections and Molecular Epidemiology</i>		
14:00–14:20	Outbreak in Afghanistan	Dr H. Asghar, WHO/EMRO
14:20–14:50	Outbreak in Somalia and Yemen	Dr. Steve Oberste, CDC
14:50–15:15	Discussion	
15:30–16:00	VDPVs in Iran	Dr S. Shahmahmoodi, Iran
16:00–16:30	VDPVs in Egypt	Dr L. Baisouni, Egypt
16:30–17:00	Update on the long-term poliovirus excreter in the United Kingdom	Dr J. Martin, WHO/EMRO
17:00–17:30	Discussion	

Tuesday, 25 October 2011*Session 4: Laboratory quality assurance*

08:30–08:50	Accreditation status of EMR polio laboratories	Dr H. Asghar, WHO/EMRO
08:50–09:20	Summary of proficiency test performance-virus isolation and ELISA	Dr H. Avoort, WHO/EMRO

09:20–09:50	Summary of proficiency test performance-PCR Methods	Dr. Steve Oberste, CDC
09:50–10:10	Improving the quality control methods in Kuwait RRL	Dr S. Al-Mufti, Kuwait
10:10–10:30	Discussion	
11:00–11:45	Poliovirus detection sensitivity: influence of number of specimens analysed per case	Dr. Steve Oberste, CDC
11:45–12:30	A new protocol for cell authentication using real-time PCR	Dr J. Martin, WHO/EMRO
12:30–13:00	Discussion	
14:00–14:30	Results from evaluating authenticity of identity of cell lines stocked in global specialized laboratories	Dr J. Martin, WHO/EMRO
14:30–15:00	Discussion	
	<i>Session 5: FTA Cards performance</i>	
15:00–15:30	Experience of shipping of FTA cards and processing in the laboratory: presentation and practical demonstration	Mr. Hatim Babikar, Sudan Dr Eman Al Maamoun, Egypt
15:30–16:00	Shipping from national laboratory to specialized global laboratory: Ibadan Nigeria to CDC	Dr O. Diop, WHO/HQ
16:00–17:00	Discussion	

Wednesday, 26 October 2011

Session 6: Environmental surveillance for polioviruses

08:30–09:15	Progress of environmental surveillance for polioviruses detection in Pakistan	Dr F. Malik, WHO/Pakistan
	<i>Session 7: Research topics</i>	
09:15–09:45	iVDPV surveillance project	Dr H. Asghar, WHO/EMRO
09:45–10:30	Evaluation of high temperature incubation for enriching for wild polioviruses and VDPVs in cell cultures	Dr H. Asghar, WHO/EMRO
10:30–11:30	Group discussion: Biosafety campaign implementation	
11:30–12:30	Discussion	
13:00–14:00	Discussion on conclusion and recommendations	

Annex 2

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