Annual report of the Australian National Poliovirus Reference Laboratory 2005

Kerri Anne Brussen,[1] Jason Roberts,[2] Aishah Ibrahim,[2] Vicki Stambos,[2] Bruce R Thorley[3]

Abstract

In May 1988 the World Health Assembly adopted a resolution for the global eradication of poliomyelitis. Since then two target dates for eradication (2000 and 2003) have passed and the struggle to eradicate the poliovirus continues. Australia's commitment to the worldwide campaign began in December 1994 with the designation of the National Poliovirus Reference Laboratory at the Victorian Infectious Diseases Reference Laboratory and the initiation of acute flaccid paralysis (AFP) surveillance in March 1995. During 2005 the National Poliovirus Reference Laboratory did not isolate any wild or vaccine derived polioviruses from the 42 samples collected from eighteen cases of acute flaccid paralysis in Australian residents. Three Sabin–like polioviruses were isolated from three cases of acute flaccid paralysis but all were considered incidental isolations by the Polio Expert Committee and not implicated in the disease of the patients. After exceeding the World Health Organization target of one case of AFP per 100,000 children aged less than 15 years in 2004, Australia's non-polio AFP rate in 2005 fell to 0.75 cases per 100,000 children. The high number of wild poliovirus importations reported globally in 2005 into previously polio free countries, highlights the need for a sensitive AFP surveillance system within Australia and for specimens from AFP cases to be forwarded to the National Poliovirus Reference Laboratory. *Commun Dis Intell* 2006;30:334–340.

Keywords: poliovirus, acute flaccid paralysis, surveillance, enterovirus

Introduction

Acute flaccid paralysis (AFP) is the main clinical manifestation of poliomyelitis and occurs in approximately one per cent of poliovirus infections. Surveillance for AFP cases in children along with high polio immunisation coverage has been the hallmark of the World Health Organization (WHO) Global Polio Eradication Program since its inception. AFP surveillance in Australia is conducted by the National Polio Reference Laboratory (NPRL) located at the Victorian Infectious Diseases Reference Laboratory (VIDRL) in conjunction with the Australian Paediatric Surveillance Unit (APSU). All faecal specimens collected from cases of AFP in Australia, are forwarded to the NPRL for testing for poliovirus and other enteroviruses. All cases of AFP are reviewed by the Australian Polio Expert Committee (PEC). The NPRL is also the national laboratory for the Pacific Island countries and Brunei Darussalam, and is a regional reference laboratory for the WHO Western Pacific Region.

Since September 1966 the Australian Standard Immunisation Schedule has included the Sabin oral poliovirus vaccine (OPV) as the vaccine of choice for immunisation against poliovirus infection.[1] OPV is a trivalent vaccine comprising all three poliovirus serotypes. After administration of the vaccine, the viruses multiply in the gut of the recipient and can be excreted for up to six weeks from immunocompetent individuals.[2] Longer excretion times for immunocompromised recipients have been documented.[3]

In November 2005 the Australian National Immunisation Program introduced inactivated poliovirus vaccine (IPV) as the recommended vaccine.[4] The introduction of IPV into the schedule will eliminate the risk of vaccine associated poliomyelitis (VAPP) which occurs in one in 2.5 million administered doses of OPV.[5] Vaccination with IPV will also eliminate the isolation of incidental polioviruses in Australian virology laboratories as vaccine viruses will no longer be excreted by poliovirus vaccine recipients.

1. Scientist and Co-ordinator AFP Surveillance, Victorian Infectious Diseases Reference Laboratory, Carlton South, Victoria
2. Scientist, Victorian Infectious Diseases Reference Laboratory, Carlton South, Victoria
3. Head, Poliovirus Reference Laboratory, Victorian Infectious Diseases Reference Laboratory, Carlton South, Victoria

Corresponding author: Mrs Kerri Anne Brussen, Scientist and Co-ordinator AFP Surveillance, Victorian Infectious Diseases Reference Laboratory, Locked Bag 815, Carlton South, VIC 3053. Telephone: +61 3 9342 2607. Facsimile: +61 3 9342 2665. Email: kerrianne.brussen@mh.org.au

The activities of the Australian National Poliovirus Reference Laboratory in 2005 are summarised in this annual report which also includes a comparison of AFP surveillance in Australia against the major targets nominated by WHO.

