Country Report - Hong Kong

Public Health Laboratory Services Branch
Centre for Health Protection
Department of Health
December 2012

AFP Surveillance

- 1948: Poliomyelitis notifiable
- 1963: Polio vaccination introduced
- 1996: National Committee for Certification of Wild Poliovirus Eradication of HK established
- 1997: AFP surveillance introduced
  National Polio Laboratory accredited

Affiliation corresponding to Table of Contents

Notification rate of acute poliomyelitis

Classification of AFP cases

Polio Vaccination

<table>
<thead>
<tr>
<th>Age</th>
<th>Before Feb 2007</th>
<th>After Feb 2007</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td>OPV type 1</td>
<td></td>
</tr>
<tr>
<td>2 months</td>
<td>OPV*</td>
<td>IPV</td>
</tr>
<tr>
<td>2-4 months</td>
<td>OPV*</td>
<td>IPV</td>
</tr>
<tr>
<td>4-6 months</td>
<td>OPV</td>
<td>IPV</td>
</tr>
<tr>
<td>6 months</td>
<td>OPV</td>
<td>IPV</td>
</tr>
<tr>
<td>12 months</td>
<td>OPV</td>
<td>IPV</td>
</tr>
<tr>
<td>18 months</td>
<td>OPV</td>
<td>IPV</td>
</tr>
<tr>
<td>6 yrs (Primary 1)</td>
<td>OPV</td>
<td>IPV</td>
</tr>
<tr>
<td>11-12 yrs (Primary 6)</td>
<td>OPV</td>
<td>IPV</td>
</tr>
</tbody>
</table>

# trivalent vaccine, ^ DTwP-IPV vaccine
Immunization coverage rates

Table 2: Immunization coverage rates for polio vaccines based on administrative data (2007 to 2011).

<table>
<thead>
<tr>
<th>Age</th>
<th>Polio Type 1</th>
<th>Coverage rate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newborn</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 - 4 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 - 6 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11 months</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary 1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary 2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Immunization coverage rates

Table 3: Immunization coverage rates for polio vaccines among children aged 2 to 5

<table>
<thead>
<tr>
<th>Year of birth</th>
<th>Born in Hong Kong (% of age group)</th>
<th>Born in Mainland China (% of age group)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Type 1</td>
<td>1st dose</td>
</tr>
<tr>
<td>2005</td>
<td>98.2</td>
<td>100.0</td>
</tr>
<tr>
<td>2004</td>
<td>98.4</td>
<td>100.0</td>
</tr>
<tr>
<td>2005</td>
<td>99.1</td>
<td>100.0</td>
</tr>
</tbody>
</table>

Note: * Data based on immunization coverage survey conducted in 2009.

Cell sensitivity test

L20B (Sabin 1)

- 0.5 log

Expected LQC titer

L20B (Sabin 2)

- 0.5 log

Expected LQC titer

L20B (Sabin 3)

- 0.5 log

Expected LQC titer

RD (Sabin 1)

- 0.5 log

Expected LQC titer
Samples received in PHLSB

<table>
<thead>
<tr>
<th></th>
<th>2010</th>
<th>2011</th>
<th>2012^</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AFP samples</strong></td>
<td>31</td>
<td>35</td>
<td>34</td>
</tr>
<tr>
<td><strong>Non-AFP other than stools</strong></td>
<td>80674</td>
<td>69362</td>
<td>65092</td>
</tr>
<tr>
<td><strong>Non-AFP stools</strong></td>
<td>2523</td>
<td>1334</td>
<td>1301</td>
</tr>
</tbody>
</table>

*as of 30th Aug 2012

Samples with Poliovirus Isolated

<table>
<thead>
<tr>
<th></th>
<th>2010</th>
<th>2011</th>
<th>2012^</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>AFP patients</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Non-AFP other than stools</strong></td>
<td>1</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td><strong>Non-AFP stools</strong></td>
<td>0</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

*as of 30th Aug 2012

ITD results

<table>
<thead>
<tr>
<th></th>
<th>2010</th>
<th>2011</th>
<th>2012^</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sabin 1</strong></td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td><strong>Sabin 2</strong></td>
<td>1</td>
<td>4</td>
<td>(100%)</td>
</tr>
<tr>
<td></td>
<td>(99.8-100%)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><strong>Sabin 3</strong></td>
<td>0</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

*as of 30th Aug 2012

％ of similarity compared to Sabin 2 VP1 region

ITD proficiency testing results (Conventional method)

<table>
<thead>
<tr>
<th></th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PT score</strong></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Results reported &lt;7 days</strong></td>
<td>100%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>
Testing Algorithm for HFMD

- Molecular tests:
  - Detection: rRT-PCR for EV (5'UTR)
  - rRT-PCR for EV71 (VP1)
  - Typing: Duplex real-time PCR for CoxA4/A6
  - Nested conventional PCR (VP1) + Seq

- Culture:
  - RD and HEp-2C
  - Enterovirus CPE → IF test with mAb
    - rRT-PCR for EV and Rhino
    - Nested PCR + Seq

