Annual report of the Australian National Enterovirus Reference Laboratory 2010-2011

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Abstract

Australia conducts clinical surveillance for cases of polio-like illness in children in accordance with the World Health Organization (WHO) recommended surveillance criteria for acute flaccid paralysis (AFP). AFP cases are ascertained either by clinicians notifying the Australian Paediatric Surveillance Unit or designated nurses enrolling cases as part of the Paediatric Active Enhanced Disease Surveillance system at four sentinel tertiary paediatric hospitals. The National Enterovirus Reference Laboratory (NERL), formerly the National Poliovirus Reference Laboratory, is accredited by the World Health Organization (WHO) for the testing of faecal specimens from cases of AFP and operates as a Poliovirus Regional Reference Laboratory for the Western Pacific Region. In 2010 and 2011, for the 3rd and 4th consecutive years, Australia met the WHO AFP surveillance performance indicator. This is indicative of a sensitive surveillance system capable of detecting an imported case of polio in children. However, the faecal collection rate for the virological investigation of AFP cases was below the WHO surveillance performance indicator in both years and represented a gap in Australia's polio surveillance. Enterovirus and environmental surveillance were established in Australia as virological surveillance to complement the clinical surveillance schemes. No poliovirus was detected by the clinical or virological surveillance schemes in 2010 or 2011 and Australia maintained its polio-free status. India was declared polio-free in January 2012, a significant step towards global polio eradication, leaving Afghanistan, Nigeria and Pakistan as the remaining countries endemic for wild poliovirus.

Keywords: poliovirus, acute flaccid paralysis, surveillance, enterovirus, poliomyelitis, eradication, vaccination

Introduction

The National Enterovirus Reference Laboratory (NERL), formerly the National Polio Reference Laboratory, is responsible for the virological testing of faecal specimens from cases with a clinical suspicion of poliomyelitis. This includes cases of acute flaccid paralysis (AFP), a major clinical presentation of poliomyelitis, in children less than 15 years of age and cases of suspected poliomyelitis in patients of any age. The World Health Organization (WHO) recommends that two faecal specimens be collected for virological investigation at least 24 hours apart and within 14 days of the onset of paralysis from cases of AFP to exclude poliovirus as the causative agent. It is a requirement of the WHO polio eradication program that the specimens are tested in a WHO accredited laboratory, which for Australia is the NERL at the Victorian Infectious Diseases Reference Laboratory (VIDRL).

Enterovirus and environmental surveillance programs were established to provide virological surveillance for poliovirus to complement the clinical surveillance program focussed on AFP cases in children. Enteroviruses other than poliovirus have been associated with AFP and poliovirus infection may manifest clinically without paralysis. The Enterovirus Reference Laboratory Network of Australia (ERLNA) was established in 2009, bringing together public diagnostic virology laboratories. ERLNA aims to identify the types of enteroviruses detected in clinical specimens to exclude poliovirus and establish the epidemiology of non-polio enteroviruses in Australia. WHO supports environmental surveillance as another aspect of polio surveillance through the testing of sewage samples. Clinical and virological surveillance schemes for poliovirus serve to monitor Australia's polio-free status.

From November 2005, inactivated poliomyelitis vaccine (IPV) replaced oral poliomyelitis vaccine (OPV) in the National Immunisation Program.[1] IPV is administered to children at 2, 4 and 6 months of age, with a booster dose at 4 years of age. With the removal of OPV (containing live attenuated virus) from the immunisation schedule, any poliovirus identified by Australian virology laboratories requires further investigation to determine its origin, as it potentially represents an importation event.

It is important that Australia maintains high levels of polio vaccine coverage to avoid a resurgence of poliomyelitis in the event of a wild poliovirus importation. In 2010, China reported an outbreak of polio due to wild type virus in Xinjiang province, which borders Pakistan and from where the importation originated. A total of 21 cases of polio were reported ranging in age from 6 months to 42 years before the outbreak was controlled through mass immunization programs.[2] The WHO provides a weekly update of the global polio eradication situation including a list of countries reporting cases due to wild poliovirus (http://www.polioeradication.org/Dataandmonitoring/Poliothisweek.aspx).

