Annual report of the Australian National Poliovirus Reference Laboratory, 2008

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Abstract

The Australian National Poliovirus Reference Laboratory (NPRL) is accredited by the World Health Organization (WHO) for the testing of stool specimens from cases of acute flaccid paralysis (AFP), a major clinical presentation of poliovirus infection. The NPRL, in collaboration with the Australian Paediatric Surveillance Unit, co-ordinates surveillance for cases of AFP in children in Australia, according to criteria recommended by the WHO. Clinical specimens are referred from AFP cases in children and suspected case of poliomyelitis in persons of any age. The WHO AFP surveillance performance indicator for a polio-free country such as Australia, is 1 non-polio AFP case per 100,000 children less than 15 years of age. In 2008, the Polio Expert Committee (PEC) classified 62 cases as non-polio AFP, or 1.51 non-polio AFP cases per 100,000 children aged less than 15 years. Poliovirus infection is confirmed by virus culture of stool specimens from AFP cases as other conditions that present with acute paralysis can mimic polio. While no poliovirus was reported in Australia from any source in 2008, the non-polio enteroviruses echovirus 25, coxsackievirus B2 and echovirus 11 were isolated from stool specimens of AFP cases. The last report of a wild poliovirus in Australia was due to an importation from Pakistan in 2007. With 4 countries remaining endemic for poliomyelitis–Afghanistan, India, Nigeria and Pakistan–and more than 1,600 confirmed cases of wild poliovirus infection in 18 countries in 2008, Australia continues to be at risk of further importation events. *Commun Dis Intell* 2009;33(3):291–297.

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

Introduction

The global disease burden of poliomyelitis was reduced from 350,000 cases in 1988, when the World Health Assembly passed a resolution to eradicate polio, to less than 1,700 in 2008. The World Health Organization (WHO) polio eradication program is based on maintaining high levels of polio vaccine coverage, clinical surveillance for cases of acute flaccid paralysis (AFP) in children less than 15 years of age and laboratory confirmation of poliovirus infection by testing stool specimens from AFP cases at a laboratory accredited by the WHO for the purpose. In Australia, the National Polio Reference Laboratory (NPRL) is located at the Victorian Infectious Diseases Reference Laboratory (VIDRL).

Surveillance for AFP cases in children less than 15 years of age in Australia is co-ordinated by the NPRL in collaboration with the Australian Paediatric Surveillance Unit (APSU). Clinicians who treat a case of AFP are requested to arrange for collection of 2 stool specimens, due to intermittent virus shedding, within 14 days of the onset of paralysis, notify the case and complete a clinical questionnaire. The Polio Expert Committee (PEC) reviews the clinical and laboratory data from AFP cases in children less than 15 years of age, and suspected polio in persons of any age, to determine if the case is compatible with poliovirus infection.

Countries no longer endemic for polio, such as Australia, will continue to report cases of AFP as other clinical conditions mimic poliovirus infection. WHO considers an AFP surveillance scheme sufficiently sensitive to detect a wild poliovirus importation if 1 case of non-polio AFP per 100,000 children less than 15 years of age is reported each year. Based on Australia's population in 2008, the WHO AFP surveillance performance indicator was 41 AFP cases in children less than 15 years of age.

It is important that Australia maintains high levels of polio vaccine coverage to avoid a resurgence of poliomyelitis in the event of a wild poliovirus importation. The National Immunisation Program of Australia recommends immunisation with inactivated polio vaccine at 2, 4 and 6 months of age, with a booster dose at 4 years of age.[1] Multiple vaccinations ensure seroconversion occurs to each of the 3 poliovirus serotypes present in the vaccine. People travelling to polio endemic countries and countries with recent wild poliovirus importations should receive a booster polio vaccine prior to departure, or a full course of vaccination if they are unsure of their vaccination history. Individuals who are at continuing risk of infection, such as health care workers, are recommended to have a booster polio vaccine every 10 years. The WHO provides weekly updates of global wild poliovirus cases at http://www.polioeradication.org/

With the removal of the live, attenuated Sabin oral poliovirus vaccine (OPV) from the Australian immunisation schedule from November 2005, virol-

ogy laboratories in Australia are no longer expected to routinely isolate OPV-derived polioviruses from clinical specimens. Any poliovirus isolated within Australia may be an importation event and requires further investigation.