Challenges

- Establishing real-time RT-PCR for ITD and VDPV detection
- Establishing sequencing of mixed poliovirus

Thank you
Country Report - New Zealand

National Poliovirus and Enterovirus Reference Laboratory
Institute of Environmental Science and Research
National Centre for Biosecurity and Infectious Disease
Wellington, New Zealand

WHO-WPRO Realtime PCR Workshop, Manila, 3-7 December 2012

Specialist Science Solutions

© ESR 2006

Outline

• AFP surveillance system
• Data on testing for AFP cases 2010-2012
• Data on NPEV rates 2010-2012
• Cell sensitivity testing chart
• National enterovirus surveillance system
• Data on EV71 surveillance 1986-2012

NZ population: ~4.4 million
Children <15 years: 891,000
Expected AFP cases: 8-9/year

Acute Flaccid Paralysis Surveillance System in New Zealand

• Ministry of Health: overall responsibility for polio surveillance, laboratory and immunization activities.

• NZ Paediatric Surveillance Unit (NZPSU):
  - AFP case: Paediatrician is required to report to NZPSU immediately
  - monthly reports: All paediatricians (163) are required to provide monthly report to NZPSU.

• ESR Clinical Virology Lab: The only WHO accredited lab to conduct lab tests for all AFP cases.

Testing data for AFP cases, 2010-2012

<table>
<thead>
<tr>
<th>Year</th>
<th>2010</th>
<th>2011</th>
<th>2012 Jan-Oct</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of AFP cases</td>
<td>4</td>
<td>4</td>
<td>6</td>
</tr>
<tr>
<td>AFP Stool Samples</td>
<td>8</td>
<td>6</td>
<td>14</td>
</tr>
<tr>
<td>Poliovirus</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>NPEV</td>
<td>1xEV71, 1xEV74</td>
<td>1xEV71</td>
<td>0</td>
</tr>
</tbody>
</table>

NPEV isolation rates, 2010-2012

<table>
<thead>
<tr>
<th>Year</th>
<th>2010</th>
<th>2011</th>
<th>2012 Jan-Oct</th>
</tr>
</thead>
<tbody>
<tr>
<td>Clinical Stool Samples</td>
<td>16</td>
<td>19</td>
<td>52</td>
</tr>
<tr>
<td>NPEV</td>
<td>6</td>
<td>7</td>
<td>6</td>
</tr>
<tr>
<td>NPEV isolation rate</td>
<td>37.5%</td>
<td>36.8%</td>
<td>11.5%</td>
</tr>
</tbody>
</table>
**RD sensitivity Chart, 2011-2012**

- **Polio 1 acceptable range LQC titre:** 7.7-8.7

- **Polio 2 acceptable range LQC titre:** 7.5-8.5

- **Polio 3 acceptable range LQC titre:** 6.9-7.9

---

**RD sensitivity Chart, 2011-2012**

- **Polio 1 acceptable range LQC titre:** 7.7-8.7

- **Polio 2 acceptable range LQC titre:** 7.5-8.5

- **Polio 3 acceptable range LQC titre:** 6.9-7.9

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**L20B sensitivity Chart 2011-2012**

- **Polio 1 acceptable range LQC titre:** 7.5-8.5
- **Polio 2 acceptable range LQC titre:** 7.0-8.0
- **Polio 3 acceptable range LQC titre:** 6.6-7.6

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**Polio ITD PT results 2010-2012**

- **2010 - 100 %**
- **2011 - 100 %**
- **2012 - 100 %**
**National Enterovirus Surveillance System in New Zealand**

- Identify circulation patterns of enterovirus serotypes and predominant strains
- Describe clinical diseases associated with circulating serotypes
- Detect enterovirus outbreaks to assist public health intervention
- Supplement polio surveillance until eradication of wild polio and cessation of OPV use

**Enterovirus Laboratory Network**

- Weekly, Quarterly, Annual reports to Ministry of Health

**Enterovirus type 71 detections**

- Plot showing number of enterovirus type 71 detections over time.

**Enterovirus 68**

- First time identified in 2010 in New Zealand.
- EV68 is associated with respiratory illness more common on young children and shares biological and molecular properties with both the enteroviruses and rhinoviruses
- Out of the 15 samples isolated with EV 68, 11 (73%) are from children less than 2 years of age. Majority came from South Auckland (87%, 13/15) and a small proportion from Waikato (13%, 2/15).
Enterovirus 74

- Enterovirus 74 identified in NZ for the first time in 2011.
- First isolated from a faecal sample of a 2-year old boy with Acute Flaccid Paralysis from Auckland.
- Also associated with patients with a variety of illnesses, including respiratory tract infections, neonatal disease and unspecified central nervous system disease.