The Australian Immunisation Handbook recommends that individuals who are at continuing risk of infection, such as health care workers should have a polio vaccine booster every 10 years.[1]

The last wild poliovirus isolated in India was in January 2011. The country was declared polio-free 12 months later reducing the number of countries that have never interrupted wild poliovirus transmission to three; Afghanistan, Nigeria and Pakistan.[2] It was only in 2009 that India reported 741 cases of polio due to wild type poliovirus which accounted for 46% of the cases worldwide. India's polio-free status is a significant public health achievement that supports the feasibility of the global polio eradication strategy.

This report summarises the polio surveillance program in Australia for 2010 and 2011, encompassing AFP surveillance in children and virological surveillance.

Methods

AFP Surveillance

AFP surveillance was initiated by the Australian Government in 1995 in collaboration with the Australian Paediatric Surveillance Unit (APSU) as part of Australia's commitment to the WHO poliomyelitis eradication program. Since 2000, AFP surveillance has been co-ordinated by VIDRL in collaboration with the APSU. In late 2007, the Paediatric Active Enhanced Disease Surveillance (PAEDS) surveillance scheme was established as a collaboration between the APSU and the National Centre of Immunisation Research and Surveillance. PAEDS is a hospital based surveillance system for paediatric conditions of public health interest, including AFP, at four tertiary paediatric hospitals in Adelaide, Melbourne, Perth and Sydney.[3] In April 2011 the Polo Expert Committee which is responsible for reviewing AFP cases to determine if they are compatible with polio was renamed the Polio Expert Panel (PEP) by the Communicable Disease Network Australia (CDNA).

The strategy adopted for AFP surveillance is as follows:

- Paediatricians reviewing a patient less than 15 years of age who presents with AFP, or clinicians reviewing a patient of any age suspected of poliomyelitis, are requested to notify the NERL (telephone 03 9342 2607, email polio@mh.org.au). Notification of the AFP case is also included on the paediatrician's monthly report card to the APSU (http://www.apsu.org.au/). Upon receipt of the notification the AFP National Surveillance Co-ordinator, based at VIDRL, forwards a clinical questionnaire for the clinician to complete.
- Alternatively, AFP cases are ascertained by PAEDS nursing staff from medical records and with parental agreement are enrolled in the surveillance system.
- Two faecal specimens are collected 24 to 48 hours apart and within 14 days of onset of paralysis. The collection of specimens within these time frames enables them to be classified as adequate by WHO.
- The faecal specimens are tested free of charge by the NERL, which is accredited by WHO for this purpose.
- The PEP which is convened by the Department of Health (DoH), reviews the clinical and laboratory data for all notified cases of AFP, irrespective of whether they are an eligible or ineligible case. An eligible case is: an Australian child under 15 years of age with AFP (including Guillain-Barré syndrome) or an Australian of any age with paralytic illness if polio is suspected. Examples of ineligible cases are where the patient is aged 15 years or older, an overseas resident and cases notified in error or later determined to be non-AFP. The PEP classifies cases of AFP as:
    - Poliomyelitis due to wild poliovirus, vaccine-derived poliovirus (VDPV) or vaccine associated paralytic poliomyelitis (VAPP)
    - Non-polio AFP or
    - Non-AFP.

A follow-up questionnaire is sent to notifying clinicians if the PEP requires more information regarding the AFP case before a final classification can be made.

After each PEP meeting the Australian AFP data is forwarded to WHO for inclusion in the global AFP surveillance data published in the Weekly Epidemiological Record (available at http://www.who.int/wer/en/). Ineligible cases are not reported to WHO.

The WHO AFP surveillance performance indicator for a polio non-endemic country is 1 case of non-polio AFP per 100,000 children aged less than 15 years each year. For Australia in 2009 this equated to 41 cases, based on the Australian Bureau of Statistics (ABS) population data released in December 2008. An AFP surveillance scheme that satisfies the surveillance performance indicator is deemed sufficiently sensitive to detect a wild poliovirus importation in children of that country.

The WHO surveillance performance indicator for laboratory testing is that at least 80% of notified AFP cases have adequate faecal specimens collected and tested in a WHO accredited laboratory.

