

Annual reports

ANNUAL REPORT OF THE AUSTRALIAN NATIONAL POLIOVIRUS REFERENCE LABORATORY, 2006

Jason Roberts, Kerri Anne Brussen, Aishah Ibrahim, Bruce Thorley

Abstract

The Australian National Poliovirus Reference Laboratory (NPRL), located within the Victorian Infectious Diseases Reference Laboratory, is the national laboratory for Australia, the Pacific Islands and Brunei Darussalam, and is accredited by the World Health Organization (WHO) as the Regional Reference Laboratory for the WHO Western Pacific Region. The NPRL, in collaboration with the Australian Paediatric Surveillance Unit, co-ordinates surveillance for acute flaccid paralysis (AFP), the major clinical presentation of poliovirus infection. After classification of AFP cases by the Polio Expert Committee, the non-polio AFP rate for Australia in 2006 was 1.1, meeting the WHO surveillance requirement of detecting more than one AFP case per 100,000 children aged less than 15 years. During 2006, 80 specimens were referred to the NPRL, 59 from AFP cases and 21 from other sources. Poliovirus type 3 was isolated from two patients without AFP and the isolates were characterised as Sabin-like using WHO accredited methodologies. Echovirus 30 was isolated from two cases of AFP and coxsackievirus B5 and adenovirus were isolated from individual cases. During 2006, 1,998 cases of poliomyelitis due to wild poliovirus infection were reported world-wide, of which, only 6.8% (127) were due to importation of wild poliovirus. *Commun Dis Intell* 2007;31:263–269.

Keywords: acute flaccid paralysis, disease surveillance, laboratory testing, poliomyelitis

Introduction

The World Health Organization's (WHO) polio eradication program is the largest public health initiative ever undertaken. In 1994, the Australian Government established the Australian National Poliovirus Reference Laboratory (NPRL) as part of Australia's commitment to the polio eradication program. Based within the Victorian Infectious Diseases Reference Laboratory (VIDRL), the NPRL is the WHO accredited facility for the isolation and characterisation of poliovirus from clinical specimens within Australia, the Pacific Islands and Brunei Darussalam. The NPRL is also designated as a Regional Reference Laboratory for the WHO Western Pacific Region.

In 1995, the Australian Federal Government initiated a surveillance program for the most serious clinical syndrome associated with poliovirus infection, acute flaccid paralysis (AFP). Since 2000, co-ordination of this surveillance program has been undertaken by the NPRL, in collaboration with the Australian Paediatric Surveillance Unit (APSU). All reported cases of AFP and suspected poliomyelitis are reviewed by the Australian Polio Expert Committee (PEC).

Polio vaccination in Australia is given at 2, 4 and 6 months and at 4 years of age, prior to school entry. From November 2005, the Australian immunisation program changed to exclusive use of inactivated poliovirus vaccine (IPV) in place of the live attenuated Sabin oral poliovirus vaccine (OPV).¹ Immunisation with OPV has been linked to vaccine associated paralytic poliomyelitis (VAPP), which is estimated to occur in one in 2.4 million doses. After administration of OPV, the recipient will shed live poliovirus intermittently for up to six weeks. In immunosuppressed persons who receive OPV, virus excretion can persist in excess of six weeks.^{2,3} The exclusive use of IPV in the vaccination schedule eliminates the possibility of VAPP and the laboratory isolation of OPV polioviruses from recently vaccinated persons in Australia.⁴ Any poliovirus isolated within Australia is now most likely indicative of importation and requires careful investigation.⁵

The performance of AFP surveillance in Australia and the laboratory activities of the NPRL in 2006 are described in this report.

Methods

The current system of AFP surveillance used by the NPRL in collaboration with the APSU is as follows:

- Clinicians reviewing patients presenting with AFP are advised to notify the NPRL. In keeping with WHO guidelines, the AFP surveillance program requires that all AFP cases involving children aged less than 15 years be reported. However, the NPRL tests specimens from cases of suspected poliomyelitis involving patients of all ages. Notification of AFP cases in children aged less than 15 years are also included on monthly report cards and emails submitted by paediatricians to the APSU.