Methods

The approach adopted by Australia for AFP surveillance is as follows:

- paediatricians reviewing a patient aged less than 15 years and presenting with AFP or a clinician reviewing a patient of any age suspected of poliomyelitis are requested to notify the national AFP surveillance co-ordinator at VIDRL. Notification of the case is also included on the paediatrician's monthly report card to the APSU;
- two faecal specimens should be collected 24 to 48 hours apart and within 14 days of onset of paralysis;
- the faecal specimens should be referred for testing to the WHO accredited NPRL located at VIDRL;
- clinicians are requested to complete a clinical questionnaire upon notification of the case;
- the PEC convened by the Australian Government Department of Health and Ageing reviews the clinical and laboratory data for all notified cases of AFP; irrespective of whether they are an eligible or ineligible case;
  - the PEC, case definition for AFP is: 'An eligible case 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 polio is suspected.
  - An ineligible case is an AFP case outside the case definition: patients aged greater than 15 years, an overseas resident, or a case notified 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 if the PEC requires more information regarding the AFP case before a final classification can be made;
- Australian AFP data is forwarded to WHO on a quarterly basis 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 are not classified by the PEC as clinical and laboratory data are not available from the notifying clinician.

On receipt at the NPRL, faecal specimens are extracted in a 10 per cent v/v chloroform solution in Modified Essential Medium with Earles salts and inoculated onto a series of continuous cell lines. The main WHO cell line utilised for the isolation of poliovirus is L20B,—a mouse epithelial cell line with cell surface expression of the human poliovirus receptor, CD155.[6,7] The NPRL employs three other cell lines for the isolation of poliovirus and other enteroviruses. They are RD (human rhabdomyosarcoma) also recommended by the WHO, HEp2 Cincinnati (human epidermoid carcinoma) and HEL (human embryonic lung). Other laboratories within Australia 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). ITD distinguishes between wild and vaccine strains of poliovirus. ITD involves a genetic based method, [polymerase chain reaction (PCR)] and an antigenic based method, [enzyme–linked immunosorbent assay (ELISA)]. These methods have been described in detail in previous annual reports.[8,9]

Polioviruses isolated from Australian AFP cases and those with discordant ITD results are sequenced routinely by the NPRL. Two regions of the poliovirus genome are sequenced. The VP1 capsid gene where greater than one per cent changes compared to the parental OPV strain, indicates the presence of a vaccine-derived poliovirus as defined by the WHO.[10] A portion of the 3D gene is also sequenced to provide information on whether the virus has undergone a recombination event with another poliovirus or enterovirus during replication.

The NPRL is accredited annually as a national and regional polio reference laboratory, through proficiency testing and an on-site visit by the WHO.

Results

AFP surveillance

In 2005, no Australian AFP cases were due to wild poliovirus, VDPV or VAPP. There were a total of 59 notifications, 36 eligible cases, 15 duplicate notifications and eight ineligible cases.

Polio Expert Committee reviews

Clinical and laboratory information was available for review by the PEC for 37 of the 44 eligible and ineligible AFP notifications. This included 30 of

the 36 eligible cases, and seven ineligible cases. Six eligible cases and one ineligible case, remain unclassified by the PEC due to lack of clinical and laboratory data.

The WHO target for AFP surveillance in a non-endemic country is one case of AFP per 100,000 children aged less than 15 years.[11] Australia's non-polio AFP notification rate in 2005 for Australian residents was 0.75 cases per 100,000 children.

Notification rates in States and Territories

Three of the unclassified cases were from Western Australia with the others located in New South Wales and Queensland. The differences in the rates of notification of AFP cases between states and territories continued in 2005 as reported previously,[8] with only New South Wales and Tasmania reaching the expected target. AFP data for Australian states and territories is presented in Table 1.

Faecal collection

Adequate faecal collection at 19 per cent was well below the expected WHO target of 80 per cent of notified cases[11] (Table 2). Adequate faecal collection is defined by WHO as two faecal specimens collected 24 hours apart and within 14 days of onset of paralysis. Four of the seven (57%) AFP cases with adequate faecal collection were from New South Wales with the remaining three cases from Queensland. Six of the seven cases with adequate faecal collection were first notified to the NPRL, but 38 (64%) of all notifications were first notified to the APSU.