This report summarises the activities of the Australian NPRL and the performance of AFP surveillance in Australia in 2008.

Methods

Notification of cases of suspected poliomyelitis is mandatory in all Australian states and territories, whereas AFP in children less than 15 years of age is only notifiable in Queensland. Australia follows the criteria recommended by the WHO for AFP surveillance. Namely, cases of AFP in children less than 15 years of age are notified and stool specimens arranged for collection and testing at a WHO accredited laboratory. AFP surveillance is co-ordinated by the NPRL in collaboration with the Australian Paediatric Surveillance Unit and is implemented as follows:

- In keeping with WHO guidelines, clinicians are requested to notify all cases of AFP in children less than 15 years of age and cases of suspected poliomyelitis in patients of all ages.
- Clinicians notify AFP cases by contacting the NPRL (telephone: 03-9342 2607, email: polio@mh.org.au) while paediatricians also complete a monthly report card submitted to the APSU (http://www.apsu.org.au/).
- Two faecal specimens should be collected 24 to 48 hours apart, due to intermittent shedding of virus, and within 14 days of onset of paralysis for optimal virus isolation.
- Faecal specimens are referred to the NPRL at VIDRL for testing without charge.
- 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 a paralytic illness if poliomyelitis is suspected.
  - In accordance with the WHO guidelines an ineligible case for Australia involves a patient aged 15 years or greater, a resident of another country, or a case notified as AFP in error by a clinician.
- The PEC case classifications are as follows:
  1. AFP as poliomyelitis due to poliovirus (wild type or vaccine);
  2. non-polio AFP or;
  3. non-AFP.
- If the PEC requires more information regarding an AFP case before a final classification can be made, a follow-up clinical questionnaire is sent to the notifying clinicians 60 days after the onset of paralysis.
- 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/).

Upon receipt at the NPRL, faecal specimens are treated with Minimum Essential Medium containing Hank's Salts, chloroform (9.1% v/v) and foetal bovine serum (2%). The suspension is clarified and the supernatant inoculated onto a series of mammalian cell lines. Following the WHO recommendation, the cell lines used for the isolation of poliovirus are L20B (a transgenic mouse epithelial cell line expressing the human poliovirus receptor, CD155)[2] and RD-A (human rhabdomyosarcoma). The NPRL utilises 2 additional cell lines for the isolation of poliovirus and non-polio enteroviruses: BGMK (buffalo green monkey kidney) and HEL (human embryonic lung). Diagnostic laboratories in Australia are encouraged to refer enteroviruses of unknown serotype to the NPRL for further characterisation as poliovirus infection can lead to clinical presentations without paralysis such as aseptic meningitis.

All polioviruses, whether isolated from AFP cases or other sources, are further characterised to distinguish between wild and vaccine strains of poliovirus, a process known as intratypic differentiation. Polioviruses are characterised by reverse transcriptase polymerase chain reaction (RT-PCR) and sequencing the VP1 genomic region. The VP1 genomic region encodes one of the virus capsid proteins containing a major antigenic determinant. One per cent or more change in this region of the genome compared with the respective prototype OPV strain is, by definition, a vaccine-derived poliovirus (VDPV). A fragment of the 3D genomic region is often sequenced in order to determine whether the poliovirus has undergone a recombination event with another poliovirus serotype or non-polio enterovirus. The VP1 nucleotide sequence is also used following published methods to identify non-polio enteroviruses.[3,4]

The NPRL is also accredited, through proficiency testing and periodic on-site inspections by WHO staff as a Regional Reference Laboratory for the Western Pacific Region.

