Annual report of the Australian National Poliovirus Reference Laboratory and summary of acute flaccid paralysis surveillance, 2001

Bruce R Thorley, Kerri Anne Brussen, Vicki Stambos, Lilly KW Yuen, Heath A Kelly,
Victorian Infectious Diseases Reference Laboratory

Abstract

The National Poliovirus Reference Laboratory at the Victorian Infectious Diseases Reference Laboratory (VIDRL) is responsible for poliovirus testing for Australia and the Pacific Island countries. It is also a regional reference laboratory for the Western Pacific Region of the World Health Organization. Surveillance for acute flaccid paralysis, a clinical manifestation of poliomyelitis, is coordinated at VIDRL in collaboration with the Australian Paediatric Surveillance Unit. There were 60 unique notifications of acute flaccid paralysis (AFP) in 2001, of which 44 were classified by the polio expert committee as eligible non-polio AFP cases, that is, from patients resident in Australia and aged less than 15 years. Polioviruses were isolated from one AFP patient and characterised as Sabin oral poliovirus vaccine-like for all 3 serotypes. In the same period, the National Poliovirus Reference Laboratory identified 40 Sabin-like viruses from 74 referred isolates and specimens, and an additional five non-Sabin-like polioviruses as part of the laboratory containment of poliovirus. The Western Pacific Region, of which Australia is a member nation, was declared free of circulating wild poliovirus in October 2000. However, during 2001, viruses derived from the Sabin oral poliovirus vaccine caused 3 cases of poliomyelitis in the Philippines, also a member nation of the Western Pacific Region. The identification of circulating vaccine-derived poliovirus in the Philippines has emphasised the necessity of maintaining a high level of vaccination coverage within Australia and an effective surveillance system to detect cases of poliomyelitis. *Commun Dis Intell* 2002;26:419-427.

Keywords: poliovirus, vaccine-derived poliovirus, acute flaccid paralysis, surveillance

Introduction

The National Poliovirus Reference Laboratory at the Victorian Infectious Diseases Reference Laboratory (VIDRL) is responsible for the characterisation of all poliovirus isolates in Australia. This includes Sabin oral polio vaccine-like viruses, isolated incidentally from nasopharyngeal aspirates taken from recently immunised infants when investigating other illnesses such as bronchiolitis. The laboratory is also responsible for testing stool samples from all patients with acute flaccid paralysis (AFP) in Australia.

Acute flaccid paralysis is the major clinical manifestation of poliovirus infection, occurring in 0.1 per cent to one per cent of infections. Clinical surveillance for cases of AFP and the subsequent laboratory testing of faecal specimens for poliovirus will also detect cases of poliomyelitis caused by imported wild-type poliovirus, circulating vaccine-derived poliovirus (cVDPV) or vaccine-associated paralytic poliomyelitis (VAPP). In Australia, AFP surveillance with laboratory support was initiated in March 1995 to meet the certification standards of the World Health Organization (WHO) poliomyelitis eradication program[1] and since 2000, has been coordinated at VIDRL in collaboration with the Australian Paediatric Surveillance Unit (APSU).

In 1994, the World Health Organization declared the region of the Americas to be free of circulating wild poliovirus. This was followed by a similar declaration for the Western Pacific Region in October 2000 and represented a milestone in the WHO program for the global eradication of poliomyelitis.[2] Despite these achievements, outbreaks of poliomyelitis have recently occurred in both regions due to viruses derived from the Sabin oral polio vaccine.[3,4,5] This has focussed attention on the need for quality surveillance and extensive characterisation of all poliovirus isolates.

Corresponding author: Dr Bruce R Thorley, Head Poliovirus Reference Laboratory, Victorian Infectious Diseases Reference Laboratory, Locked Bag 815, Carlton South, VIC 3053. Telephone: +61 3 9342 2607. Facsimile: +61 3 9342 2666. E-mail: bruce.thorley@mh.org.au.

