Annual report of the Australian National Poliovirus Reference Laboratory, 2004

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Abstract

The Australian National Poliovirus Reference Laboratory at the Victorian Infectious Diseases Reference Laboratory is the World Health Organization designated laboratory for the isolation and testing of poliovirus from clinical specimens within Australia, the Pacific Island countries and Brunei Darussalam. Surveillance for acute flaccid paralysis (AFP) within Australia, the main clinical manifestation of poliomyelitis, is also coordinated at the Victorian Infectious Diseases Reference Laboratory in conjunction with the Australian Paediatric Surveillance Unit. The annual non-polio acute flaccid paralysis rate after classification of cases by the Polio Expert Committee was 1.0 per 100,000 population, reaching the expected World Health Organization annual target for a non-polio endemic country. During 2004, 64 specimens from 30 AFP cases were referred to the National Polio Reference Laboratory. A mixture of poliovirus types 1 and 2 was isolated from an infant with AFP from New South Wales. Both isolates tested as Sabin-like and the case was subsequently classified as infant botulism by the Polio Expert Committee. The laboratory isolated adenoviruses from seven AFP cases. A coxsackievirus B5 and an echovirus 18 were identified from a further two AFP cases. During 2004, 1,266 cases of poliomyelitis due to wild poliovirus were reported world-wide. Many of these resulted from wild poliovirus importations, which continued in 2005, including to Indonesia. This highlights the need for maintaining high poliovirus vaccination coverage to prevent the transmission of poliovirus and high quality AFP and laboratory surveillance for the detection of poliomyelitis due to an imported wild poliovirus. *Commun Dis Intell* 2005;29:263–268.

Keywords: poliovirus, acute flaccid paralysis, surveillance, enterovirus

Introduction

The National Poliovirus Reference Laboratory (NPRL) located at the Victorian Infectious Diseases Reference Laboratory (VIDRL) is responsible for the testing of specimens from patients with acute flaccid paralysis (AFP). AFP is the main clinical manifestation of poliovirus infection and occurs in approximately one per cent of infections. In addition to poliovirus, other viruses and microorganisms can cause AFP. Non-polio enteroviruses such as echoviruses 11, 18 and enterovirus 71 have been associated with AFP.[1] Other diseases presenting as AFP include transverse myelitis, Guillain-Barré syndrome and infant botulism.[2]

Surveillance for AFP is coordinated at VIDRL and conducted in collaboration with the Australian Paediatric Surveillance Unit. The World Health Organization (WHO) target for a non-polio-endemic country such as Australia is one AFP case per 100,000 children aged below 15 years. Based on this figure, we would anticipate 40 AFP cases per annum in Australia.[3]

The Australian standard immunisation schedule recommends administration of Sabin oral poliovirus vaccine (OPV) at two, four and six months of age with an additional booster dose prior to school entry. OPV contains live attenuated strains of all three poliovirus serotypes that replicate in the gut and are excreted in the faeces. Therefore, it is possible to isolate strains of poliovirus from individuals recently immunised with OPV. These may be considered as incidental isolations during routine specimen testing. Laboratories with uncharacterised polioviruses or enterovirus isolates may refer them to the NPRL for further characterisation. This will ensure that no poliovirus remains undetected and any poliovirus isolated in Australia, has been tested to differentiate between wild and vaccine strains.

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Methods

The NPRL is responsible for coordinating AFP surveillance in collaboration with the Australian Paediatric Surveillance Unit. Doctors are requested to notify by telephone, all AFP cases in children aged less than 15 years and residing in Australia, or a person of any age suspected of an acute poliomyelitis infection, to the AFP co-ordinator at VIDRL. AFP cases are also reported to the Australian Paediatric Surveillance Unit via a monthly reporting system. The clinicians, who notify a case, are requested to complete a questionnaire, which is reviewed in conjunction with laboratory results, by the Polio Expert Committee (PEC). Cases are classified by the committee as either (i) non-polio AFP, (ii) poliomyelitis due to wild poliovirus, vaccine-derived poliovirus or vaccine-associated paralytic poliovirus or (iii) non-AFP.