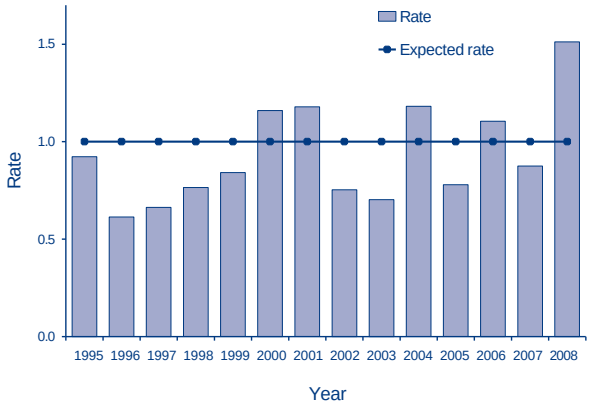
Results

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

A total of 71 AFP cases with onset of symptoms in 2008, were notified to the APSU or the NPRL. Sufficient data were available to classify 62 cases involving children less than 15 years of age as non-polio AFP. In 2008, the non-polio AFP rate for Australia was 1.51 (62/41) per 100,000 children aged less than 15 years, which exceeds the WHO AFP surveillance performance indicator (Table 1). The classification of eligible AFP cases from 1995 to 2008 is presented in the Figure. Nine cases did not meet the WHO criteria; 8 cases involved Australian residents aged 15 years or more while 1 case was a patient from the United Kingdom.

In addition to the 71 AFP cases for which the date of onset is known, a further 8 cases remain pending classification by the PEC due to insufficient information being available for the committee to review. The 8 cases were notified as AFP cases with no further details supplied, such as the patient's name and date of symptom onset, which would allow more thorough investigation.

Figure: Classification of eligible acute flaccid paralysis cases by the Polio Expert Committee from 1995 to 2008



World Health Organization (WHO) acute flaccid paralysis (AFP) surveillance performance indicator = 1 non-polio AFP case per 100,000 population <15 years.

WHO AFP surveillance performance indicator for Australia in 2008 = 41 AFP cases in children aged less than 15 years of age.

Notification of acute flaccid paralysis cases by state and territory

In 2008, the AFP case notification rates for all states and territories exceeded the AFP surveillance performance indicator of 1 case per 100,000 children except for the Northern Territory and Tasmania, who did not notify any AFP cases. (Table 2). The 2 most populous states, New South Wales and Victoria, which account for more than 56% of expected AFP cases in Australia, met the surveillance performance indicator based on final classification of cases by the PEC. Queensland is the only jurisdiction in Australia where AFP is notifiable. While the notification rate of AFP cases in Queensland was 2.0 per 100,000 children less than 15 years of age, sufficient information was available to classify eight of the 18 cases notified, which represented a non-polio AFP rate of 0.9.

Faecal specimen collection from acute flaccid paralysis cases

The WHO has a further surveillance performance indicator that adequate faecal specimens be collected from 80% of eligible AFP cases. The WHO

Table 1: Surveillance for acute flaccid paralysis cases in children aged less than 15 years, Australia 2008, compared with the World Health Organization acute flaccid paralysis surveillance performance indicators

WHO surveillance performance indicator for AFP cases in children less than 15 years*	Australia's surveillance for AFP cases in children with onset of paralysis in 2008	Australia's AFP surveillance performance in 2008
Non-polio AFP case rate of 1.0 per 100,000 children (41 cases for Australia in 2008).	71 unique cases of AFP notified 62 cases classified by the PEC as non-polio AFP	AFP notification rate: 1.93 per 100,000 children. Non-polio AFP case rate: 1.51 per 100,000 children.
More than 80% of classified AFP cases with 2 adequate faecal specimens collected at least 24 hours apart and within 14 days of onset of paralysis.	19 AFP cases with 2 or more adequate specimens	Referral of adequate specimens from AFP cases: 31% (19/61) of the eligible cases.