At the end of each calendar year, a number of AFP notifications remain unclassified if insufficient clinical and laboratory data were available to enable the PEP to review the cases. The PEP classifies the remaining AFP notifications as "polio compatible-zero evidence" if a final review reveals no evidence of clustering amongst the unclassified cases.

Virus Culture

Upon receipt at the NERL, faecal specimens are treated with Minimum Essential Medium containing Hank's salts, chloroform (9.1% v/v) and foetal bovine serum (2%). The suspension is clarified and the supernatant inoculated onto a series of mammalian cell lines. Two WHO recommended cell lines are used for the isolation of poliovirus; L20B (a transgenic mouse epithelial cell line expressing the human poliovirus receptor, CD155) and RD-A (human rhabdomyosarcoma).[4,5] Up to September 2011 the NERL utilised two additional cell lines for the isolation of poliovirus and non-polio enteroviruses: Buffalo Green Monkey Kidney (BGMK) and human embryonic lung (HEL).

Diagnostic laboratories in Australia are encouraged to refer enteroviruses of unknown serotype to the NERL for further characterisation as poliovirus infection can lead to clinical presentations without paralysis such as aseptic meningitis.

A series of tests known as intratypic differentiation (ITD) are performed on poliovirus isolates to determine whether the virus is a wild poliovirus strain, OPV strain (Sabin-like) or a VDPV. In 2009 WHO introduced diagnostic poliovirus real time reverse transcriptase PCR (rRT-PCR) developed by the Centers for Disease Control and Prevention USA, as the primary ITD method.[6] The Australian NERL sequences the complete poliovirus VP1 genomic region, which contains a major neutralizing antibody binding site. The VP1 genomic sequence provides valuable biological information, including the number of mutations within a significant region of the OPV virus strain and enables the phylogenetic analysis of wild poliovirus to rapidly determine the likely source of the virus, as utilised in the 2007 importation.[7,8]

Enterovirus Surveillance

The ERLNA was established primarily as a means of detecting imported poliovirus amongst untyped enteroviruses from clinical specimens. The network consists of 10 public sector diagnostic virology laboratories:

Australian Capital Territory

Canberra Hospital: Prof. Peter Collignon, Dr Karina Kennedy, Ms Jennifer Ridgway

New South Wales

Infectious Diseases and Immunology, the University of Sydney: Prof. Peter McMinn

Queensland

Queensland Health and Scientific Services: Dr Russell Simmons, Dr Bruce Harrower, Dr David Warrilow

South Australia

Microbiology and Infectious Diseases, Flinders Medical Centre: Prof. David Gordon

SA Pathology, Institute of Medical and Veterinary Science: Dr Tuck Weng, Ms Kok, Ms Lyn Payne

Victoria

Department of Microbiology, Royal Children's Hospital: Dr Andrew Daley, Ms Poppy Adamopoulos

National Enterovirus Reference Laboratory, VIDRL: Dr Bruce Thorley, Mr Jason Roberts

Viral Identification Laboratory, VIDRL: Dr Chris Birch, Ms Gina Papadakis

Western Australia

Department of Clinical Microbiology, Sir Charles Gairdner Hospital: Dr Avram Levy, Dr Simon Williams, Dr David Williams, Dr David Speers

Department of Microbiology, Princess Margaret Hospital for Children: Dr Leanne Sammels, Ms Katie Lindsay, Prof. Tony Keil

The NERL encourages members of the ERLNA to perform their own virus typing. It has advised members of the ERLNA on enterovirus detection, supplied laboratory and computer analysis protocols and performed tests in parallel with other laboratories for quality assurance purposes. The NERL receives untyped enteroviruses from three laboratories for typing on a regular basis. The other laboratories perform their own enterovirus typing and report the results to the NERL for inclusion in the National Enterovirus Database.

The NERL screens clinical specimens for enterovirus using a semi-nested RT-PCR directed to highly conserved sequence in the five non-translated region (NTR).[9] Enterovirus typing is

primarily performed by amplifying a fragment of the VP1 genomic region according to a published method,[10] but the complete nucleotide sequence of VP1 is required to type some enteroviruses. The enterovirus typing RT-PCR is directed to a region of sequence divergence that allows differentiation between enterovirus genomes. As a consequence, the enterovirus sequence based typing assay is not as sensitive as the pan-enterovirus detection assay. This can result in an enterovirus being detected by pan-enterovirus RT-PCR in a clinical specimen without subsequent identification by the VP1 enterovirus typing assay.