Enterovirus 109

- Identified in New Zealand in 2011 for the first time.
- Isolated from a nasopharyngeal aspirate from a 1Yr old male child with wheezing and bronchiolitis.

iPrep™ Purification Instrument

Automated purification of nucleic acids using magnetic beads

Thank you
2nd Regional Hands-on Training Course to Implement Real-time Polymerase Chain Reaction Technique for Rapid Detection and Characterization of Polioviruses
December 3-6, 2012
Manila, Philippines

Country Report
PHILIPPINES

OUTLINE

- RITM structure/set-up
- AFP surveillance performance indicators
- Cell sensitivity results
- Result of ITD testing
- Other enterovirus works (HFMD)

RITM structure/set-up

- Research arm of the Department of Health
- Provides laboratory support for DOH for surveillance and outbreak investigation

the Department of Virology...

- NRL Polio and other Enteroviruses
- NRL Measles and other Exanthema
- NIC Influenza and other Respiratory Viruses
- NRL Dengue and other Arboviruses

4 National Reference Laboratories

WHO Accredited National Laboratories
AFP surveillance

National Polio Laboratory performance indicators using the new algorithm

<table>
<thead>
<tr>
<th>PERFORMANCE INDICATOR</th>
<th>2012</th>
<th>2013 (as of Nov)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Test results reported on at least 80% of AFP specimens ≤ 14 days of receipt</td>
<td>95.3%</td>
<td>95.5%</td>
</tr>
<tr>
<td>virological tests performed on at least 80% stool specimens annually</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>The accuracy of TV detection and identification among all stool isolates is at least 90%</td>
<td>100% (1)</td>
<td>100% (1)</td>
</tr>
<tr>
<td>At least 80% of AFP isolates from AFP cases and forwarded to WHO in 7 days of receiving the sample</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Internal of procedures, including cell culture sensitivity, are implemented at least quarterly in accordance with the WHO protocol</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>The site on the most recent WHO/PAHO proficiency test is at least 80%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>At least 90% of AFP laboratories report laboratory operating procedures and practices is at least 80%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>At least 90% of laboratories report laboratory operating procedures and practices is at least 80%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Strategy on Global Eradication of Polio

In the Philippines, nationwide, human-based surveillance

AFP surveillance

Invasive Nonpolio Enterovirus (NPEVs) - 3/4s of total isolates from 1992-2008

Objectives

To describe the occurrence, diversity and pattern of circulation of NPEV genotypes implicated in acute flaccid paralysis surveillance

Identification of Isolates

N = 750
Results: Diversity of isolates

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HEV-A</th>
<th>HEV-B</th>
<th>HEV-C</th>
</tr>
</thead>
<tbody>
<tr>
<td>Important EV71</td>
<td>EV71</td>
<td>EV71</td>
<td>EV71</td>
</tr>
<tr>
<td>Prescient EV90</td>
<td>EV90</td>
<td>EV90</td>
<td>EV90</td>
</tr>
<tr>
<td>Recent EV73, EV77, EV91, EV96, EV99</td>
<td>EV73, EV77, EV91, EV96, EV99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rare EV93, EV94, EV95, EV96, EV99</td>
<td>EV93, EV94, EV95, EV96, EV99</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

HEV-B: N=625
HEV-C: N=108

Results: Trends

- NPEV rate: ~8%
- Similar isolation pattern throughout the Philippines

Results: Circulation patterns

Endemic
Cyclic
Epidemic

- Very high degree of enterovirus diversity
- Evolutionary patterns: rare serotypes
- Isolation trend follows all tropical countries
- HEV B > HEV C > HEV A
- Multiple patterns observed: ENDEMIC, CYCLIC, EPOCHAL

Limitations
- Overall data may be underrepresented (only in API)
- Overrepresentation of neurotropic NPEV types
- Virus isolation (underdetected for some CVs vs favored serotypes)

- Although EV detection is only a side product of the PV surveillance
- Different patterns of circulation of different NPEV serotypes were detected
- Emergence of new genetic lineages of EV which might cause outbreaks
- Continued monitoring of NPEVs; genetic characterization is encouraged

More to go... 2009 – 2012!

Cell sensitivity
ITD RESULTS

100% in congruence with RRL result

<table>
<thead>
<tr>
<th>Technical</th>
<th>Virus type</th>
<th>Specimen</th>
<th>Genotype</th>
<th>Result</th>
<th>Genotype</th>
<th>Result</th>
<th>Result</th>
<th>Result</th>
<th>Result</th>
<th>Result</th>
<th>Result</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>2013-0820</td>
<td>3a (1)</td>
<td>P31</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
</tr>
<tr>
<td>2013-0821</td>
<td>3a (1)</td>
<td>P31</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
</tr>
<tr>
<td>2013-0821</td>
<td>3a (1)</td>
<td>P31</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
</tr>
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<td>3a (1)</td>
<td>P31</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
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<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
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<tr>
<td>2013-0821</td>
<td>3a (1)</td>
<td>P31</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
<td>EV71</td>
<td>P0</td>
</tr>
</tbody>
</table>

FEEDBACK ON LAB AND FIELD PERFORMANCE

- Issuance of Quarterly Polio and Measles Bulletins
- Weekly transmission to WHO and NEC
- Attendance to the quarterly Expert Panel Review meeting

Other enterovirus works (HFMD)