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

AFP Acute flaccid paralysis.

PEC Polio Expert Committee.

defines adequate specimens for poliovirus culture, as 2 faecal specimens collected 24 to 48 hours apart and within 14 days of onset of symptoms.

In 2008, faecal specimens from 37 of the 62 eligible cases were tested at the NPRL:

- 19 (31%) cases had adequate specimens;
- 13 (21%) cases had at least 1 specimen collected within 14 days of onset of symptoms;
- 5 (8%) cases had 2 specimens collected after 14 days of onset of symptoms;
- no faecal specimens were received from the remaining 25 (40%) eligible cases.

While the number of AFP cases with adequate faecal specimen collection did not meet the WHO AFP surveillance performance criterion, at least 1 specimen was collected within 14 days of the onset of symptoms from 52% of the eligible cases.

Laboratory testing of specimens

Acute flaccid paralysis cases

Between 1 January and 31 December 2008, 99 specimens were referred to the NPRL from AFP cases involving patients of all ages with onset of paralysis in 2007–2008. No poliovirus was isolated from any specimens. Seventy-nine specimens were referred from the 37 cases classified as non-polio AFP involving Australian children less than 15 years of age with onset of paralysis in 2008. The non-polio enteroviruses, coxsackievirus B2, echovirus 11 and echovirus 25, were identified from 3 separate AFP cases by sequencing a fragment of the VP1 genomic region. Adenovirus type 1 and type 5 were identified from 2 separate AFP cases by sequencing a fragment of the hexon gene. No enterovirus was isolated from the remaining faecal specimens (Table 3).

Table 2: Unique notifications of eligible acute flaccid paralysis cases, onset of symptoms between 1 January and 31 December 2008, by state or territory of residence

State or territory	Estimated population aged <15 years*	Expected number of cases per year	Total number of notifications	Eligible cases classified by PEC 1 January to 31 December 2008	Non-polio AFP rate per 100,000 population aged <15 years
ACT	63,874	0.5	2	1	2.0
NSW	1,332,066	13	30	23	1.8
NT	52,253	0.5	0	0	0.0
Qld	861,002	9	18	8	0.9
SA	289,309	3	8	5	1.7
Tas	97,012	1	0	0	0.0
Vic	995,096	10	22	16	1.6
WA	426,476	4	11	9	2.3
Australia	4,117,612	41	91	62	1.5

* Australian Bureau of Statistics, estimated resident population, preliminary – 30 June 2007. ABS publication 3201.0, December 2007.

AFP Acute flaccid paralysis.

PEC Polio Expert Committee.

Table 3: Results from specimens referred to the Australian National Poliovirus Reference Laboratory from within Australia, 1 January to 31 December 2008

Result	Isolations from AFP cases*	Isolations from non-AFP referred samples	Total
Non-polio enterovirus†	8	12	20
Adenovirus‡	5	1	6
No enterovirus isolated	86	0	86
Total	99	13	112

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

† Genetic sequence results of non-polio enterovirus isolates from acute flaccid paralysis (AFP) and non-AFP sources identified coxsackieviruses B2 and B5 and echoviruses 6, 11, 18, 21, 25 and 30.

‡ Genetic sequence results of adenovirus isolates from AFP and non-AFP sources identified adenovirus types 1, 2 and 5.

A total of 10 faecal specimens involving Australian patients greater than 15 years of age were referred from 6 AFP cases with onset of paralysis in 2008. A total of 4 specimens from 2 cases of AFP with onset of symptoms in 2007 were referred in January 2008. No enterovirus was isolated from any of the specimens.

Sources other than acute flaccid paralysis

Thirteen specimens and isolates were received by the NPRL from sources other than AFP in 2008 (Table 3). Coxsackievirus B5 was identified from an adult with aseptic meningitis. Adenovirus type 2 was identified from a case with unspecified aetiology. Untyped enteroviruses were referred from a virology laboratory and the following non-polio enteroviruses were identified by nucleotide sequencing: coxsackievirus B5, echovirus 6, echovirus 11, echovirus 18, echovirus 21 and echovirus 30.