- Two faecal specimens should be collected 24 to 48 hours apart and within 14 days of onset of paralysis.
- Faecal specimens are referred to the NPRL for testing.
- Reporting clinicians are supplied with a clinical questionnaire immediately upon notification of an AFP case.
- The PEC, convened by the Australian Government Department of Health and Ageing, reviews clinical and laboratory data for all notified cases of AFP, regardless of case eligibility.
 - The PEC case definition for AFP is: Any child under 15 years of age with acute flaccid paralysis (including Guillain-Barré syndrome) or any person of any age with paralytic illness if poliomyelitis is suspected.
 - In accordance with the WHO guidelines an ineligible case is a patient aged greater than 15 years, an overseas resident, or a case notified as AFP in error by a clinician.
- The PEC classifies cases of AFP as poliomyelitis due to wild poliovirus, vaccine-derived poliovirus (VDPV) or vaccine associated poliomyelitis; non-polio AFP; or non-AFP.
- A follow-up questionnaire is sent to notifying clinicians 60 days after the onset of paralysis in the patient if the PEC requires more information regarding the AFP case before a final classification can be made.
- Australian AFP data are forwarded to WHO for inclusion in the global AFP surveillance data published in the *Weekly Epidemiological Report*, (available from: <http://www.who.int/wer/en/>).
- At the end of each calendar year, a small number of eligible cases may remain un-classified by the PEC if no clinical or laboratory data were available from the notifying clinician.

Upon receipt at the NPRL, faecal specimens are extracted in a 7.7% v/v chloroform solution in Minimum Essential Medium containing 2% foetal bovine serum and inoculated onto a series of mammalian cell lines. In keeping with WHO requirements, cell lines used for the isolation of poliovirus are L20B (a transgenic mouse epithelial cell line expressing the human poliovirus receptor, CD155)^{6,7} and RD-A (human rhabdomyosarcoma). These two cell lines are inoculated in duplicate to increase the sensitivity of virus isolation. The NPRL also utilises two additional cell lines for the isolation of poliovirus and non-polio enteroviruses (NPEVs): Hep2 Cincinnati (human epidermoid carcinoma) and HEL (human embryonic lung). Laboratories throughout Australia are encouraged to refer enteroviruses of unknown serotype to the

NPRL for further characterisation. All polioviruses, whether isolated from AFP cases or other sources, undergo a process known as intratypic differentiation (ITD) to distinguish between wild and vaccine strains of poliovirus. ITD involves a nucleic acid detection method, [polymerase chain reaction (PCR)] and an antigenic method, [enzyme-linked immunosorbent assay (ELISA)]. These methods have been described in detail in previous annual reports.^{8,9} In place of the ELISA, the NPRL is now sequencing portions of the poliovirus genome.

Two regions of the poliovirus genome are routinely sequenced from all poliovirus isolates. These regions are the VP1 capsid genomic region, where greater than 1% change compared to the prototype OPV strain is indicative of a vaccine-derived poliovirus as defined by WHO,¹⁰ and the 3D genomic region, which is sequenced in order to determine whether the virus has undergone a recombination event with another poliovirus or enterovirus.

The NPRL is accredited as a Polio Regional Reference Laboratory, through proficiency testing and on-site inspections by WHO staff.

Results

Notification of acute flaccid paralysis cases and Polio Expert Committee case classifications

In 2006, no AFP cases due to wild poliovirus, VDPV or VAPP were reported in Australia. A total of 48 eligible AFP cases were notified in Australia between 1 January and 31 December 2006 (Table 1).

Clinical and laboratory information was available for the PEC to review 43 of the 48 eligible AFP notifications. The WHO target for AFP surveillance in a polio non-endemic country is one case of AFP per 100,000 children aged less than 15 years. For Australia, this correlates to 40 cases per year (Table 1). Australia's non-polio AFP rate was 1.2, based on 48 eligible notifications. The non-polio AFP rate, based on the 43 eligible cases classified by the PEC, was 1.1 (Table 2).

The PEC was unable to provide final classification for five AFP notifications due to insufficient clinical information.