Table 1. Notified acute flaccid paralysis cases, aged less than 15 years, 2005, by Australian state or territory of residence

State or territory	Estimated population aged <15 years*	Expected number of AFP cases per year	Number of eligible notifications	Number of eligible cases classified by the PEC	Notification rate per 100,000 population aged <15 years for eligible cases	Notification rate per 100,000 population aged <15 years for cases classified by the PEC
ACT	62,448	0.5	0	0	0.00	0.00
NSW	1,319,450	13	18	17	1.40	1.30
NT	50,521	0.5	0	0	0.00	0.00
Qld	807,065	8	9	7	1.10	0.88
SA	283,610	3	0	0	0.00	0.00
Tas	96,516	1	1	1	1.00	1.00
Vic	958,596	10	4	4	0.40	0.40
WA	390,274	4	4	1	1.00	0.25
Australia	3,978,221	40	36	30	0.90	0.65

* Australian Bureau of Statistics, estimated population, preliminary – 30 June 2005. ABS publication 3201.0, June 2005.

AFP Acute flaccid paralysis.

PEC Polio Expert Committee.

Table 2. AFP surveillance compared with WHO indicator targets for children less than 15 years, Australia, 2005

WHO indicator target for AFP cases of children less than 15 years	Australia's surveillance for AFP cases with onset in 2005	Australia's AFP surveillance rates for 2005
Non-polio AFP case rate of 1.0 per 100,000 children (40 cases for Australia in 2005).	36 eligible AFP cases notified.	AFP notification rate: 0.9 cases per 100,000 children.
	30 eligible AFP cases classified by the PEC as non-polio AFP.*	Non-polio AFP notification rate: 0.75 cases 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.	7 eligible AFP cases with 2 or more adequate specimens per case.	Referral of adequate specimens from AFP cases: 19% (7/36) of the eligible cases.

* Six cases require clinical information from the referring doctor before final classification by the PEC.

Polio Expert Committee classification of acute flaccid paralysis cases

Gullian-Barré Syndrome continued to be the most common diagnosis of non-polio AFP cases classified by the PEC (30% of cases) in 2005, followed by transverse myelitis (14%) and infant botulism (8%). Poliovirus type 3 (PV3), Sabin-like, was isolated from one case of transverse myelitis and one case of infant botulism. Poliovirus type 2 (PV2), Sabin-like, was isolated from one case of transverse myelitis in a patient aged greater than 15 years. All isolations were considered incidental by the PEC.

Laboratory testing of specimens

AFP cases

The NPRL received a total of 42 samples from 18 AFP cases within Australia in 2005. This included 32 faecal specimens from 13 cases of AFP and one faecal extract from one case of AFP in children aged less than 15 years. A further seven faecal specimens and two swabs were collected from four AFP cases aged greater than 15 years. Results of testing are presented in Table 3.

Isolations of poliovirus

In June 2005, PV3 was isolated from three faecal specimens from a six-month-old child from Queensland. The specimens were collected 69, 70 and 71 days post-vaccination with OPV. The virus was characterised as Sabin-like by the WHO approved methods of ITD. Three further specimens, collected 106, 113 and 162 days post-vaccination, did not yield any poliovirus. The VP1 gene was sequenced for all three poliovirus isolates. The nucleotide homology for the VP1 gene to the parental Sabin strain was greater than 99 per cent for all three poliovirus isolates confirming their classification as Sabin-like. No evidence of a recombination event was detected in the 3D gene.

The PEC classified the case as non-polio AFP, diagnosed as transverse myelitis with the isolation of a Sabin-like PV3 that may have a possible association.

In August 2005, PV3 was isolated from two faecal specimens collected from a four-month-old infant in Queensland. The onset of symptoms occurred seven days post-vaccination with OPV. Faecal specimens were collected 14 and 15 days post-vaccination. The viruses were characterised as Sabin-like by the WHO approved methods of ITD. The VP1 gene was sequenced and the nucleotide homology for the VP1 gene to the parental Sabin strain was greater than 99 per cent, confirming the classification as Sabin-like.

Faecal specimens were tested with mice, and a type B/E toxin producing *Clostridium botulinum* was detected. Based on this evidence the PEC classified the case as non-polio AFP, diagnosed as infant botulism.