Methods

AFP surveillance

The approach adopted in Australia for AFP surveillance has been presented in detail elsewhere[1] and is briefly outlined here. Any paediatrician seeing a patient aged less than 15 years and presenting with AFP is requested to notify the surveillance coordinator by telephone, complete a detailed questionnaire and arrange for the collection of 2 faecal samples 24 hours apart and within 14 days of the onset of paralysis. A follow-up questionnaire requesting clinical details 60 days after the onset of paralysis is required for cases where a definitive diagnosis cannot be made from the first questionnaire and available laboratory results. In addition, all paediatricians are asked to return a reply-paid survey card to the APSU each month, indicating the number of patients seen with a range of rare conditions, including AFP.

The Polio Expert Committee, appointed by the Australian Commonwealth Department of Health and Ageing, reviews all notifications of AFP. Cases may be classified as poliovirus due to wild poliovirus, VDPV or VAPP; non-polio AFP; or non-AFP. Australia's population aged under 15 years was estimated at approximately 3,922,000 in 2001. In order for Australia to fulfil the WHO surveillance target of one AFP case per 100,000 population in this age group,[6] 40 notifications of AFP cases would have been expected during 2001.

Laboratory investigations

The National Poliovirus Reference Laboratory at VIDRL characterises all viruses isolated from AFP cases and all incidental isolations of polioviruses, for instance, viruses isolated from the nasopharynx of infants following vaccination. Results of laboratory investigations in this report are for tests performed in the calendar year 2001, irrespective of the date of notification of an AFP case or receipt of a referred incidental poliovirus. The laboratory also tests untyped enteroviruses referred from other Australian laboratories.

All polioviruses isolated by cell culture are tested to determine whether they are Sabin or non-Sabin-like. The WHO global network of poliovirus reference laboratories uses standardised methods for enzyme-linked immunosorbent assay (ELISA), nucleic acid probe hybridisation and polymerase chain reaction (PCR) for the intratypic differentiation of the 3 serotypes of Sabin and wild-type polioviruses. To maintain standards, all laboratories within the WHO poliovirus global network undergo accreditation on an annual basis and participate in quality assurance programs.

Intratypic differentiation of poliovirus isolates

Nucleic acid probe hybridisation and PCR are directed to highly conserved regions of the poliovirus genome and differentiate the 3 poliovirus serotypes from one another and Sabin-like from non-Sabin-like viruses. Differentiation of viruses by the ELISA is based on polyclonal antisera to polioviruses. The ELISA test is capable of detecting minor amino acid changes to the antigenic regions within the poliovirus capsid protein. A Sabin virus that has accumulated mutations may result in a VDPV with a test result of non-Sabin-like by ELISA but Sabin-like by the nucleic acid probe hybridisation and PCR tests. WHO laboratory protocols require all polioviruses isolated on or after 1 January 2001 to be tested by two methods of intratypic differentiation, one of which must be the ELISA.

Viral isolation and neutralisation

Faecal specimens are transported cold to the poliovirus reference laboratory to ensure virus viability. Specimens are extracted in chloroform and cell culture media and added to a panel of continuous cell lines. The main cell line for isolation of poliovirus is L20B, a mouse fibroblast line with a stable genetic integration of the poliovirus receptor.[7] Other cell lines, including RD-A (human rhabdomyosarcoma) and HEp2C (human epithelium carcinoma) are used for the isolation of non-polio enteroviruses as well as polioviruses. Identification of both polioviruses and non-polio enteroviruses is confirmed by antisera neutralisation.

Nucleic acid probe hybridisation

Inactivated viral ribonucleic acid (RNA) is immobilised on nylon membranes and individually tested with two digoxigenin-labelled oligonucleotide probes.[8] One probe is specific for a highly conserved sequence within the 5' non-translated region of enteroviruses and the other a Sabin serotype specific probe directed to the VP1 gene. A positive result with both probes indicates the isolate is Sabin-like, while a positive result only with the enterovirus probe may indicate a wild-type poliovirus.