A summary of laboratory testing at the NPRL for the period 1995 to 2008 is presented in Table 4.

Polio serology

Poliovirus serology is only performed for cases with a clinical suspicion of acute poliovirus infection. Sixty-two requests for polio serology were cancelled after discussion with the referring doctor, as the requests related to the patient's immune status for work or travel purposes.

Regional reference laboratory activities

In addition to the Australian samples, 239 specimens and isolates were received from countries of the Western Pacific Region in 2008. The specimens referred for testing included 73 faecal specimens from 36 cases of AFP from Pacific island countries, Papua New Guinea and Brunei Darussalam; 103 specimens and isolates from Malaysia; 54 specimens and isolates from the Philippines; and 9 enterovirus isolates from the Republic of Korea.

Quality assurance program

The laboratory retained full accreditation status as a WHO Polio Regional Reference Laboratory for 2008 after an on-site review by WHO headquarters in September 2007. The main recommendation from the review was to implement all aspects of the new WHO test algorithm introduced to the regions with endemic wild poliovirus in 2006. The new algorithm was designed to shorten the time for issuing laboratory reports from 28 to 14 days, mainly by reducing the period for virus isolation by cell culture incubation from 14 to 10 days and for poliovirus intratypic differentiation from 14 to 7 days.

The NPRL retained full accreditation for poliovirus isolation from and identification of specimens and poliovirus diagnostic RT-PCR after completing the respective WHO proficiency panels for 2008. The poliovirus isolation and identification proficiency

Table 4: Summary of enterovirus testing at the Australian National Poliovirus Reference Laboratory, 1995 to 2008

Year	Poliovirus		Non-polio enterovirus	No enterovirus detected	Total samples tested
	Sabin-like	Non-Sabin-like			
1995	190	0	200	13	403
1996	224	0	198	9	431
1997	124	0	76	0	200
1998	52	0	15	4	71
1999*	60	1	9	9	79
2000	45	0	44	47	136
2001*	46	5	33	75	159
2002	36	0	21	49	106
2003	9	0	15	47	71
2004	6	0	26	61	93
2005	18	0	10	39	67
2006	2	0	6	71	79
2007†	0	2	32	115	149
2008	0	0	20	92	112

* Untyped enterovirus or uncharacterised poliovirus isolates were referred for further testing after completion of a laboratory inventory. The 6 isolates tested as non-Sabin-like and were subsequently identified as wild type poliovirus prototype strains and were destroyed.

† Wild poliovirus type 1 was imported from Pakistan.

panel was subsequently distributed to the national polio reference laboratories throughout the Western Pacific Region by the Australian NPRL, as part of the terms of reference as a WHO regional reference laboratory.

The NPRL was accredited to AS 4633:2004 (ISO 15189:2003) for quality and competence as a medical testing facility as part of an institutional review by the National Association of Testing Authorities (NATA) in October 2008.

Discussion

In 2008, Australia met the WHO AFP surveillance performance indicator for the 5th time since the program commenced in 1995. The non-polio AFP rate of 1.51 in 2008, is the highest reported by Australia. The establishment of the Paediatric Active Enhanced Disease Surveillance (PAEDS) pilot study in 2007, co-ordinated by the APSU and the National Centre for Immunisation Research and Surveillance of Vaccine Preventable Diseases contributed to the high number of cases classified by the PEC in 2008. PAEDS is being trialled as a hospital based surveillance system in 4 major hospitals to collect clinical data and specimens for four childhood conditions including AFP.[5] While the system led to more cases classified by the PEC, the proportion of cases with adequate stool collection (31%) still did not reach the WHO performance indicator of 80%. It is important that stool specimens are tested from cases of AFP to exclude poliovirus infection as part of the differential diagnosis; however these results indicate the difficulties faced in establishing a system for the efficient referral of specimens.