HFMD Surveillance, started in June 2012 (Routine Diagnostics)

- Mild and severe cases
  - Cases: 1100
  - Specimens: 1196

- Samples
  - Swabs (oropharyngeal, nasopharyngeal, vesicular)
  - Cerebrospinal fluid (CSF)
  - Stool

- Method of testing
  - Screening: pan-EV nested PCR
  - Confirmatory: EV71-specific RT-PCR


- Much has been known about its epidemiology of EV 71 in the Asia-Pacific Region, but little is known about its prevalence, diversity or spectrum of disease in the Philippines.
- There is no specific surveillance for EV71 being implemented in the Philippines.
- AFP surveillance has been implemented to detect poliovirus (PV) & other EVs.
- Since 1992, it has been detecting large number of EVs, including wild-type & vaccine-derived PVs as well as other known EVs and untypable NPEVs.
- Since 1997, 2 genetically distinct major lineages (B,C) of EV71 have circulated in Asia-Pacific Region. Group B predominates in Southeast Asia, whereas Group C have prevailed in northern Asia.


8 EV71 were isolated and detected

- This provides the first epidemiologic and virologic survey of EV71 in the Philippines.
- Similar to the situation in other countries, EV71 infection was associated with a subset of AFP cases.
- Ongoing epidemiologic surveillance will be necessary to determine whether this pattern of AFP and enterovirus activity occurs on a regular basis.
- Subgenogroup C2 was identified
  - C2 has a wide circulation both in the tropics and temperate countries.

Discussion/Conclusion
National Polio Laboratory in Korea
- The Acute Flaccid Paralysis Surveillance and Enterovirus Surveillance in Korea

Korea Centers for Disease control and Prevention
Division of Vaccine Research
Sang Gu Yeo

New campus in Osong, Korea.
- Bio complex
- KCDC(KNIH), KFDA, KOHI, KHIDI

Contents
1. National Polio Laboratory
2. Enterovirus Surveillance in Korea
3. Development EV71 vaccine in Korea
Serology of polio vaccine

Country background
- Estimated total population: 48,875,000
  - 21 million people are concentrated in Seoul(10m) and Kyeonggido(11m)
- Estimated population under 15 years of age: 7,559,063
- Polio vaccination included in National vaccination program since 1960's
- Last wild poliomyelitis case reported in 1983
- Introduced IPV only schedule in 2005
- Estimated Immunization rate: over 95%
- non-polio AFP rate: 0.97 in 2012
- Average Annual non-polio AFP rate: 0.34

Progress of polio eradication: Korea

2002 2003 2004 2005
Annual rate
- OPV, IPV
AFP surveillance in Korea

Flow chart of diagnosis of enteroviruses

Quality assurance measures: Cell sensitivity test

EV and EV71 real time PCR

AFP cases in Korea (2003-2012.11.)
### AFP cases in Korea (2003-2012.11.)

<table>
<thead>
<tr>
<th>Year</th>
<th>Expected AFP cases</th>
<th>Total AFP cases (15A yrs)</th>
<th>Non-polio AFP rate</th>
<th>AFP cases with adequate stool samples (n, %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>97</td>
<td>23</td>
<td>0.23</td>
<td>23 (100%)</td>
</tr>
<tr>
<td>2004</td>
<td>96</td>
<td>27</td>
<td>0.29</td>
<td>27 (100%)</td>
</tr>
<tr>
<td>2005</td>
<td>92</td>
<td>16</td>
<td>0.17</td>
<td>16 (100%)</td>
</tr>
<tr>
<td>2006</td>
<td>90</td>
<td>33</td>
<td>0.37</td>
<td>33 (100%)</td>
</tr>
<tr>
<td>2007</td>
<td>88</td>
<td>26</td>
<td>0.3</td>
<td>26 (100%)</td>
</tr>
<tr>
<td>2008</td>
<td>84</td>
<td>8</td>
<td>0.1</td>
<td>8 (100%)</td>
</tr>
<tr>
<td>2009</td>
<td>83</td>
<td>20</td>
<td>0.24</td>
<td>20 (100%)</td>
</tr>
<tr>
<td>2010</td>
<td>79</td>
<td>70</td>
<td>0.88</td>
<td>70 (98.6%)</td>
</tr>
<tr>
<td>2011</td>
<td>77</td>
<td>31</td>
<td>0.4</td>
<td>31 (90.6%)</td>
</tr>
<tr>
<td>2012</td>
<td>77</td>
<td>75</td>
<td>0.97</td>
<td>75 (98.6%)</td>
</tr>
</tbody>
</table>

