

# Annual report of the Australian National Poliovirus Reference Laboratory, 2002

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## Abstract

Acute flaccid paralysis is the main clinical manifestation of poliomyelitis. Faecal specimens from cases of acute flaccid paralysis in Australia are referred to the National Poliovirus Reference Laboratory for virus culture to determine if poliovirus is the causative agent. Isolations of poliovirus are tested to determine whether they have characteristics of the Sabin oral polio vaccine virus strains or wild type polioviruses. In 2002, a poliovirus type 3, which tested as Sabin vaccine-like, was isolated from an Australian patient with acute flaccid paralysis. A non-polio enterovirus, Echovirus type 18, was isolated from the faecal specimens of another case of acute flaccid paralysis. In the same period, the laboratory identified 35 Sabin-like polioviruses from 52 referred specimens and isolates from cases without acute flaccid paralysis. Australia is a member nation of the World Health Organization's Western Pacific region that was declared free of endemic wild poliovirus in October 2000. Poliomyelitis remains endemic in three of the WHO regions of the world and wild poliovirus may be re-introduced to Australia. While the number of polio-endemic countries has been reduced to seven, the total number of wild polioviruses identified increased in 2002 compared to 2001 due to a sharp rise in isolations of wild virus from Northern India. Until global eradication of poliomyelitis is achieved, it is essential that a high level of poliovirus vaccination coverage, and surveillance for cases of acute flaccid paralysis, be maintained in Australia. *Commun Dis Intell* 2003;27:352–356.

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## Introduction

The Australian National Poliovirus Reference Laboratory at the Victorian Infectious Diseases Reference Laboratory was established in 1994 and has played a major role in Australia's commitment to the World Health Organization's (WHO) program for the global eradication of poliomyelitis. The laboratory is responsible for virological testing of specimens from patients in Australia with acute flaccid paralysis (AFP), the predominant clinical manifestation of poliomyelitis. Diseases such as Guillain-Barré syndrome and transverse myelitis are the most common presentations of AFP in countries free of endemic polio, such as Australia.<sup>1</sup> Members of the enterovirus family, other than poliovirus, can also cause AFP. Non-polio enteroviruses isolated from specimens of Australian AFP cases since 2000 include Echovirus types 11 and 18 and Enterovirus type 71.

The laboratory has worked closely with the AFP clinical surveillance program since its establishment in 1995. Since 2000, the AFP surveillance program has been coordinated at the Victorian Infectious Diseases Reference Laboratory and is conducted in collaboration with the Australian Paediatric Surveillance Unit. The WHO target for notification of AFP cases in children less than 15 years is one per 100,000 population, equivalent to 40 cases for Australia in 2002. A further target nominated by WHO is for two faecal specimens to be referred for laboratory investigation from 80 per cent of the notified AFP cases. The referral of faecal specimens from AFP cases throughout Australia through the clinical surveillance program facilitates the detection of cases of poliomyelitis due to vaccine associated paralytic poliomyelitis (VAPP), circulating vaccine-derived poliovirus (cVDPV) or imported wild-type poliovirus.

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The Australian standard vaccination schedule recommends that the live attenuated Sabin oral polio vaccine (OPV) be administered to children at 2, 4 and 6 months of age with a booster dose at 4 to 5 years of age. OPV is a live attenuated vaccine and replication of poliovirus takes place in the gut of a recently immunised person. The virus is shed in faecal specimens up to six weeks post-vaccination, and for longer periods from immunocompromised people.<sup>2</sup> Thus, poliovirus may be isolated from clinical specimens of cases other than AFP as an incidental finding during routine laboratory testing.

This annual report provides a summary of the activities of the Australian National Poliovirus Reference Laboratory in 2002 and includes a comparison of AFP surveillance in Australia against the major targets nominated by WHO.