Polymerase chain reaction and nucleotide sequencing

An alternative to the nucleic acid probe hybridisation method is to amplify viral RNA by PCR. The oligonucleotide primers are directed to the same genomic regions and interpretation of results is comparable to the probe hybridisation method. Sequencing of poliovirus isolates is performed using primers directed to the 5' non-translated region, VP1 and 3D subgenomic regions. Sequencing of non-polio enteroviruses is based on the method of Oberste.[9]

Enzyme-linked immunosorbent assay

The poliovirus ELISA differentiates between Sabin and wild-type strains using serotype specific antisera to intact virus particles of one strain that have been cross-adsorbed against the heterologous strain.[10] Results may indicate a Sabin-like virus, a non-Sabin-like virus or one with properties common to both strains (double reactive), arising from antigenic drift due to mutation of the Sabin virus genome.

Results

AFP surveillance in Australia

During 2001, 81 AFP notifications from 60 cases were received and reviewed by the Polio Expert Committee. Forty-six (76%) cases were first notified to the National Poliovirus Reference Laboratory at VIDRL with the balance notified through the APSU monthly reporting system. There were no cases of poliomyelitis and 44 cases were classified as non-polio AFP occurring among patients aged less than 15 years and resident in Australia (Figure 1). Other notifications were duplicates; from cases aged 15 years or more; from non-Australian residents; with date of paralysis onset in 2000; or were classified as non-AFP. As in previous years, Guillain-Barré syndrome was the most common single diagnosis, accounting for almost one third of all AFP cases (Table 1).

The WHO has defined AFP surveillance targets and Australia's AFP surveillance for 2001 is compared with these targets in Table 2. In 2001, the target of one notified and confirmed case per 100,000 population aged less than 15 years was exceeded (1.1/100,000 population). However, the proportion of cases with adequate stool samples was still below target, with only 36 per cent of notified cases having adequate stool samples, compared with a target of at least 80 per cent. Summaries of Australia's AFP surveillance from 1995 to 2001 are given in Table 3 and Figure 2. Figure 2 demonstrates the increasing proportion of duplicate notifications in the 7 years of surveillance.

Table 1. Classification of all cases notified through AFP surveillance, Australia, 2001

Case classification by Polio Expert Committee	Number	Per cent of non-polio AFP cases	Per cent of all cases
Polio AFP	0		
Non-polio AFP diagnosed as:	44		73
Guillain-Barré syndrome	14	32	
Transverse myelitis	6	14	
Acute demyelinating encephalomyopathy	3	7	
Non-polio enterovirus*	9	20	
Infant botulism	2	4	
Other	10	23	
Non-AFP	2		3
Non Australian resident/age >15 years/onset in 2000	10		17
Insufficient information for classification	4		7
Total cases	**60**		**100**

* Non-polio enterovirus isolations at the National Poliovirus Reference Laboratory: Coxsackie A24 = 1; Echovirus 11 = 2; Enterovirus 71 = 1; Enterovirus 71 identified in other laboratory = 5.

Figure 1. AFP notifications and testing of stool samples from AFP cases at the National Poliovirus Reference Laboratory, 2001