A patient aged greater than 15 years presented with AFP 10 days post-vaccination with OPV in September 2005. Vaccine had been administered prior to travel to Indonesia, where the onset of symptoms occurred. PV2 was isolated from a faecal specimen collected 23 days post-vaccination upon return to Australia. The virus was characterised as Sabin-like by the WHO approved methods of ITD. The VP1 gene was sequenced and the nucleotide homology for the VP1 gene to the parental Sabin strain was greater than

Table 3. Test results of specimens and isolates referred to the Australian National Poliovirus Reference Laboratory, Australia, 2005

Result	Isolations from AFP cases*	Isolations from non-AFP referred samples	Total
Poliovirus Sabin-like type 1	0	6	6
Poliovirus Sabin-like type 2	2†	4	6
Poliovirus Sabin-like type 3	5	1	6
Adenovirus	2	0	2
Rhinovirus	0	1	1
NPEV‡	0	10	10
No virus isolated	33	3	36
Total	42	25	67

* Includes eligible and ineligible cases.

† Isolated from an ineligible case.

‡ NPEV: non-polio enterovirus. Molecular sequence results of NPEV from non-AFP sources identified coxsackievirus B2 (3 isolates), echovirus 11 (1 isolate), echovirus 18 (2 isolates), echovirus 25 (1 isolate) and echovirus 30 (3 isolates).

AFP Acute flaccid paralysis.

99 per cent, confirming the classification as Sabin-like. The 3D gene had 100 per cent homology to the parental Sabin strain and therefore no evidence of a recombination event. PV2 was also isolated from a rectal swab received by the NPRL in October 2005. The PEC classified the case as non-polio AFP, diagnosed as transverse myelitis with the isolation of an incidental Sabin-like PV2.

Adenovirus was isolated and confirmed by PCR from two faecal specimens from one case of AFP from New South Wales. No serotyping was performed on this isolate. The increase in adenovirus isolations from AFP cases observed during 2004[8] did not continue in 2005.

No enterovirus was isolated after 14 days in culture, from the remaining 33 faecal specimens, faecal extract and swab received from all AFP cases.

Polio serology

Polio serology testing is available through the NPRL for patients with a suspected case of acute poliomyelitis.

Polio serology was performed on paired sera from a nine-year-old child from South Australia with onset of paralysis in September 2004. The titres determined for poliovirus type 1, 2 and 3 in the acute and convalescent sera, were consistent with evidence of past infection or immunisation with poliovirus type 1, 2 and 3 but there was no evidence of seroconversion to any of the three poliovirus serotypes. The PEC classified the case as non-polio AFP diagnosed as anterior horn cell disease (motor neuropathy) causing monoplegia. No faecal specimens were available for this case.

Samples from sources other than AFP cases

Eleven polioviruses were identified from 23 samples referred from sources other than AFP cases during 2005 (Table 3).

A laboratory in South Australia referred 20 untyped enteroviruses to the NPRL for further identification. Nine polioviruses were isolated from the referred isolates and eight tested as Sabin-like with the WHO approved methods of ITD. One further poliovirus type 1 was Sabin-like by PCR but did not react in the ELISA test. The virus was sequenced and confirmed as Sabin-like with a 99.7 per cent nucleotide homology to the parental Sabin strain in the VP1 gene, and no evidence of recombination was detected in the 3D region with 100 per cent homology to the parental Sabin strain. This result was confirmed by the Global Specialised Laboratory in Japan according to WHO protocol for polioviruses with discordant ITD results. This virus referred to the NPRL by the laboratory in South Australia was isolated from a three-month-old infant from the Northern Territory. The infant had no clinical evidence of AFP.

Sequencing of 10 of the other referred isolates from South Australia identified coxsackievirus B2, and echovirus 11, 18, 25 and 30. One further isolate from a nasopharyngeal aspirate was confirmed as a rhinovirus, and two isolates failed to passage, which may have been due to loss of virus titre.

A bowel specimen was referred from a four-month-old infant who had died of sudden infant death. PV3 was isolated from the bowel specimen and classified as Sabin-like by WHO approved methods of ITD.

A faecal specimen from a three-month-old infant from New South Wales with asthma but thought to have an enteroviral co-infection was referred to the NPRL. A PV2 was isolated from the specimen and subsequently tested as Sabin-like by WHO approved methods of ITD. No further investigation of this case was undertaken.