Environmental surveillance

The laboratory cell culture protocol implemented by the NERL is based on a two-phase separation procedure published by WHO. Further advice was obtained from the Enterovirus Laboratory at the National Public Health Institute, Finland, a Global Specialised Laboratory in the WHO Polio Laboratory Network.[11] In brief, 800 mL of sewage is collected prior to any biological or chemical treatment and referred to the NERL within 24 hours. At the laboratory, 500 mL of the sample is centrifuged and the supernatant vigorously shaken at 4°C with dextran, polyethylene glycol and sodium chloride. The mixture is incubated overnight at 4°C in a separating funnel and the lower organic phase is collected the next day and used to re-suspend any pellet stored after the initial centrifugation. The final solution is clarified as for a faecal specimen and inoculated onto the L20B and RD-A cell lines and observed microscopically for cytopathic effect.

Results

Classification of AFP cases

A total of 57 notifications of AFP were received in 2010 (Table 1) and 78 notifications in 2011 (Table 2). The PEP classified 41 cases as non-polio AFP involving children less than 15 years of age with onset of paralysis in 2010, and 60 cases in 2011 (Tables 1 and 2). This equates to a non-polio AFP rate of 1.0 case per 100,000 children less than 15 years of age in 2010 and a rate of 1.4 per 100,000 in 2011. Thus, the WHO AFP surveillance performance criterion for a polio-free country of one case of non-polio AFP per 100,000 children less than 15 years of age was met in both years (Table 3).

In 2009, the PEP resolved to follow a WHO recommendation and report AFP notifications that could not be classified due to a lack of clinical information as "polio compatible – zero evidence". In 2010-11, a total of three AFP notifications, one each from New South Wales, Queensland and Victoria were reported to WHO as polio compatible-zero evidence (Tables 1 and 2).

Four AFP cases were notified by more than one clinician in 2010 and were regarded as duplicate notifications while nine cases were duplicated in 2011 (Tables 1 and 2). Ten AFP notifications in 2010 and eight cases in 2011, did not meet the criteria for an eligible case, either involving patients greater than 14 years of age or the cases were later reported as non-AFP. The cases involving patients greater than 14 years of age were all classified by the PEP as non-polio AFP but were not reported to the WHO as the global polio surveillance program focuses on AFP in children less than 15 years of age as an age group at high risk of poliovirus infection.

Notification of AFP cases by state and territory

In 2010 and 2011, AFP cases were reported from all jurisdictions in Australia except the Australian Capital Territory (Tables 1 and 2). After excluding duplicate notifications and ineligible cases, the non-polio AFP rates per jurisdiction exceeded the WHO AFP surveillance performance indicator of 1.0 case per 100,000 children in New South Wales, South Australia and Victoria in both years. Western Australia did not reach the surveillance indicator in 2010, with a non-polio AFP rate of 0.5 per 100,000, but it was well exceeded in Western Australia in 2011, with a rate of 2.0 per 100,000. The increase in AFP cases notified in Western Australia in 2011 may indicate a surveillance failure in 2010, and/or the year-to-year variation in incidence of a rare childhood condition in a relatively small population. This is further demonstrated in Tasmania, which did not notify any AFP cases in 2010 but reported a non-polio AFP rate of 2.0 per 100,000 in 2011 (Tables 1 and 2).

Faecal collection from AFP cases

WHO defines adequate specimens for poliovirus culture as being two faecal specimens collected at least 24 hours apart and within 14 days of the onset of paralysis. A further surveillance criterion set by WHO is for adequate faecal collection from 80% of the eligible AFP cases.

In 2010, a total of 54 faecal specimens from 29 of the 41 eligible cases were tested at the NERL. Twelve (29%) of the eligible cases had adequate specimens collected while another 12 (29%) cases had only one specimen collected within 14 days of onset.

In 2011, a total of 69 faecal specimens were received from 36 of the 60 eligible cases. Nineteen (32%) of the non-polio AFP cases had adequate specimens collected, and a further 13 (22%) cases had one specimen collected within 14 days of onset.