*Methods*

The methods for AFP surveillance and laboratory testing of faecal specimens and poliovirus isolates were described in detail in the 2001 National Poliovirus Reference Laboratory annual report.<sup>3</sup> In brief, doctors treating AFP patients are requested to collect two faecal specimens, 24 hours apart and within 14 days of the onset of paralysis, and forward them to the National Poliovirus Reference Laboratory. Faecal specimens are extracted with chloroform and inoculated onto continuous cell lines for virus isolation. Polioviruses are tested by enzyme-linked immunosorbent assay (ELISA), nucleic acid probe hybridisation and polymerase chain reaction (PCR) to determine whether they are a strain of wild type virus or the Sabin vaccine. This process is known as intra-typic differentiation. The test results for the poliovirus ELISA are reported as either Sabin-like, non-Sabin-like or double reactive, depending on their

reactivity with the respective antisera; double reactive isolates bind the Sabin-like and non-Sabin-like antisera with similar avidity. All polioviruses isolated from Australian AFP patients have portions of the genome sequenced to characterise the isolate more fully.

Poliovirus serology is used only for cases compatible with acute polio infection to test the ability of acute and convalescent serum to neutralise each of the three poliovirus serotypes. The serology test is not able to differentiate between an immune response to a wild or vaccine strain of poliovirus. The patient serum is serially diluted and incubated with a standard dose of each poliovirus serotype to determine the titre of neutralising antibody. For an individual who has not been exposed to poliovirus (wild type or vaccine), a conversion from seronegative to seropositive is regarded as significant. With regard to a person who has been previously exposed to poliovirus, antibodies would be expected to be present in both acute and convalescent sera. To determine if that person has an illness consistent with acute polio infection, a fourfold increase in neutralising antibody titre to any of the three serotypes would be required.<sup>4</sup>

*Results*

The National Poliovirus Reference Laboratory received a total of 106 specimens and isolates from all sources within Australia for the year 2002.

**AFP surveillance**

Forty-six cases of AFP in patients of all ages were notified with onset of paralysis in 2002, of which 33 were from children less than 15 years, representing 83 per cent of the WHO notification target (Table 1). Two faecal specimens from six AFP cases and three specimens from a further two cases of children aged

**Table 1. AFP surveillance in Australia 2002, compared with WHO indicator targets for children less than 15 years**

WHO indicator target for AFP cases of children less than 15 years	Australia's surveillance for AFP cases with onset in 2002	Australia's AFP surveillance rates for 2002
Non-polio AFP case rate of 1 per 100,000 population (40 cases for Australia in 2002).	33 cases of AFP notified.  30 cases classified by the Polio Expert Committee as non-polio AFP.*	AFP notification rate: 0.83 per 100,000 population.  Non-polio AFP case rate: 0.75 per 100,000 population.
More than 80% of notified AFP cases with 2 adequate stool samples collected at least 24 hours apart within 14 days of onset of paralysis.	Seven AFP cases with 2 or more specimens per case.	Referral of adequate specimens from AFP cases: 21% of case notifications (7/33).

\* Three cases require further information from the referring doctor before final classification.

less than 15 years with onset of symptoms in 2002, were tested at the National Poliovirus Reference Laboratory. Seven cases met the WHO criteria of collection of two or more specimens within 14 days of onset of paralysis representing 21 per cent of notified AFP cases. Two specimens from one case were collected more than 14 days after onset of paralysis and hence did not meet the WHO criteria. Single specimens were referred from five cases with onset in 2002. A further three faecal specimens from a child less than 15 years were referred from an AFP case with onset of paralysis in 2001. Two faecal specimens were referred from five patients 15 years or older and single faecal specimens and a throat swab from a further five cases.

### AFP cases

Laboratory test results of specimens from AFP cases in this report includes specimens from patients with symptom onset late in 2001 and 2002. Forty faecal specimens and one throat swab were tested in 2002 from 24 cases of AFP (Table 2).