At the end of each calendar year a small number of AFP notifications remain unclassified by the PEC as no clinical or laboratory data are available for the cases. After consultation with the WHO, the PEC resolved that AFP notifications remaining pending after an appropriate period, would be classified as 'polio compatible-zero evidence' and to consider them as surveillance failures. As a consequence, the PEC classified a total of 12 cases notified from 2005 to 2007 as 'polio compatible-zero evidence' in December 2008. The 8 cases listed as pending in this report may be classified according to this criterion if further information is not forthcoming before the end of 2009.

The WHO AFP surveillance performance indicator of 1 case of non-polio AFP per 100,000 children less than 15 years of age was based on the incidence of Guillain-Barré syndrome as the most common cause of AFP in the absence of wild poliovirus circulation in the Americas.[6] The year-to-year fluctuation in Australia meeting this surveillance indicator led to a 2-source capture-recapture investigation of the incidence of AFP from 1998 to 2000 in the state of Victoria, which had consistently not met the WHO criteria.[7] Whereas 0.5 AFP cases per 100,000 children less than 15 years of age were reported in Victoria via the national surveillance scheme, the retrospective study determined the average annual incidence to be 1.4 per 100,000 children less than 15 years of age. The improved performance of AFP notifications in 2008, through the establishment of a hospital based surveillance system in New South Wales, South Australia, Victoria and Western Australia, supports the validity of the WHO AFP surveillance performance indicator and that it should be considered a minimum at the national level. Thus, the years that Australia does not meet the AFP surveillance performance indicator represent gaps in surveillance for the detection of imported cases of polio.

The identification of enteroviruses from clinical specimens is important. Apart from excluding poliovirus, non-polio enteroviruses are also associated with the onset of AFP and their serotype identification will ascertain the viral aetiology of non-polio AFP. Furthermore, poliovirus infection can cause clinical symptoms without paralysis and may not be considered as part of the differential diagnosis unless techniques to identify the specific serotype are employed. Therefore, identification of enteroviruses from clinical specimens is important as part of the laboratory surveillance for poliovirus and also to establish the epidemiology of enteroviruses in Australia. With this in mind, the NPRL has been in discussion with other virology laboratories to establish an enterovirus reference laboratory network. The NPRL has introduced the CODEHOP method for the identification of non-polio enteroviruses isolated from clinical specimens and untyped enteroviruses referred from other virology laboratories.[4] This method has the benefit of being able to identify enteroviruses from original clinical specimens or cell culture isolates, which in 2008 included 2 enterovirus 71 and a coxsackievirus A16 that could not be identified by standard PCR methods.

It is well established that in areas of poor sanitation and low vaccine coverage, the Sabin live attenuated oral polio vaccine can produce vaccine derived polioviruses, defined as having more than 1% variation from the prototype serotype nucleotide sequence.[8,9] In such settings, sustained person-to-person transmission results in the accumulation of viral genome mutations with a loss of attenuation and the potential to cause outbreaks of paralytic polio. In 2008, the largest vaccine derived poliovirus outbreak was reported from Nigeria, which stands at 276 cases as at July 2009.[10] The outbreak is all the more concerning since it involves oral polio vac-

cine type 2 poliovirus, whereas wild poliovirus type 2 was last isolated in 1999 and its eradication was considered a success of the global polio eradication program.[11]

Australia last reported a wild poliovirus importation in 2007.[12] Since 2005, the number of polio cases worldwide caused by wild poliovirus has not dropped below 1,300.[13] The continued presence of type 1 and type 3 wild poliovirus in the four endemic countries (Afghanistan, India, Nigeria and Pakistan) and importations in 17 countries so far in 2009, coupled with the circulation of type 2 vaccine derived poliovirus in Nigeria underscores the need for continued awareness for imported cases of polio in Australia.

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