Table 1: Notification of AFP cases 2010, by state or territory

State/ Territory	Estimated population aged <15 years*	Expected number of AFP cases in 2010†	Total number of notifications	Ineligible notifications	Duplicate notifications	Polio compatible-zero evidence	Eligible cases with final classification by PEP	Non-polio AFP rate per 100,000 children
ACT	64,981	1	0	0	0	0	0	0
NSW	1,343,184	13	23	3	4	1	15	1.2
NT	52,857	1	0	0	0	0	0	0
QLD	886,584	8	6	0	0	0	6	0.8
SA	291,569	3	4	0	0	0	4	1.3
TAS	97,579	1	0	0	0	0	0	0
VIC	1,008,841	10	20	5	0	1	14	1.4
WA	438,532	4	4	2	0	0	2	0.5
Australia	4,184,127	41	57	10	4	2	41	1.0

* Australian Bureau of Statistics, estimated population at 30 June 2009. Available at www.abs.gov.au.

† Based on a non-polio AFP rate of 1 case per 100,000 children less than 15 years of age

PEP Polio Expert Panel

AFP Acute flaccid paralysis

Table 2: Notification of AFP cases 2011, by state or territory

State/ Territory	Estimated population aged <15 years*	Expected number of AFP cases in 2011†	Total number of notifications	Ineligible notifications	Duplicate notifications	Polio compatible-zero evidence	Cases classified by the PEP as non- polio AFP	Non-polio AFP rate per 100,000 children
ACT	66,077	1	0	0	0	0	0	0
NSW	1,355,128	13	24	2	2	0	20	1.5
NT	53,079	1	2	1	0	0	1	1
QLD	901,689	9	5	0	0	1	4	0.4
SA	293,041	3	4	0	0	0	4	1.3
TAS	97,626	1	2	0	0	0	2	2
VIC	1,017,432	10	30	4	5	0	21	2.1
WA	446,058	4	11	1	2	0	8	2
Australia	4,230,130	42	78	8	9	1	60	1.4

* Australian Bureau of Statistics, estimated population at 30 June 2010. Available at www.abs.gov.au.

† Based on a non-polio AFP rate of 1 case per 100,000 children less than 15 years of age

PEP Polio Expert Panel

AFP Acute flaccid paralysis

Australia has never achieved the WHO criterion of collection of adequate specimens from 80% of AFP cases nationally (Figure 2). At the jurisdictional level, Western Australia was the only state to reach the WHO target, with adequate specimens collected from seven of the eight cases (88%) classified in 2011.

Laboratory testing of specimens

AFP cases

In 2010, a total of 54 faecal specimens were referred from 31 cases of AFP involving patients aged less than 15 years, while in 2011, 69 faecal specimens were referred (Table 4). The specimens included faeces, faecal extracts, swabs and cell culture isolates. No poliovirus was isolated from any of these specimens.

Non-polio enteroviruses (NPEV) were reported from three AFP cases in 2010. In the first case, echovirus 19 was isolated from an unimmunised three year old with a diagnosis of anterior horn cell disease confirmed by a MRI consistent with myelitis.[12] The patient had an upper respiratory tract infection three weeks prior to presentation. There was no history of recent travel or contact with an overseas visitor. Initial laboratory investigation was hampered by the CSF being inhibitory by RT-PCR. The local hospital isolated enterovirus by virus culture from a nasopharyngeal aspirate and faeces, which was confirmed by RT-PCR. The original specimens and virus isolates were referred to the NERL and echovirus 19 was identified from the virus isolates by sequencing a fragment of the capsid encoding region of the virus genome.

Echovirus 3 was identified from the first of two faecal specimens referred from the second AFP case involving a 12 year old patient diagnosed with meningitis. The specimen was collected nine days after the onset of symptoms while the second specimen was collected 13 days after onset.

In the third case, enterovirus 68 (EV68) was identified from a single faecal specimen received by the NERL from a patient with spinal cord ischaemia. The virus was detected by RT-PCR from the faecal extract while virus culture was negative.