### Summary of AFP cases

<table>
<thead>
<tr>
<th>Year</th>
<th>Total AFP cases</th>
<th>GBS*</th>
<th>Meningo</th>
<th>Transverse</th>
<th>ADEM*</th>
<th>Otherb</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004</td>
<td>37</td>
<td>13</td>
<td>12</td>
<td>8</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>2005</td>
<td>16</td>
<td>4</td>
<td>3</td>
<td>3</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>2006</td>
<td>33</td>
<td>10</td>
<td>4</td>
<td>7</td>
<td>4</td>
<td>8</td>
</tr>
<tr>
<td>2007</td>
<td>26</td>
<td>6</td>
<td>4</td>
<td>5</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td>2008</td>
<td>8</td>
<td>5</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>2009</td>
<td>20</td>
<td>10</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>2010</td>
<td>70</td>
<td>3</td>
<td>4</td>
<td>4</td>
<td>5</td>
<td>55</td>
</tr>
<tr>
<td>2011</td>
<td>31</td>
<td>4</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td>7</td>
</tr>
<tr>
<td>2012</td>
<td>75</td>
<td>12</td>
<td>9</td>
<td>5</td>
<td>6</td>
<td>33</td>
</tr>
</tbody>
</table>

* GBS = Guillain-Barré Syndrome, ADEM = acute disseminated encephalomyelitis
b Others include 1 idiopathic IICP, 1 myopathy, 2 multiple sclerosis, 3 HFMD with severe complication, 4 Menigitis, 5 Encephalitis

### Genotypes of AFP cases (2012)

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>No of cases</th>
<th>Symptoms</th>
</tr>
</thead>
<tbody>
<tr>
<td>CA4</td>
<td>1</td>
<td>acute cerebellar ataxia</td>
</tr>
<tr>
<td>CA6</td>
<td>3</td>
<td>ADEM, Meningoencephalitis</td>
</tr>
<tr>
<td>CB3</td>
<td>2</td>
<td>GBS, Meningoencephalitis</td>
</tr>
<tr>
<td>Echo7</td>
<td>2</td>
<td>encephalitis</td>
</tr>
<tr>
<td>Echo10</td>
<td>1</td>
<td>Others</td>
</tr>
<tr>
<td>EV71</td>
<td>16</td>
<td>GBS, HFMD, meningocerephalitis, transversemyelitis</td>
</tr>
</tbody>
</table>

* GBS = Guillain-Barré Syndrome, ADEM = acute disseminated encephalomyelitis
b Others include 1 idiopathic IICP, 1 myopathy, 2 multiple sclerosis, 3 HFMD with severe complication, 4 Menigitis, 5 Encephalitis

### WHO-Proiciency Test

**Contents of PT (New Algorithm)**

- **PT excellence**
  - 10 stool samples
  - to be tested on L20B and RD as is according to new algorithm
- **Real samples should mimic real life situations and may therefore contain**
  - no virus
  - polioviruses (as single virus or in poliovirus mixtures)
  - enteroviruses plus Simian viruses only growing on HEp-2
  - combinations of these viruses
- **Possible final results reported per sample:**
  - negative
  - L20B-positive
  - NPEV
  - L20B-positive + NPEV
- **Result:**
  - 3 negatives / 5 polioviruses(L20B+) / 2 NPEVs

### WHO-Proiciency Test (cont.)

**Poliovirus Isolation New Algorithm**

- **Input:** Stool samples
- **Output:** Identification of polioviruses and enteroviruses
- **Steps:**
  1. **Sample preparation:**
     - Homogenize stool
     - Centrifuge
  2. **Initial screening:**
     - L20B and RD cell culture
  3. **Poliovirus detection:**
     - L20B positive
     - NPEV
  4. **Combination assay:**
     - L20B positive + NPEV
  5. **Final confirmation:**
     - Additional testing if necessary

- **Results:**
  - Negative
  - L20B positive
  - NPEV
  - L20B positive + NPEV

### Regional distribution of AFP cases in Korea (As of Nov 2012)

- Seoan (12)
- Kyeong (29)
- Jeonbuk (1)
- Busan (11)
- Daejeon (1)
- Incheon (11)
- Ulsan (1)
- Daegu (1)
- Chungbuk (3)
- Chungnam (3)
- Gumi (1)
- JeJu (1)
Enterovirus Surveillance in Korea

**Enterovirus Laboratory Surveillance Networks**

- Enterovirus Laboratory Surveillance Networks (ELSN) in Korea were composed of NPL, 38 surveillance hospitals, 7 public health regional institutes since 2006.
- Nearly 2,000 enterovirus cases are reported annually by ELSN.
- Most cases were recruited from Seoul, Busan, Kyeonggi, and Gwangju regions.

### Enterovirus Detection in Korea (1999-2012.11)

![Graph showing enterovirus detection from 1999 to 2012.11](image)

- All polioviruses detected were Sabin-strain
- Major outbreak of EV71 infection occurred and two patients and one patient had deceased with EV71 infection in 2009 and 2012, respectively.