Notifications of acute flaccid paralysis by state or territory

New South Wales, Queensland and Victoria reached the expected WHO target of 1 case per 100,000 children aged less than 15 years for the reporting period (Table 1). This is the first time that Victoria has reached the WHO target since the initiation of AFP surveillance in Australia.

Faecal specimen collection from acute flaccid paralysis cases

WHO defines adequate specimens for laboratory testing, as two faecal specimens collected at least 24 hours apart and within 14 days of onset of paralysis. WHO recommends that specimens be tested in an accredited polio reference laboratory.

Faecal specimens were collected from 21 of the 43 eligible AFP cases with onset of symptoms in 2006 of which:

- Nine cases had two or more adequate specimens as defined by WHO.
- Seven cases had one specimen collected within 14 days of onset.
- Five cases had one or more specimens collected after 14 days of onset.

- No faecal specimens were referred to the NPRL from the remaining 22 eligible cases.

The proportion of eligible cases meeting the WHO criteria for adequate faecal specimen collection in the reporting period was 21% (9/43), well below the target of 80%.¹¹

Laboratory testing of specimens

Acute flaccid paralysis cases

Forty-nine faecal specimens were received from 24 cases of AFP in Australian children less than 15 years of age. This included specimens from three AFP cases with onset of symptoms in late 2005, received by the laboratory in early 2006. An additional 10 specimens were referred from AFP patients aged greater than 15 years.

Table 1. Unique notifications of eligible acute flaccid paralysis cases by state or territory of residence with onset of symptoms between, 1 January to 31 December 2006

State or territory	Estimated population aged <15 years*	Expected number of cases/year	Unique notified eligible cases 1 January to 31 December 2006	Notification rate per 100,000 population aged <15 years
ACT	62,430	0.5	0	0.0
NSW	1,309,104	13	23	1.8
NT	50,674	0.5	0	0.0
Qld	816,566	8	11	1.4
SA	283,763	3	2	0.7
Tas	96,318	1	0	0.0
Vic	961,410	10	11	1.2
WA	404,349	4	1	0.3
Australia	3,984,614	40	48	1.2

* Australian Bureau of Statistics, estimated resident population, preliminary – 30 June 2006. ABS publication 3201.0, June 2006.

Table 2. Acute flaccid paralysis surveillance compared with WHO indicator targets for children less than 15 years, Australia, 2006

WHO indicator target for AFP cases of children less than 15 years*	Australia's surveillance for AFP cases with onset in 2006	Australia's AFP surveillance rates for 2006
Non-polio AFP case rate of 1.0 per 100,000 children (40 cases for Australia in 2006).	48 unique cases of AFP notified 43 cases classified by the Polio Expert Committee as non-polio AFP	AFP notification rate: 1.2 per 100,000 children Non-polio AFP case rate: 1.1 per 100,000 children
More than 80% of notified AFP cases with 2 adequate stool specimens collected at least 24 hours apart, within 14 days of onset of paralysis.	9 AFP cases with 2 or more adequate specimens	Referral of adequate specimens from AFP cases: 21% (9/43) of the eligible cases

* Based on data supplied by the Australian Bureau of Statistics, estimated resident population, preliminary – 30 June 2006. ABS publication 3201.0, June 2006.

AFP Acute flaccid paralysis.

No polioviruses were isolated from the specimens of AFP cases in the reporting period. Non-polio enteroviruses were isolated from three cases of AFP: echovirus 30 was isolated from two cases and coxsackievirus B5 from one case. Adenovirus, which is not a member of the enterovirus family, was isolated from one case of AFP. No enterovirus was isolated from the faecal specimens of the remaining 17 eligible cases (Table 3).

A throat swab was received from an overseas resident aged greater than 15 years, who was admitted to hospital with AFP seven days after arriving in Australia. No enterovirus was isolated from the swab and the patient discharged themselves from hospital without further follow-up.

Four rectal swabs and a faecal specimen from an overseas resident with AFP who was aged less than 15 years, were referred to the NPRL. Adenovirus was isolated from the faecal specimen.