A faecal specimen from a six-month-old infant with monoclonal proliferation was referred for enteroviral studies. No enterovirus was isolated from this specimen.

A summary of enteroviruses tested at the NPRL between 1995 and 2005 is presented in Table 4.

Regional reference laboratory activities

As a WHO regional reference laboratory, the NPRL received a total of 252 specimens and isolates during January to December 2005, from national poliovirus laboratories and hospitals in the Western Pacific Region. This included six specimens from three AFP cases from the Pacific Islands, four specimens from two cases of AFP from Brunei Darussalam, 36 specimens and isolates from the Philippines and 61 specimens and isolates from Malaysia. A further 145 specimens and isolates from Papua New Guinea, were referred for retesting as part of an ongoing laboratory quality assurance program.

Laboratory accreditation

The NPRL retained its full accreditation status for 2005 as a national laboratory for Australia, the Pacific Island countries and Brunei Darussalam and as a regional reference laboratory for the Western Pacific Region.

Discussion

In 2005, there were 0.75 cases of AFP per 100,000 children aged less than 15 years, detected in Australia. This is less than the WHO standard target

Table 4. Summary of enterovirus testing at the Australian National Poliovirus Reference Laboratory, 1995 to 2005

Year	Poliovirus		Non-polio enterovirus	Non-enterovirus detected or no virus 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

* Untyped enterovirus or uncharacterised poliovirus isolates were referred for further testing after completion of a laboratory inventory.

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

for AFP surveillance in a polio non-endemic country, of one case of AFP per 100,000 children. Since the establishment of AFP surveillance in Australia in 1995, the WHO rate has only been reached or exceeded in 2000, 2001 and 2004.[8,9]

Adequate faecal sampling in 2005 was achieved in only 19 per cent of eligible AFP cases—well below the WHO target of 80 per cent and the lowest level recorded since the introduction of AFP surveillance in Australia.[8,9] An increased awareness of the need to collect faecal specimens is required amongst notifying paediatricians and may increase Australia's rate of collection of adequate faecal specimens to enable Australia to meet the WHO requirement of at least 80 per cent of notified AFP cases with two adequate faecal specimens.[11] During the next year the PEC will also be implementing changes to facilitate the completion of the clinical questionnaire and the collection of faecal specimens.

With the removal of OPV, and the introduction of IPV into the Australian National Immunisation Program, laboratories will see a gradual decrease in the number of incidental poliovirus isolations to zero. Consequently the isolation of a poliovirus will represent a potentially imported virus as will untyped enteroviruses. Between 1 January and 31 December 2005, 40 uncharacterised polioviruses, and 185 untyped enteroviruses were reported to LabVISE.[12] Australian virology laboratories are therefore encouraged to forward any untyped enteroviruses and uncharacterised polioviruses to the NPRL for further characterisation. Thus from 2006, the isolation of any poliovirus by an Australian virology laboratory needs to be fully investigated, as demonstrated by two reports from the United States of America during 2005. In March 2005 an imported case of VAPP occurred in an unvaccinated adult on return from a student exchange program in Cost Rica.[13] In August 2005 a VDPV was isolated from an unvaccinated, immunocompromised seven-month-child presenting without AFP.[14] In both cases the detection of the poliovirus was due to thorough laboratory investigation and serotyping of isolated viruses.

Globally, the number of wild poliovirus confirmed cases reported, increased from 1,266 in 2004 to 1,962 in 2005 with the number of polio cases in non-endemic countries increasing from 256 in 2004 to 1,034 in 2005. A majority of cases were due to wild poliovirus importations originating mainly from India and Nigeria.[15] Sixteen countries reported importations of wild poliovirus while the number of polio endemic countries remained at six. Egypt and Niger interrupted transmission of indigenous poliovirus during 2005.[16] After 10 years of 'polio free' status, Indonesia detected 303 cases of poliomyelitis during 2005. Genetic analysis of the poliovirus isolated from the index case linked the outbreak to polioviruses circulating in Sudan, which originated from Nigeria.[17]

With an increase in the number of countries reporting importations, it is imperative Australia maintains a sensitive AFP surveillance system able to detect an imported case of poliomyelitis.[18] As we move closer

to global eradication, the classification of all AFP case notifications by the PEC will become crucial to maintaining Australia's polio free status.

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