### Outbreak of Neurologically Complicated EV71 Infection in Korea (2010-2012.11)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (months), mean±SD</td>
<td>37±4.5</td>
</tr>
<tr>
<td>Gender, male</td>
<td>113 (57.07%)</td>
</tr>
<tr>
<td>Clinical complicated presentation</td>
<td></td>
</tr>
<tr>
<td>Encephalitis</td>
<td>33 (16.4%)</td>
</tr>
<tr>
<td>Meningitis</td>
<td>126 (62.7%)</td>
</tr>
<tr>
<td>Others</td>
<td>36 (17.9%)</td>
</tr>
<tr>
<td>Death</td>
<td>6 (3.0%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Presenting symptoms</th>
<th>No. (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever &gt;37°C</td>
<td>83 (45.9%)</td>
</tr>
<tr>
<td>Headache</td>
<td>80 (41.1%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>74 (37.9%)</td>
</tr>
<tr>
<td>Neck stiffness*</td>
<td>93 (47.1%)</td>
</tr>
<tr>
<td>Seizure</td>
<td>11 (5.6%)</td>
</tr>
<tr>
<td>Decreased mentality</td>
<td>40 (20.6%)</td>
</tr>
</tbody>
</table>

[Korea Centers for Disease Control and Prevention logo]

![Graph showing enterovirus laboratory surveillance from 2010 to 2012.11](image)
Genotypes of Enteroviruses from Hand Foot and Mouth Disease Patients with Neurological Complication (2010-2012.11)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Number of Cases (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EV71</td>
<td>121 (78.1%)</td>
</tr>
<tr>
<td>CA6</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>CA10</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>CA16</td>
<td>8 (5.2%)</td>
</tr>
<tr>
<td>CB2</td>
<td>3 (1.9%)</td>
</tr>
<tr>
<td>CB3</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>CB4</td>
<td>4 (2.6%)</td>
</tr>
<tr>
<td>CB5</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>Echo6</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>Echo7</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>Echo9</td>
<td>2 (1.3%)</td>
</tr>
<tr>
<td>Echo18</td>
<td>1 (0.6%)</td>
</tr>
<tr>
<td>other Evs</td>
<td>10 (6.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>155</td>
</tr>
</tbody>
</table>

Outbreak of Neurologically Complicated EV71 Infection In Korea (2010-2012.11)

<table>
<thead>
<tr>
<th>No. examined samples</th>
<th>No. positive samples</th>
<th>Detection Rate (%)</th>
<th>Genotypes (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CSF</td>
<td>93</td>
<td>10</td>
<td>10.8% EV71(6), EV71(UT)(1), Echo6(1), Echo9(1), CB3(1)</td>
</tr>
<tr>
<td>Stool</td>
<td>174</td>
<td>148</td>
<td>85.1% EV71(93), EV71(UT)(23), EV71(UT)(9), Echo7(1), Echo9(1), Echo18(1), CA6(1), CA10(1), CA16(8), CB2(3), CB3(1), CB4(4), CB5(1)</td>
</tr>
<tr>
<td>Throat swabs</td>
<td>26</td>
<td>9</td>
<td>34.6% EV71(7), EV71(UT)(3), CA6(1)</td>
</tr>
<tr>
<td>Others</td>
<td>11</td>
<td>3</td>
<td>27.3% EV71(2), EV71(UT)(2)</td>
</tr>
<tr>
<td>Total</td>
<td>304</td>
<td>170</td>
<td>55.9%</td>
</tr>
</tbody>
</table>

Development of EV71 vaccines and Serology of polio vaccine
**Ev71 vaccine candidates**

**Recent EV71 outbreaks**

- 2009: China ~1,000,000 cases with 257 deaths by Jul-09
- 2008: Singapore >29,600 cases
- 2007: Singapore >15,000 cases with school closures
- 2006: Malaysia Schools & Playgrounds closed for 2 weeks
- 2005: Taiwan 30 deaths
- 2004: Malaysia 4 deaths
- 2003: Australia 44 severe cases
- 2002: Taiwan 78 deaths
- 2001: Taiwan 58 deaths
- 2000: Malaysia 34 deaths
- 1999: Australia 29 severe cases
- 1998: Taiwan 7 deaths
- 1997: Malaysia 34 deaths
- 1996: Taiwan 29 deaths

**Humoral Immunity: Cross NT (Neutralizing Test)**

Effectiveness of vaccination against poliovirus

- Aged 6-90 years
- Collection date: 2012.04.23 - 2012.07.02
- Total 720 cases - P1, P2, P3

**Cellular Immunity: ICS by FACS assay**

- MOCK
- Ev71

**Banking vero cell line for development of EV71 vaccine**

- Less 1. Standard: Vero cell line (Vero, Monza)
- Less 2. Control: Vero cell line (Vero, Human)
- Less 3. Vero: Vero Cell (Vero, E8)

**Ev71 VLP vaccine candidates**

We think:
- about 200,000-400,000 patients/year
- 50% of EV71 patients
- severe cases about 0.1-0.5% (100-1000)
- <2% of death (2-30/ year)

**EMR based Incidence**

- CD4/CD8 population
- IFN-g+

**Korean Center for Disease Control and Prevention**
Effectiveness of vaccination against poliovirus (cont.)

Results of effectiveness of vaccination against poliovirus
~ 2012 Korea – 720 cases

Current status of laboratory data
Reporting to WPRO and Sharing Data with National EPI or Surveillance

-We have web based enterovirus reporting system including AFP reporting system and report the data through E-mail to WPRO
-Through the AFP enhancement research project, we collect the AFP samples and Share the data.