Due to intermittent shedding of the virus, two faecal specimens are collected 24 to 48 hours apart and up to 14 days after onset of paralysis for virological testing in a WHO accredited laboratory. The specimens are extracted in a 10 per cent v/v chloroform solution and inoculated onto a series of continuous cell lines. The main cell line employed for the isolation of poliovirus is L20B,—a mouse epithelial cell line with cell surface expression of the poliovirus receptor, CD155.[4] Another cell line used by the WHO network for the isolation of poliovirus and other enteroviruses is RD (human rhabdomyosarcoma). Other laboratories within Australia refer enteroviruses of unknown serotype to the NPRL for further characterisation. Polioviruses identified amongst these isolates are tested to differentiate between wild and vaccine strains.

All polioviruses, whether isolated from AFP cases or other sources, are tested by a WHO-accredited process known as intratypic differentiation (ITD) that 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)]. The ELISA utilises cross-absorbed polyclonal antisera for the specific detection of wild and OPV strains of poliovirus. The poliovirus ELISA is sensitive to mutations within the capsid of the OPV strains, resulting from nucleotide substitutions during virus genome replication. The mutations can result in vaccine strains with discordant ITD results; for example, Sabin-like by PCR and non-Sabin-like or double reactive by ELISA. A poliovirus strain that displays equal avidity for the Sabin and non-Sabin-like cross-absorbed antisera in the ELISA is described as double reactive. Sequencing of the VP1 capsid gene is performed for poliovirus isolates with discordant ITD results. Sabin polioviruses with more than one per cent nucleotide changes from the parental OPV strain within the VP1 gene, are classified as vaccine-derived polioviruses by the WHO.[5]

The NPRL is accredited annually by the WHO as a national and regional polio reference laboratory. The accreditation process includes proficiency panels for the main laboratory techniques of poliovirus isolation and identification, ELISA and PCR. In addition, an annual on-site laboratory review by WHO is conducted.

Results

Acute flaccid paralysis surveillance

According to the WHO criteria, eligible AFP cases are patients who are Australian residents and aged less than 15 years at the onset of paralysis. However, the PEC reviews cases of suspected poliomyelitis in people of any age. Sixty-two notifications of AFP in Australia were received in 2004. Forty-nine of the AFP cases notified were from patients aged less than 15 years, four cases were patients 15 years or older and nine duplicate notifications were received. Of the 49 eligible cases, the PEC classified 45 as non-polio AFP (Table 1). No clinical information has

Table 1. AFP surveillance in Australia compared with WHO indicator targets for children aged less than 15 years, 2004

WHO indicator target for AFP cases of children less than 15 years	Australia's surveillance for AFP cases with onset in 2004	Australia's AFP surveillance rates for 2004
Non-polio AFP case rate of 1 per 100,000 population (40 cases for Australia in 2004).	49 unique cases of AFP notified. 45 cases classified by the PEC as non-polio AFP.*	AFP notification rate: 1.2 per 100,000 population. Non-polio AFP case rate: 1.1 per 100,000 population.
More than 80 per cent of notified AFP cases with 2 adequate stool specimens collected at least 24 hours apart within 14 days of onset of paralysis.	18 AFP cases with 2 or more specimens per case.	Referral of adequate specimens from AFP cases: 40 per cent (18/45) of the cases classified by the PEC.

* Four cases require clinical information from the referring doctor before cases can be classified by the PEC.

AFP Acute flaccid paralysis.

been received for the remaining four cases. Thus, the annual AFP notification rate in Australia was 1.2 cases per 100,000 children aged less than 15 years. The annual non-polio AFP rate in 2004 after classification of cases by the PEC was 1.1 cases per 100,000 children aged less than 15 years, reaching the expected WHO target for a non-polio endemic country.[6] The WHO target for the non-polio AFP rate has only been met on two previous occasions, in 2000 and 2001. Twenty of the 45 (44%) cases classified as non-polio AFP by the PEC were diagnosed as Guillain- Barré syndrome.