No other virus isolations were reported from the specimens of the remaining AFP cases (Table 3).

Isolations from non-acute flaccid paralysis samples

In January 2006, five faecal specimens, a throat swab, a rectal swab, and cerebrospinal fluid were referred to the NPRL from an infant who had received routine immunisation of OPV in October 2005, followed by a booster of IPV in January 2006. Poliovirus type 3 (PV3) was isolated from one of the three faecal specimens initially forwarded to the NPRL. The PV3 was classified as Sabin-like using WHO approved methods for ITD. The VP1 genomic region was sequenced and had 99.4% nucleotide sequence identity to the prototype PV3 OPV strain. The isolation of a poliovirus, 107 days post-vaccination, is within the upper limits of 42–137 days for the excretion of poliovirus from a

recently vaccinated patient.² No enteroviruses were isolated from a further two specimens that had been requested to confirm the clearance of the virus from the patient.

Although vaccine-associated paralytic polio (VAPP) was considered as a potential diagnosis by the PEC, the length of time between the administration of OPV and onset of symptoms (106 days) was outside the accepted range of 4–35 days for an OPV recipient. Acute and convalescent sera were also available for testing by the NPRL. There was evidence of immunity to all three poliovirus serotypes, with no detectable rise in titre observed between the acute and convalescent sera. The case, initially reported as post-trauma to a lumbar puncture, was subsequently diagnosed as osteomyelitis and classified as non-AFP by the PEC based on the available clinical information.

Two faecal specimens were received from a patient who was administered a low dosage of methotrexate and had received OPV. PV3 Sabin-like was isolated from one of the two initial specimens. A further three specimens were referred over a six week period to determine if there was prolonged virus excretion but no enterovirus was isolated from the specimens. A summary of enteroviruses tested at the NPRL between 1995 and 2006 is presented in Table 4.

Possible importation of wild poliovirus

On 19 October 2006, the importation of a wild poliovirus type 1 was reported in Kenya.¹² Virus genome sequencing and phylogenetic analysis traced the origin of this virus to Nigeria, an African country endemic for wild poliovirus. It was later determined that 12 people who arrived in Australia from Kenya between August and October, may have been in contact with the index case. Three people were tested as part of this investigation.

Table 3. Results from specimens referred to the Australian National Poliovirus Reference Laboratory, 2006

Result	Specimens from AFP cases*	Specimens from non-AFP referred samples	Total
Poliovirus Sabin-like type 3	0	2	2
NPEV†	4	2	6
Adenovirus	3	0	3
No virus isolated	52	16	68
Total	59	20	79

* Includes specimens from patients of all ages and nationalities referred from within Australia.

† NPEV: non-polio enterovirus. A coxsackievirus B5 (1 AFP case) and echovirus 30 (2 AFP cases) and coxsackievirus A17 (1 non-AFP case) were identified using either micro-neutralisation or molecular serotyping methods.

AFP Acute flaccid paralysis.

A non-polio enterovirus, coxsackievirus A17, was isolated from two faecal specimens from one of the people, while no enterovirus was isolated from the specimens collected from the others. Phylogenetic analysis of the VP1 nucleotide sequence with other coxsackievirus A17 sequences available through international databases did not identify a link with recent global isolations, thus providing no evidence as to whether the person was infected with the coxsackievirus before or after arrival in Australia.

Regional reference laboratory activities

In addition to the Australian samples, 155 specimens and isolates were received from countries of the Western Pacific Region. The referred samples included 30 specimens from 16 cases of AFP from the Pacific Islands with a non-polio enterovirus isolated from five of the cases. Ten specimens from five cases of AFP were referred from Brunei Darussalam and enterovirus 71, the cause of severe outbreaks of hand, foot and mouth disease in East and South Asia, was isolated from two cases of AFP. Fifty-nine specimens and isolates from Malaysia, and 32 specimens and isolates from the Philippines were also referred for ITD. A further 24 specimens and isolates from the National Polio Reference Laboratory of Papua New Guinea were tested in parallel as part of an ongoing laboratory quality assurance program.