Biosafety practice in the polio Laboratory and institution

-We inform and train monthly to the NPL members about Biosafety in the Lab and we have biosafety manuals and biosafety officer.
-We have under the investigation by institutional Biosafety team regularly and we learned a lot of biosafety practice through education.
Challenges or problems, request
- Reinforcement of EV71 surveillance.
- Development of EV71 vaccine.
- Wild poliovirus sero prevalence investigation.

Thank you for attention!
Surveillance & diagnosis activities of the national polio reference laboratory in northern Vietnam

RITM, Alabang, Muntinlupa, Philippines
3 – 7 December 2012.

Nguyen Thi Thu Trang (MSc.)
Laboratory of enteroviruses
National Institute of Hygiene & Epidemiology, Hanoi, Vietnam

Laboratory introduction

- Staff: 6 persons (1 Asso. Prof.; 1 Dr.; 1 MSc.; 3 BSc.)
- Workload:
  - AFP surveillance
  - HFMD surveillance
  - Rotavirus surveillance (from hospitalized paediatric acute diarrhea)
- Lab space: 3 experiment rooms and 2 office room
- Member of WHO GPLN
- Work closely with NEPI to build a good surveillance system for polio and rotavirus.

AFP surveillance 2010 – 2012

<table>
<thead>
<tr>
<th>YEAR</th>
<th>AFP cases</th>
<th>No. of Specimens tested</th>
<th>Laboratory result</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polio</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2010</td>
<td>191</td>
<td>382</td>
<td>2 (P2)</td>
</tr>
<tr>
<td>2011</td>
<td>194</td>
<td>384</td>
<td>1 (P3)</td>
</tr>
<tr>
<td>10/11/2012</td>
<td>194</td>
<td>371</td>
<td>2 (P1)</td>
</tr>
</tbody>
</table>

Polio vaccination updates (2010-2012)

<table>
<thead>
<tr>
<th>YEAR</th>
<th>NORTHERN VIETNAM</th>
<th>THE WHOLE VIETNAM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Children &lt;1yr</td>
<td>Children Immunized</td>
</tr>
<tr>
<td>2010</td>
<td>712021</td>
<td>711499</td>
</tr>
<tr>
<td>2011</td>
<td>724007</td>
<td>731314</td>
</tr>
<tr>
<td>10/2012</td>
<td>757069</td>
<td>625689</td>
</tr>
</tbody>
</table>

Virus isolation using the new algorithm

<table>
<thead>
<tr>
<th>YEAR</th>
<th>% results reported within 14 days</th>
<th>Pending specimens (*)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 14 days/ PVs</td>
<td>≥ 15 days/ EVs</td>
</tr>
<tr>
<td>2010</td>
<td>100%</td>
<td>0 0</td>
</tr>
<tr>
<td>2011</td>
<td>100%</td>
<td>0 0</td>
</tr>
<tr>
<td>10/11/2012</td>
<td>96.23%</td>
<td>16 0</td>
</tr>
</tbody>
</table>

(*) Pending specimens = No. of specimens from AFPs – No. of specimens tested.

CELL SENSITIVITY TESTING (2011 – 2012)
STANDARD OF VIRUS TITRE IN L20B CELL LINE

<table>
<thead>
<tr>
<th>Poliovirus Type</th>
<th>Polio 1</th>
<th>Polio 2</th>
<th>Polio 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected LQC titer (Mean)</td>
<td>7.38</td>
<td>7</td>
<td>7.63</td>
</tr>
</tbody>
</table>

Acceptable range for LQC titer
6.82 - 8.62 | 7.4 - 8.4 | 7.33 - 8.33

STANDARD OF VIRUS TITRE IN RD CELL LINE

<table>
<thead>
<tr>
<th>Poliovirus Type</th>
<th>Polio 1</th>
<th>Polio 2</th>
<th>Polio 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Expected LQC titer (Mean)</td>
<td>8</td>
<td>7.9</td>
<td>7.83</td>
</tr>
</tbody>
</table>

Acceptable range for LQC titer
6.88 - 8.88 | 6.5 - 7.5 | 6.6 - 7.6

CHART 1: RD Cell sensitivity to Polio 1, 2011-2012

CHART 2: RD Cell sensitivity to Polio 2, 2011-2012


CHART 4: L20B Cell sensitivity to Polio 1, 2011-2012

CHART 5: L20B Cell sensitivity to Polio 2, 2011-2012
Hand-foot-and mouth disease surveillance

- Clinical sample type: throat swab; vesicle fluid, and faeces.
- Diagnostic method: snRT-PCR/ sequencing; virus isolation (RD, Vero)
- Investigation of etiology during outbreak of HFMD
- Regular and sentinel surveillance of HFMD in north Vietnam

Diagnosis of HFMD cases

<table>
<thead>
<tr>
<th>YEAR</th>
<th>No. of samples collected</th>
<th>No. of EV71 detected</th>
<th>No. of CA16 detected</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td>1053</td>
<td>262</td>
<td>30</td>
</tr>
<tr>
<td>30/10/2012</td>
<td>716</td>
<td>223 (*)</td>
<td>18</td>
</tr>
</tbody>
</table>

(*) 49/223 were sequenced C4; 5/223 was sequenced C5. Isolation in RD only for these positive samples seemed less sensitive: 49C4 [18 (+), 6 pending, 25 (-)]; 5C5 [1 (+), 5 (-)].