A poliovirus type 3 (P3) was isolated from a faecal specimen of a fully immunised child with AFP, who had been in contact with a sibling recently immunised with OPV. The virus tested as Sabin vaccine-like (Table 2) and the case was the subject of an extensive clinical and laboratory procedural review as a potential case of VAPP. No significant variation from the Sabin type 3 vaccine strain was identified by sequencing of three subgenomic regions of the

isolate. Poliovirus serology determined no significant increase in antibody titre between acute and convalescent serum for P3. The final classification of the case was an acute focal neuropathy and isolation of an incidental Sabin-like P3.

Echovirus type 18 was isolated from both faecal specimens of one case of AFP in a child less than 15 years. No virus was isolated from the specimens of the remaining cases of AFP, from patients of all ages (Table 2).

### Specimens and isolates referred for enterovirus typing

Thirty-five polioviruses were identified from specimens and isolates from sources other than cases of AFP (Table 2). The Sabin OPV includes the three serotypes of poliovirus and mixtures of virus types may be identified from the one specimen or isolate. A total of 17 poliovirus type 1 (P1), 14 poliovirus type 2 (P2) and four P3 isolates were identified from the referred enterovirus samples. This included two mixtures of P1 and P2 type viruses identified from two referred samples.

A total of 19 non-polio enteroviruses were identified from the specimens and isolates referred from Victoria and South Australia. The viruses were identified by partial genomic sequencing and confirmed by antisera neutralisation. Table 3 summarises the activities of the laboratory from 1995 to 2002 for specimens and isolates referred from within Australia.

**Table 2. Testing of specimens and isolates referred to the Australian National Poliovirus Reference Laboratory for the year 2002**

Result	Results of testing from AFP cases		Isolations from referred samples	Total samples
	< 15 years	15 years		
Poliovirus Sabin-like type 1			15	15
Poliovirus Sabin-like type 1 & 2			2	2
Poliovirus Sabin-like type 2			12	12
Poliovirus Sabin-like type 3	1		4	5
Non-polio enterovirus <sup>†</sup>	2		19	21
No virus isolated	23	15	11 <sup>‡</sup>	49
Total	26	15	65	106

\* Includes polioviruses isolated from recently immunised infants.

† NPEV: non-polio enterovirus. Echovirus type 18 was isolated from both faecal specimens of one AFP case. Testing of the referred NPEVs identified six Echovirus type 6 viruses, two Echovirus type 11, two Coxsackie type A16, two Coxsackie types B2, four Coxsackie types B4, two Coxsackie type B5 and an Enterovirus type 71.

‡ Viruses may not have been isolated from some referred samples due to loss of titre in transit and/or not passaging between different cell lines.

**Table 3. Summary of enterovirus testing at the National Poliovirus Reference Laboratory, 1995 to 2002**

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

### Poliovirus isolates with incongruent intra-typic differentiation results

Two isolates (one P1 and one P2) from separate patients with clinical conditions other than AFP tested as double reactive by the ELISA in 2002. Both isolates were Sabin-like by nucleic acid probe hybridisation and thus gave an incongruent result for intra-typic differentiation. The VP1 region of the isolates was sequenced and determined to be Sabin-like with 99.6 per cent and 99.8 per cent homology to the Sabin P1 and P2 strains respectively. The first case was from a patient with immune deficiency who had received Sabin vaccine 10 days prior to the onset of symptoms. A second set of faecal specimens was collected six weeks after the first to determine if viral shedding was ongoing. No virus was isolated. The second case was from a suspected cytomegalovirus infection and the poliovirus isolation was regarded as incidental.

### Poliovirus serology

Serum specimens from two cases that were compatible with an acute polio infection were referred in 2002. The first was the fully immunised child with AFP, who had been in contact with a sibling recently immunised with OPV, described in the section on AFP.