Quality assurance program

As part of the accreditation procedure for a WHO polio reference laboratory, proficiency panels relating to the isolation, molecular detection and antigenic characterisation of poliovirus were received in February, June and November respectively. All proficiency panels were successfully completed. The annual laboratory accreditation site-visit to the NPRL was waived by WHO in 2006. The NPRL submitted documentation outlining the laboratory's activities to WHO Headquarters, Geneva and received notification that full accreditation status was retained.

Discussion

In 2006, Australia exceeded the WHO standard for AFP surveillance of one case of AFP per 100,000 children under the age of 15 years. Since the inception of the Australian AFP surveillance system in 1995, the WHO AFP surveillance standard has been achieved in 2000, 2001 and 2004. In 2006, adequate faecal sampling was obtained for only 21% of eligible AFP notifications, well below the 80% target established by WHO.

With the introduction of IPV into the standard immunisation schedule in Australia from November 2005, no further isolations of OPV strains of poliovirus are expected in Australian-born AFP cases without overseas travel. This was proven to be the case in 2006, with the last reported laboratory isolations of a poliovirus occurring after

Table 4. Summary of enterovirus testing at the Australian National Poliovirus Reference Laboratory, 1995 to 2006

Year	Poliovirus		Non-polio enterovirus	No enterovirus detected	Total samples tested
	Sabin-like	Non-Sabin-like*			
1995	190		200	13	403
1996	224		198	9	431
1997	124		76	0	200
1998	52		15	4	71
1999	60	1	9	9	79
2000	45		44	47	136
2001	46	5	33	75	159
2002†	36		21	49	106
2003	9		15	47	71
2004	6		26	61	93
2005	18		10	39	67
2006	2		6	71	79

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

† Two poliovirus isolates had discordant results by ITD. Sequencing confirmed the isolates as Sabin-like, with <1.0% variation from the parental Sabin strain.

two infants were vaccinated with OPV at the end of 2005. It is imperative that all poliovirus isolations after November 2005 be rigorously investigated, as they are potentially an importation from countries still using OPV or a wild poliovirus from one of the four endemic countries. While no polioviruses were reported to the Laboratory Virology and Serology Reporting Scheme in 2006, there were 101 untyped enteroviruses reported.¹³ With pan-enterovirus PCR methods replacing routine cell culture in many diagnostic laboratories, the ability to determine enterovirus serotype is limited, thus increasing the risk of silent transmission of imported polioviruses and other enteroviruses of public health significance. As the characterisation of enteroviruses is both costly and time consuming, Australian virology laboratories are strongly encouraged to forward any untyped enteroviruses to the NPRL for further characterisation. Cases of imported VAPP and the isolation of VDPVs has been documented in countries that use IPV^{14,15} and the monitoring of circulating enteroviruses in Australia is essential for the detection of such cases.

Globally, the number of poliomyelitis cases due to wild poliovirus infection in 2006 increased slightly to 1,998 in comparison to the 2005 case total of 1,979.¹⁶ Although this may seem discouraging, the number of wild poliovirus cases reported by endemic countries in 2005 was 943 (47.7% of the total) and the number of imported cases was 1,036 (52.3%), which included a major outbreak in Indonesia.¹⁷ In 2006, the number of endemic cases rose to 1,871 (93.6%), while the number of imported cases plummeted to 127 (6.8%).¹⁶ This indicates that control measures instituted by WHO are proving successful in their capacity to contain poliovirus transmission within endemic countries and the focus now is to eradicate the last remaining pockets of circulating wild poliovirus.

Acknowledgements

We would like to thank the Australian Paediatric Surveillance Unit for their ongoing collaboration with AFP surveillance, members of the Polio Expert Committee and Dr Heath Kelly as chief investigator of AFP surveillance. We would also like to acknowledge the notifying clinicians and laboratories for their assistance throughout 2006. The Australian National Poliovirus Reference Laboratory is funded by the Australian Government Department of Health and Ageing and the Victorian Government Department of Human Services.