Differences between the rates of notification of AFP by the various Australian states and territories[7] noted in previous years, were not as striking in 2004. New South Wales was responsible for 23/49 (47%) eligible notifications involving children aged less than 15 years. This is equivalent to an annual notification rate of 1.8 per 100,000 New South Wales residents aged less than 15 years. All Australian states and territories except for Western Australia, the Australian Capital Territory and Victoria reached or exceeded the target rate. Paediatricians in Victoria notified nine cases, equivalent to 0.9 cases per 100,000 children aged less than 15 years, the highest rate the state has achieved since the introduction of AFP surveillance in 1995.

Laboratory testing of specimens

Acute flaccid paralysis cases

During the reporting period, 62 specimens from 30 AFP cases within Australia were referred to the NPRL. This included six specimens collected from two AFP cases aged greater than 15 years, which is outside the WHO standard criteria for AFP surveillance.

A mixture of poliovirus types 1 and 2 was isolated from an infant with AFP from New South Wales (Table 2). While the poliovirus type 1 isolate tested as Sabin-like by PCR, it was double reactive by ELISA. Sequencing of the VP1 gene revealed 99.7 per cent nucleotide homology compared with the prototype Sabin strain and the isolate was therefore classified as Sabin-like. The poliovirus type 2 tested as Sabin-like by both methods of ITD. The Polio Expert Committee classified the case as infant botulism based on the detection of *Clostridium botulinum* serotype B toxin and isolation of *C. botulinum* serotype B organism from a faecal specimen of the infant.

A coxsackievirus B5 was isolated from two specimens of a 12-year-old child with AFP from Queensland. The virus was identified by nucleotide sequencing and confirmed by monovalent antisera neutralisation. Specimens were collected from a six-year-old child with AFP from New South Wales. The first specimen yielded a non-polio enterovirus that was subsequently sequenced and identified as echovirus 18. No virus was isolated from the second specimen of the same patient.

In 2004, adenoviruses were isolated from seven AFP cases, with confirmation by PCR (Table 2). The seven cases represented 23 per cent of the 30 Australian AFP cases that were tested by the laboratory in the reporting period. This, and the fact that five of the seven cases were from Victoria, led us to consider whether a particular serotype was circulating or had an association with AFP. Monovalent antisera were used to type the adenoviruses from four of the cases from Victoria and one from Queensland. The viruses from the Victorian cases belonged to adenovirus species C – types 1, 2 (two cases) and 5 – while adenovirus type 4 of species E was identified from the Queensland case.

Table 2. Test results of specimens and isolates referred to the Australian National Poliovirus Reference Laboratory, 2004

Result	Isolations from AFP cases	Isolations from referred samples	Total
Poliovirus Sabin-like type 1	–	1	1
Poliovirus Sabin-like type 1 & 2	1	–	1
Poliovirus Sabin-like type 2	–	3	3
Poliovirus Sabin-like type 3	–	1	1
Adenovirus*	11	1	12
NPEV	3	23	26
No virus isolated	47	2	49
Total	62	31	93

* Eleven adenoviruses were isolated from a total of 15 specimens collected from seven acute flaccid paralysis cases.

NPEV Non-polio enterovirus. Coxsackievirus B5 was isolated from two faecal specimens of one AFP case and echovirus 18 from another case. Nucleotide sequence homology results of NPEV from sources other than AFP identified coxsackievirus A16 (4 isolates), coxsackievirus B4 (2 isolates), echovirus 7 (1 isolate), coxsackievirus B5 (3 isolates), echovirus 3 (2 isolates) and echovirus 11 (5 isolates).

AFP Acute flaccid paralysis.

No virus was isolated after 14 days in culture from a total of 47 specimens, including specimens collected from the remaining 20 AFP cases.