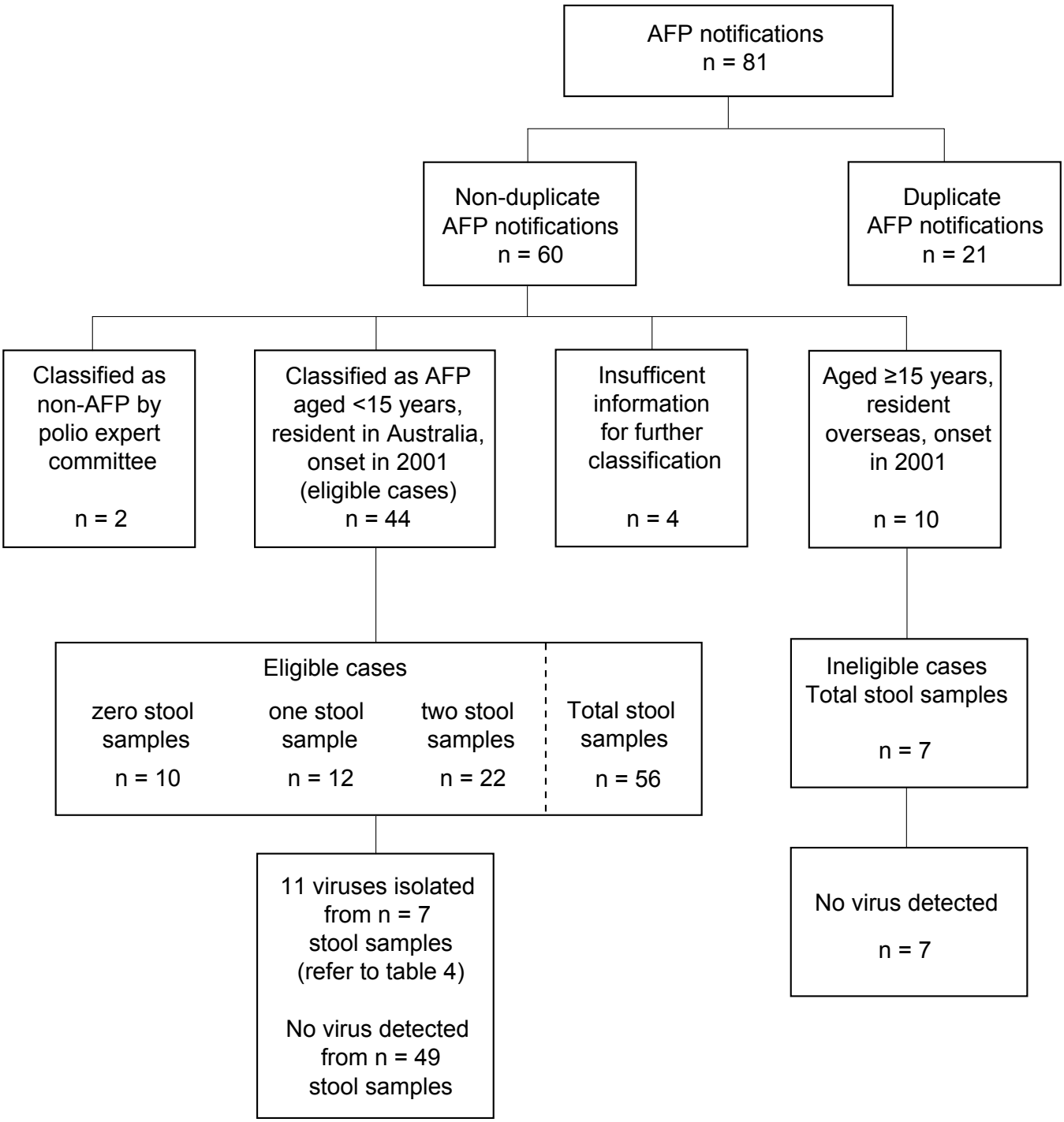


Table 2. AFP surveillance in Australia, 2001, compared with WHO indicator targets

WHO recommendation	Indicator	AFP surveillance 2001
Non-polio AFP cases per 100,000 population aged < 15 years	1/100,000	48 notified - 1.2/100,000 population 44 classified by the Polio Expert Committee 1.1/100,000 population
Percentage of routine surveillance sites that provide routine reports including (zero reports) on time	>80%	98% (completed monthly reports from the Australian Paediatric Surveillance Unit)
Percentage of AFP cases that are investigated	>80%	92%
Percentage of AFP cases that are investigated within 48 hours of notification	>80%	57% (investigated for questionnaire completion and stool collection within 48 hours of notification)
Percentage of AFP cases with a follow-up examination for residual paralysis at 60 days after the onset of paralysis	>80%	92% (only cases whose diagnosis and outcome could not be established by the initial questionnaire and laboratory tests required a 60 day follow-up)
Percentage of AFP cases with 2 adequate stool samples collected at least 24 hours apart within 14 days of onset of paralysis	>80%	36%