Author details

Mr Jason Roberts, Medical Scientist¹

Mrs Kerri Anne Brussen, Medical Scientist¹

Mrs Aishah Ibrahim Medical Scientist¹

Dr Bruce Thorley, Head^{1,2}

1. Poliovirus Reference Laboratory
2. Victorian Infectious Diseases Reference Laboratory, North Melbourne, Victoria

Corresponding author: Mr Jason Roberts, Victorian Infectious Diseases Reference Laboratory, Locked Bag 815, Carlton South, VIC 3053. Telephone: +61 3 9342 2607. Facsimile: +61 3 9342 2665. Email: Jason.Roberts@mh.org.au

References

1. Australian Government Department of Health and Ageing. Replacement of oral polio vaccine (OPV) with inactivated polio vaccine (IPV). Available from: <http://www.immunise.health.gov.au/internet/immunise/publishing.nsf/Content/opvrep-qapub> Accessed 2 August 2007.
2. Halsey NA, Pinto J, Espinosa-Rosales F, Faure-Fontenla MA, da Silva E, Khan AJ, et al. Search for poliovirus carriers among people with primary immune deficiency disease in the United States, Mexico, Brazil, and the United Kingdom. *Bull World Health Org* 2004;82:3–7.
3. Kew OM, Sutter RW, Nottay BK, McDonough MJ, Prevots DR, Quick L, et al. Prolonged replication of a type 1 vaccine-derived poliovirus in an immunodeficient patient. *J Clin Microbiol* 1998;36:2893–2899.
4. Centers for Disease Control and Prevention. Poliomyelitis prevention in the United States: Introduction of a sequential vaccination schedule of inactivated poliovirus vaccine followed by oral poliovirus vaccine: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep* 1997;46 RR-03:1–25.
5. Thorley BR, Brussen KA, Elliott EJ, Kelly HA. Vigilance is required for Australia to remain polio free *Med J Aust* 2006;184:474–475
6. Wood DJ, Hull B. L20B cells simplify culture of polioviruses from clinical samples. *J Med Virol* 1999;58:188–182.
7. Nadkarni SS, Deshpande JM. Recombinant murine L20B cell line supports multiplication of group A coxsackieviruses. *J Med Virol* 2003;70:81–85.
8. Stambos V, Brussen KA, Thorley BR. Annual report of the Australian National Poliovirus Reference Laboratory, 2004. *Commun Dis Intell* 2005;29:263–268.
9. Thorley BR, Brussen KA, Stambos V, Yuen LK, Kelly HA. Annual report of the Australian National Poliovirus Reference Laboratory and summary of acute flaccid paralysis surveillance, 2001. *Commun Dis Intell* 2002;26:419–427.
10. World Health Organization. Expanding contributions of the global laboratory network for poliomyelitis eradication, 2000–2001. *Wkly Epidemiol Rec* 2002;77:133–140.
11. WHO-recommended standards for surveillance of selected vaccine-preventable diseases. World Health Organization/Vaccines and Biologicals/03.01, 2003
12. World Health Organization. Poliomyelitis, Kenya. *Wkly Epidemiol Rec* 2006;81:409.
13. Australian Government Department of Health and Ageing. Communicable diseases surveillance tables. Table 4 Virology and serology laboratory report by state or territory for the reporting period 1 October to 31 December 2006, and total reports for the year 2006. *Commun Dis Intell* 2007;31:144.
14. Centers for Disease Control and Prevention. Poliovirus Infections in Four Unvaccinated Children—Minnesota, August—October 2005 *MMWR Morbid Mortal Wkly Rep* 2005;54,1053–1055.

15. Centers for Disease Control and Prevention. Imported vaccine-associated paralytic poliomyelitis-United States, 2005. *MMWR Morbid Mortal Wkly Rep* 2006;55:97-99.
16. The global eradication of polio. Wild Poliovirus 2000-2005. Case count by country. Available from: <http://www.polioeradication.org/casecount.asp> Accessed 12 July 2007.
17. World Health Organization. Resurgence of wild poliovirus type 1 transmission and effect of importation into polio-free countries, 2002-2005. *Wkly Epidemiol Rec* 2006;81:63-68.