Sources other than acute flaccid paralysis

Five polioviruses were identified from 31 specimens and isolates referred from sources other than AFP and all isolates tested as Sabin-like (Table 2). This included two polioviruses (type 2) isolated from faecal specimens collected from a three-month-old infant with continual diarrhoea, following vaccination with OPV.

Amongst the 31 referred samples from sources other than AFP cases (Table 2), two faecal specimens collected one month apart, were from an eight-month-old infant who had been vaccinated with OPV and had undergone a transplant. These specimens were referred to the NPRL to determine if shedding of vaccine poliovirus was ongoing. Poliovirus type 3 was isolated from the first specimen and tested as Sabin-like by ITD. The second specimen collected 32 days later, yielded an adenovirus that was confirmed by PCR and subsequently identified as adenovirus 1 by antisera neutralisation. No poliovirus was isolated from the second specimen.

Twenty-six of the 31 isolates and specimens were referred by a laboratory in South Australia for further identification. Of these, two polioviruses (types 1 and 2) were identified amongst the isolates and both tested as Sabin-like. This highlights the importance of referring untyped enteroviruses to the NPRL for the detection of polioviruses within Australia. To date, sequencing of 17 of the referred isolates has identified coxsackievirus A16, B4 and B5 and echovirus 3, 7 and 11. A further six non-polio enteroviruses are yet to be identified. One referred isolate failed to passage, which may have been due to loss of virus titre in transit.

A cerebrospinal fluid specimen collected from an adult with symptoms of meningitis, who had a child vaccinated with OPV six weeks prior to onset of symptoms, tested positive for enterovirus by PCR at the referring laboratory. The enterovirus PCR result was confirmed by the Viral Identification Laboratory at VIDRL. The cerebrospinal fluid specimen was tested by the NPRL and did not yield any enterovirus in cell culture.

A summary of enteroviruses tested at the NPRL between 1995 and 2004 is described in Table 3.

Regional reference laboratory activities

In its role as a WHO regional reference laboratory, the NPRL received a total of 406 specimens and isolates during January to December 2004, from national poliovirus laboratories and hospitals in the Western Pacific Region. This included 27 specimens from 14 AFP cases from the Pacific Islands, two specimens from an AFP case from Brunei Darussalam, three specimens and isolates from the Philippines and 48 specimens and isolates from Malaysia. A further 51 specimens and isolates from Papua New Guinea and 275 from Ho Chi Minh City, Viet Nam, were referred as part of an ongoing laboratory quality assurance program.

Table 3. Summary of enterovirus testing at the Australian National Poliovirus Reference Laboratory, 1995 to 2004

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

* 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 wildtype poliovirus prototype strains and were destroyed.

† 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.

Laboratory accreditation

The NPRL at VIDRL retained its full accreditation status for 2004 as a national and regional reference laboratory following a performance-based review by a member of the WHO. The laboratory successfully isolated and identified all viruses in a proficiency panel, referred by the National Institute of Public Health and Environmental Protection, the Netherlands, as part of a laboratory quality assurance program. In addition, the laboratory successfully completed proficiency panels referred by the National Institute of Public Health and Environmental Protection and the Centers for Disease Control and Prevention, USA, for the WHO-approved ITD methods of ELISA and PCR, respectively.

In preparation for laboratory containment of vaccine strains of poliovirus, an inventory has been completed of all specimens and isolates stored by the laboratory since 1993. This is in addition to an inventory of wild polioviruses prepared for Australia's certification as polio-free in 2000.