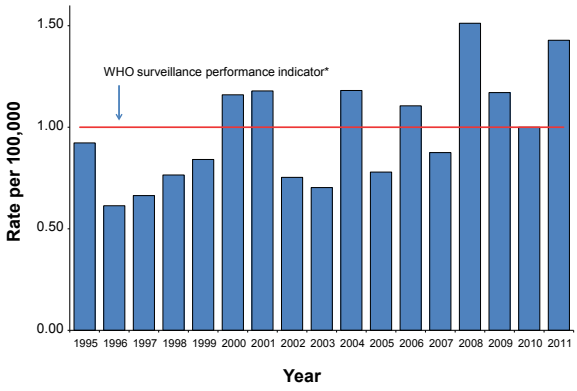
In 2011, a NPEV was detected in two faecal specimens from one AFP case. An atypical enterovirus cytopathic effect was observed in virus culture and was later confirmed by pan-enterovirus RT-PCR. The virus was typed from the stool extract by sequencing a fragment of the VP1 genomic region and identified as coxsackievirus type A24.

In 2010 and 2011, a total of 13 specimens were received from eight cases involving patients greater than 14 years of age, which is outside of the WHO AFP surveillance criterion. No enterovirus was isolated from the specimens.

Enterovirus Surveillance

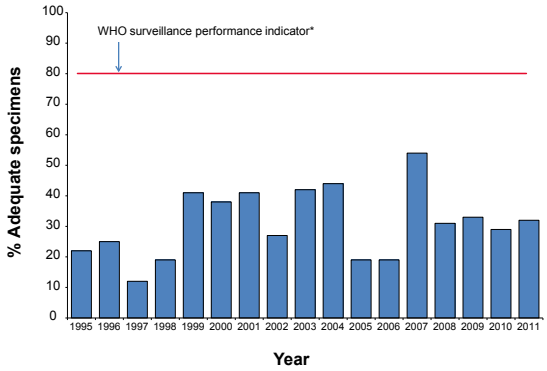
No poliovirus was detected by the ERLNA in 2010 and 2011. The ERLNA typed 234 NPEVs in 2010 and 331 in 2011. Coxsackievirus A6 (CA6) and enterovirus 71 (EV71) were amongst the leading enteroviruses detected in Australia in 2010 and 2011. Both viruses are typically associated with hand, foot and mouth disease in children. B5 was the predominant subgenogroup of EV71 detected in Australia in 2010 and 2011 and was detected in post-mortem specimens from an infant in 2010. Coxsackievirus B1 was detected in both the western and eastern states in 2009 and 2010. Echovirus 25 was the most common enterovirus identified in 2011.

Figure 1: Non-polio AFP rate classified by the PEP 1995 to 2011



* The WHO AFP surveillance performance indicator for a polio non-endemic country is one case per 100,000 children <15 years of age.

Figure 2: Percentage of AFP cases with adequate faecal specimens, 1995 to 2011



* The criterion for the WHO surveillance performance indicator is the collection of two faecal specimens more than 24 hours apart and within 14 days of the onset of symptoms.

Table 3: Surveillance for AFP cases in children less than 15 years, Australia, 2010 to 2011, compared with the WHO performance indicators

WHO surveillance performance indicator for AFP cases in children less than 15 years*	Australia's AFP surveillance performance	
	2010: 41 AFP cases expected	2011: 42 AFP cases expected
Non-polio AFP case rate of 1.0 / 100,000 children	Non-polio AFP rate 1.0 / 100,000 children (41 cases classified)	Non-polio AFP rate 1.43 per 100,000 children (60 cases classified)
More than 80% of classified AFP cases with adequate faecal specimens†	29% (adequate specimens received from 12 / 41 AFP cases)	32% (adequate specimens received from 19 / 60 AFP cases)

* Population data derived from the Australian Bureau of Statistics, estimated population, at 30 June 2009 and 2010. Available at www.abs.gov.au. Based on Australia's population less than 15 years of age.

† Adequate faecal specimens defined as 2 faecal specimens collected at least 24 hours apart and within 14 days of onset of paralysis.