Thank you for your attention!
COUNTRY REPORT

Laboratory of Enteroviruses
Pasteur Institute Ho Chi Minh City
Vietnam

Alabang, 3-7 December 2012
Presented by Nguyen Trung Kien

The Laboratory of Enteroviruses, Pasteur Institute in Ho Chi Minh City, Vietnam, is a member of the WHO Laboratory Network for the Poliomyelitis Eradication Programme in the Western Pacific Region since 1992.

We are responsible for surveillance Enteroviruses in the Southern half of Vietnam.

Year | No. of AFP cases with specimen | No. of poliovirus | No. of EV71 | No. of other enterovirus
--- | --- | --- | --- | ---
2010 | 109 | 2 PS3 | 0 | 12 (a)
2011 | 189 | 1PS1, 1PS2 | 6 | 15 (b)
2012(*) | 263 | 1 PS1+2, 3 PS3, 2 2-VDPV | 22 | 8 (c)

(*): 1/1/2012-16/11/2012

a. 1 ECHO14, 1 CA10, 1 ECHO30, 1 EV75, 1 EV84, 2 ECHO 29, 1 ECHO6, 1 CoxB4, 1 CoxB5, 1 EV81, 1 Untyped
b. 4 ECHO14, 3 EV75, 1 CoxB3, 1 ECHO9, 1 CoxB5, 1 ECHO30, 1 CoxB1, 1 ECHO25, 1 CoxA4, 1 Untyped
c. 1 CA10, 2 ECHO14, 1 ECHO3, 4 Untyped

Summary of results Acute flaccid paralysis surveillance

Summary of results VDPV cases in 2012

With two cases are positive on L20B cells, we sent the samples to NIID on April and May. Test results of NIID that the two cases are 2-VDPV.

We conducted sampling local monitoring where patients live, and the test results showed that all samples (29 cases) are negative.

Cell lines (RD-A and L20B)

- RD-A (229) and L20B (N=20) received by VDRL Australia on 17 August 2006
- Cell bank was established by following the manual WHO-POLIO.
- 35 vials of RD-A (229-3) and 27 vials of L20B (N=23) were stored in liquid nitrogen.
- Each vial of cell to use cultures with expect their use of 15 passages.
- Authenticated Sabin reference strains provided by NIBSC on 15 March 2007.
- NIBSC reference standard and Laboratory quality control standard titration results against 3 serotypes were determined (table).
- Now, there are 01 vials of RD-A (229-3) and 01 vials of L20B (N+23) were stored in liquid nitrogen.
- We got two lines new cell is RD-A 226-1 and L20B 18-1 on the day 23/2/2012.
- Now, we have create cell bank were stored in liquid nitrogen and already starting use it.

Summary of results other enterovirus surveillance

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of specimens by RT-PCR</th>
<th>No. of specimens by Cell culture</th>
</tr>
</thead>
<tbody>
<tr>
<td>2010</td>
<td>852</td>
<td>85</td>
</tr>
<tr>
<td>2011</td>
<td>2442</td>
<td>1451</td>
</tr>
<tr>
<td>2012</td>
<td>(1/1-16/11/12)</td>
<td>1022</td>
</tr>
</tbody>
</table>

(*) Specimens: Vesicle fluid, throat swab, stool, CSF

(**) Specimens from children with HFMD or central nervous system diseases
Cell Line | L20B | RD-A
---|---|---
Poliovirus Type | Polio 1 | Polio 1 |
Polio 2 | Polio 2 | Polio 2 |
Polio 3 | Polio 3 | Polio 3 |

### NIBSC reference titer (Average)
- Polio 1: 5.23
- Polio 2: 4.96
- Polio 3: 5.03
- Polio 1: 5.2
- Polio 2: 5.5
- Polio 3: 7.8

### Expected LQC titer (Average)
- Polio 1: 6.95
- Polio 2: 6.68
- Polio 3: 6.68
- Polio 1: 8.13
- Polio 2: 7.8
- Polio 3: 7.66

### Acceptable range for LQC titer
- Polio 1: 6.45 - 7.45
- Polio 2: 6.18 - 7.18
- Polio 3: 6.18 - 7.18
- Polio 1: 7.63 – 8.63
- Polio 2: 7.3 - 8.3
- Polio 3: 7.1 - 8.16

**Cells are evaluated for sensitivity to all three poliovirus serotypes midway through their use of 15 passages.**

- The results were reported to Regional Laboratory Coordinator within 48 hours.
- Results were in the expected titre.
RD-A cell sensitive with Polio 3