Discussion

In 2004, Australia achieved the WHO standard criteria for AFP surveillance in a non-polio endemic country, by detecting one case of AFP per 100,000 children aged less than 15 years. Since the introduction of AFP surveillance in 1995, the target has been reached only twice before, in 2000 (1.15/100,000) and 2001 (1.13/100,000).[8] In the intervening years, the non-polio AFP rate dropped to 0.75 per 100,000 population less than 15 years of age in 2002[9] and to 0.68 notifications per 100,000 in 2003.[7]

The rate of adequate faecal sampling in 2004 was 40 per cent, well below the WHO target of 80 per cent of notified AFP cases in children less than 15 years of age (Table 1). Nevertheless, this is the highest rate reported since the introduction of AFP surveillance in Australia. Previously, the rate of adequate faecal sampling had varied from 24 per cent to 36 per cent.[7]

A recent publication reported the isolation of adenovirus from AFP specimens and a possible link between the virus and the condition. Ooi, *et al* described an investigation of eight children who presented with AFP during an outbreak of enterovirus 71-associated hand-foot-and-mouth disease in Malaysia in 1997.[10] The laboratory identified adenovirus 21 from two of the cases and adenovirus species B, of which adenovirus 21 is a member, from a further three AFP cases. It was concluded that adenovirus 21 may cause AFP by anterior horn cell damage or neuropathy of the brachial or lumbosacral plexus. The NPRL isolated adenovirus from 23 per cent of Australian AFP cases in 2004. No single serotype or members of adenovirus species B were identified amongst the Australian isolates. Until now, the isolation of adenovirus has been considered incidental to enterovirus isolation in relation to the WHO polio eradication program. The NPRL will continue to review all adenovirus isolations from AFP cases.

The number of wild poliovirus confirmed cases reported globally increased from 784 in 2003 to 1,266 in 2004.[11] This was mainly due to the increase in wild poliovirus transmission in Nigeria, which accounted for 62 per cent of the global 2004 case count, as well as the transmission into other African countries.[12] An outbreak in Sudan, subsequently led to virus importations into Ethiopia and Saudi Arabia.[12]

In April 2005, an outbreak of polio in Indonesia, caused by wild poliovirus 1, was reported by the WHO.[13] The index case from West Java, was unimmunised and genetic analysis of the poliovirus, indicated it was imported from Sudan and was similar to recently isolated viruses in Saudi Arabia and Yemen. A wild poliovirus has not been isolated in Indonesia since 1995. Indonesia's national routine vaccination coverage is reported to be at least three doses of OPV in 74 per cent of children below one year of age.[14] Routine immunisation rates below 90 per cent increase the risk of an outbreak in the event of a polio re-introduction.[14] However, the AFP surveillance system of Indonesia continues to meet the global minimum standard of detecting at least one AFP case per 100,000 children below 15 years of age and was sufficiently sensitive to detect a wild poliovirus importation.[14]

The risk of importations of wild poliovirus into non-endemic countries remains as long as polio exists anywhere in the world. For Australia to retain its polio-free status, it is imperative that it maintains high national vaccination coverage, currently 93 per cent[15] and conducts sensitive AFP surveillance and high quality laboratory procedures for the detection of poliovirus.

The Global Polio Eradication Initiative Strategic Plan for 2004–2008 outlines the main activities required to interrupt poliovirus transmission, achieve global certification and prepare for the global cessation of OPV.[16] In order to implement the safe cessation of OPV, all six WHO regions need to be declared free of circulating wild poliovirus.[17] The WHO Biosafety Advisory Group has recommended that the strategy used for the containment of wild polioviruses be used as a basis for containment of all polioviruses, including vaccine-derived polioviruses and Sabin strains.[17] Once polio is eradicated globally, laboratories will be the only remaining source of the virus. Successful laboratory containment will prevent the transmission of poliovirus from the laboratory into the community.

The NPRL has completed an inventory of all Sabin polioviruses stored at VIDRL in preparation for the post-global certification phase.

Acknowledgements

We would like to acknowledge Aishah Ibrahim, David Tyssen and Jason Roberts for excellent technical assistance. We thank the Australian Paediatric Surveillance Unit for their ongoing collaboration with AFP surveillance, the Polio Expert Committee for the review of AFP cases and Dr Heath Kelly as the chief investigator of AFP surveillance. We would also like to thank all notifying clinicians and laboratories for forwarding the specimens and isolates during 2004. The National Poliovirus Reference Laboratory in Australia is funded by the Australian Government Department of Health and Ageing and the Victorian Department of Human Services.

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