Table 4: Test results for faecal specimens from AFP cases involving children < 15 years of age referred to the NERL, 2010 and 2011

Result	2010	2011
Non-polio enterovirus*	3	2
No enterovirus isolated	51	67
Total	54	69

* In 2010, non-polio enteroviruses identified from three AFP cases were echovirus 19, echovirus 3 and enterovirus 68. In 2011, coxsackievirus A24 was identified from two faecal specimens of one AFP case.

It was mainly associated with fever and most cases originated from Western Australia.

Environmental Surveillance

In 2010, sentinel sites for environmental surveillance for poliovirus were established at Armidale and two sites in Newcastle at Burwood Beach and Shortland. The Burwood Beach site was replaced by Byron Bay in 2011. Fifteen collections (5 from each site) were tested in 2010 and a further nine collections (3 from each site) in 2011. No poliovirus was reported from any of the 500 mL grab samples processed by the NERL.

The sewage extracts were tested in parallel by cell culture and a pan-enterovirus RT-PCR. The pan-enterovirus RT-PCR is a validated in-house test and was utilised to confirm the cell culture results as not all human enteroviruses can infect the RD-A cell line. All enterovirus isolates from cell culture and positive detections by RT-PCR were investigated to determine the virus type by nucleic acid sequencing and the results from the two methods were the same. All samples except one from each of the Newcastle sites and one from Byron Bay were positive for NPEV in virus culture, which serves as an indicator organism for the collection, transportation and laboratory procedures.

Regional reference laboratory activities

The following activities were performed as a Polio Regional Reference Laboratory in 2010 and 2011:

- Brunei Darussalam: Specimens from three AFP cases were received in each year. Sabin poliovirus types 1, 2 and 3 were isolated from one case in 2011, consistent with recent immunisation.
- Pacific Island countries: Specimens from 14 and 9 AFP cases were received in 2010 and 2011, respectively. No poliovirus was isolated from any of these but NPEVs were isolated from 6 of the 20 cases. In 2010, 14 specimens were referred from Fiji to investigate the cause of a hand, foot and mouth disease outbreak. Coxsackievirus A6 was detected in 11 of these. Twenty faecal specimens were referred from a gastroenteritis outbreak in Fiji in 2011. A NPEV was isolated from only one of the specimens, indicating that an enterovirus was unlikely to have caused the outbreak.
- Papua New Guinea: Specimens from 17 and 9 AFP cases were referred in 2010 and 2011, respectively. Sabin-like poliovirus type 3 was isolated from one case in 2010. Fourteen NPEVs were isolated by cell culture or detected by RT-PCR from 14 of the 26 cases.
- Philippines: Four poliovirus type 2 and 3 poliovirus type 3 viruses were referred from AFP cases for ITD in 2010 and all were characterised as Sabin-like. A poliovirus type 3 and a poliovirus type 1 were characterised as Sabin-like from sources other than AFP in 2010 and 2011, respectively.

Table 5: Enterovirus test results at the NERL, Australia, 1995 to 2011

Year	Poliovirus		Non-polio enterovirus	No enterovirus detected	EVID results referred §	Total samples reviewed
	Sabin-like	Non-Sabin-like				
1995	190	0	200	13	0	403
1996	224	0	198	9	0	431
1997	124	0	76	0	0	200
1998	52	0	15	4	0	71
1999*	60	1	9	9	0	79
2000	45	0	44	47	0	136
2001*	46	5	33	75	0	159
2002	36	0	21	49	0	106
2003	9	0	15	47	0	71
2004	6	0	26	61	0	93
2005	18	0	10	39	0	67
2006	2	0	6	71	29	108
2007†	0	2	32	115	107	256
2008	0	0	20	92	77	189
2009‡	1	0	63	78	113	255
2010	0	0	170	39	108	317
2011	0	0	174	61	205	440

* Untyped enterovirus or uncharacterised poliovirus isolates were referred for further testing after completion of a laboratory inventory. The six isolates tested as non-Sabin-like and were subsequently identified as wild type poliovirus prototype strains and were destroyed.

† Wild poliovirus type 1 was imported from Pakistan.

‡ A Sabin-like poliovirus type 1 was identified from an unimmunised infant.

§ Enterovirus Identification results include retrospective data made available via the ERNLA.