The second case was from an adult patient with lower limb flaccid paralysis who had no record of polio immunisation or recent travel. Acute and convalescent serum specimens were tested in parallel with detection of pre-existing immunity to all three poliovirus serotypes and no serological evidence of acute poliovirus infection. Faecal specimens for virus culture were not available from this patient.

### Regional reference laboratory activities

In addition to the Australian samples, 664 specimens and isolates were referred to the National Poliovirus Reference Laboratory from countries of the Western Pacific region in 2002. This included the retesting of specimens and isolates as part of an ongoing laboratory quality assurance program with 38 samples referred from Mongolia, 29 from Papua New Guinea and 265 from Viet Nam. Seventy-nine specimens and isolates were referred from the Philippines National Poliovirus Laboratory after national immunisation days in response to the cVDPV outbreak in 2001.<sup>5</sup> The Regional Reference Laboratory has not detected a VDPV from specimens from the Philippines since September 2001.

### Discussion

The WHO reporting system for detection of poliomyelitis focuses on the surveillance and specimen testing of AFP cases in children less than 15 years. In both 2000 and 2001, Australia achieved the WHO AFP notification target of 1 per 100,000 children less than 15 years.<sup>6,7</sup> The target was not met in 2002, with a notification rate of 0.83, a situation that had been anticipated early in the year.<sup>7</sup> The WHO target for laboratory testing of specimens from AFP cases less than 15 years of age has never been met by Australia.<sup>3,6</sup> While there were no cases of AFP caused by wild, vaccine or vaccine-derived poliovirus in Australia in 2002, the notification and referral of specimens from AFP cases continues to represent an ongoing challenge.

Mutations occur within the viral genome of the Sabin poliovirus as part of the normal replication process. These mutations may result in a loss of attenuation and, in rare circumstances, an increase in neurovirulence. A neurovirulent poliovirus derived from a vaccine strain can cause AFP in a vaccine recipient

or close contact who has not been previously immunised, or fully immunised, against polio. This is known as VAPP. The isolation of a Sabin P3 from a fully immunised child with AFP whose sibling had recently been immunised was the subject of further investigation by the Polio Expert Committee as a potential case of VAPP. The lack of a significant rise in antibody titre to P3 between acute and convalescent serum, as determined by poliovirus serology, was crucial in the final classification of the case as an acute focal neuropathy with isolation of an incidental poliovirus.

The administration of OPV is not recommended for people diagnosed with an immune deficiency in case of increased poliovirus neurovirulence and long term shedding. The ELISA result of double reactive, for the P1 isolate from the immunocompromised child who had received OPV, was indicative of mutations within the polio capsid. This was confirmed with 0.4 per cent nucleotide sequence variation from the vaccine for the VP1 genomic region and the virus was classified as Sabin-like.

The seven different types of non-polio enteroviruses identified from the referred clinical samples were from two states of Australia: Victoria and South Australia. The viruses may be considered as a representative sample of the non-polio enteroviruses circulating in south-eastern Australia during 2002. Interestingly, three types of non-polio enterovirus identified are known to be capable of causing AFP: Echovirus types 6 and 11 and Enterovirus type 71.<sup>8</sup> Echovirus type 18 was isolated from an AFP patient from Queensland in 2002 but this virus serotype was not amongst those identified in the southern states.

The WHO European region was declared free of indigenous wild poliovirus in September 2002.<sup>9</sup> Three of the six WHO regions are now designated as free of indigenous wild poliovirus: the Americas, Western Pacific and Europe. While only seven countries remain endemic for wild type poliovirus, the increased number of total wild type virus isolations in 2002 compared to 2001,<sup>10,11</sup> and an outbreak of cVDPV on the island of Madagascar<sup>12</sup> is cause for ongoing vigilance for cases of AFP. Until certification of the global eradication of poliomyelitis is declared, Australia needs to maintain high levels of polio vaccination coverage, an AFP notification scheme and virological testing of specimens from AFP cases.

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