Quality Assurance Programs

In 2010 and 2011, the NERL passed the annual quality assurance panels for poliovirus isolation by cell culture and poliovirus RT-PCR for ITD and vaccine derived poliovirus. In 2011, WHO introduced a trial poliovirus sequencing proficiency panel with the intention of making this an annual test from 2012. The NERL maintained accreditation as a Polio Regional Reference Laboratory after WHO conducted a two day on-site review of laboratory procedures and documentation in October 2010. Accreditation is assessed in the interim years by the submission of an annual checklist and subject to passing the annual laboratory proficiency tests.

Discussion

Clinical surveillance for cases of AFP in children is a sensitive means of detecting imported cases of poliomyelitis in a polio-free country by targeting an age group that is at high risk of infection if not immunised. This occurred in China when an outbreak of polio was reported in the far western province of Xinjiang due to an importation of a wild poliovirus type 1 from Pakistan in August 2011.[2] Initially four polio cases were confirmed through AFP surveillance, in children aged between four months and two years, who had had onset of paralysis the preceding month. A further 17 cases of polio were confirmed up to October 2011 and included two fatalities. Polio cases were also reported in adults. The outbreak was stopped by concerted vaccination campaigns targeting people up to 39 years of age.[2] This serves as a salutary reminder that polio can quickly spread in a population with inadequate polio vaccination coverage at any age.

Australia met the WHO AFP surveillance performance indicator of at least 1.0 non-polio AFP case per 100,000 children less than 15 years of age in 2010 and 2011, reporting a rate of 1.0 and 1.4 per 100,000 respectively. This was the fourth year in a row that Australia has met the indicator used by WHO to assess whether a national AFP surveillance system is likely to detect an imported case of polio in a child. At the state and territory level, New South Wales, South Australia and Victoria exceeded the performance indicator rate in both years, while the Northern Territory, Tasmania and

Western Australia only did so in 2011. Queensland did not reach the surveillance indicator in either year despite it being the only jurisdiction where AFP is notifiable. Six of Australia's eight jurisdictions met the WHO performance indicator in 2011, a result that reinforces the overall sensitivity of the national AFP surveillance system.

Another important aspect of AFP surveillance is the testing of stool specimens to exclude poliovirus as the causative agent. Notwithstanding the strong performance of AFP case ascertainment in Australia in recent years, the number of AFP cases with adequate stool specimens has averaged 31% for the last four years. This compares to the WHO surveillance performance indicator of 80%, a target that Australia has never met. Australia's standard of performance against this surveillance indicator is not unusual for developed nations. The reasons for poor rates of faecal specimen collection are manyfold. This could include an unwillingness or inability of the patient to provide a faecal specimen, prioritising laboratory tests of specimens from other sites such as cerebrospinal fluid and relying upon neurological diagnostic procedures such as magnetic resonance imaging and nerve conduction studies.

Virological surveillance for poliovirus was introduced in Australia to complement the clinical surveillance program for AFP cases in children. It has two components, the typing of enteroviruses through the ERLNA and environmental surveillance by testing grab samples of sewage at sentinel sites. No poliovirus was isolated through either of these surveillance systems providing additional data in support of Australia's continued polio-free status. In addition to testing for poliovirus, enterovirus typing facilitates the detection of NPEVs of public health importance, such as EV71, and will enable the epidemiology of enteroviruses circulating in Australia to be better understood. The predominant subgenogroup of EV71 detected in Australia in 2010 and 2011 was B5 and, based on reports from the ERLNA this subgenogroup was mainly associated with fever and hand, foot and mouth disease. EV71 B5 was also detected in faecal material from a post-mortem sample from an infant suffering from a suspected viral illness in 2010 but causality cannot be confirmed due to the detection of the virus from a non-sterile site. A report from New South Wales in 2011 linked EV71 meningoencephalitis with the death of a 63 year old male who had received rituximab as treatment for non-Hodgkin's lymphoma.[13] The EV71 was typed as subgenogroup C2. Anti-CD20 monoclonal antibody therapy can deplete B cells, which are required to clear enterovirus infection.

As a result of the broader focus on enterovirus surveillance in support of poliovirus surveillance, the Polio Reference Laboratory was renamed the National Enterovirus Reference Laboratory from July 2011.

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