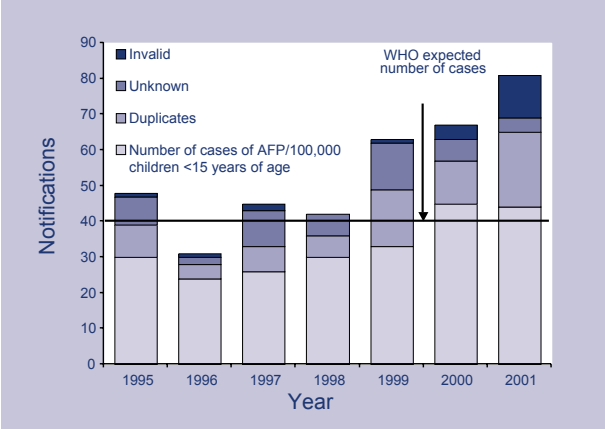
Table 3. Australia's AFP surveillance compared with WHO targets, 1995 to 2001

Year(s)	WHO target number of AFP cases detected per year in Australia and classified as eligible by the Polio Expert Committee*	Total number of Australian resident cases ascertained and classified by the Polio Expert Committee (per cent of target)	Proportion of cases with 2 stools, collected at least 24 hours apart and within 14 days of the onset of paralysis, tested for the exclusion of wild poliovirus at the National Poliovirus Reference Laboratory (WHO target > 80%)
1995-1999†	195 (average 39)	148 (76%)	25%
2000	39	45 (115%)	31%
2001	40	44 (110%)	36%

* An eligible case has acute flaccid paralysis, is aged <15 years and is resident in Australia. A minimum of 39 cases should be found in Australia per year, equivalent to 1/100,000 resident population aged <15 years.

† Data from the 7th Annual report of the Australian Paediatric Surveillance Unit.

Figure 2. Classification of notified acute flaccid paralysis cases, Australia, 1995 to 2001



Laboratory investigations

The origin of viruses isolated from AFP cases incident in 2001, is shown in Figure 1 and the origin of all isolates referred to the National Polio Reference Laboratory in 2001 is shown in Figure 3.

AFP cases

No wild poliovirus was detected in any AFP case tested in 2001. Sabin vaccine-like poliovirus serotypes 1, 2 and 3 were isolated from 2 faecal specimens of a single patient. No significant nucleotide sequence variation was detected between each of the poliovirus and corresponding Sabin vaccine serotypes. *Clostridium botulinum* type B organism and toxin were also detected from this patient at the Women's and Children's Hospital, Adelaide, and the case was classified as infant botulism. Enterovirus 71 (EV71) was isolated from 3 cases, echovirus 11 from 2 cases, Coxsackie A24 from one case and an untyped adenovirus from another case. Two of the EV71 viruses were isolated in 2001 from AFP cases with onset late in 2000. After 14 days of culture no virus was detected from the remaining 61 specimens, including stool specimens from cases incident in 2000 (Table 4).

Referred enterovirus isolates

Seventy-four viruses were identified from 79 referred isolates (Table 4). These included 40 polioviruses, generally recovered from recently immunised infants, all of which were Sabin-like. Thirteen of 27 non-polio enteroviruses referred to the National Poliovirus Reference Laboratory for identification, were characterised as EV71, Coxsackie A9 and echovirus serotypes 6, 9 and 13 (Table 4). Five uncharacterised polioviruses, referred to VIDRL for intratypic differentiation from an Australian laboratory following a review of stored untyped poliovirus isolates as part of poliovirus laboratory containment, were characterised as non-Sabin-like serotype 2. Table 5 summarises the laboratory activities from 1995 to 2001.

Figure 3. Isolates referred to the National Poliovirus Reference Laboratory, 2001

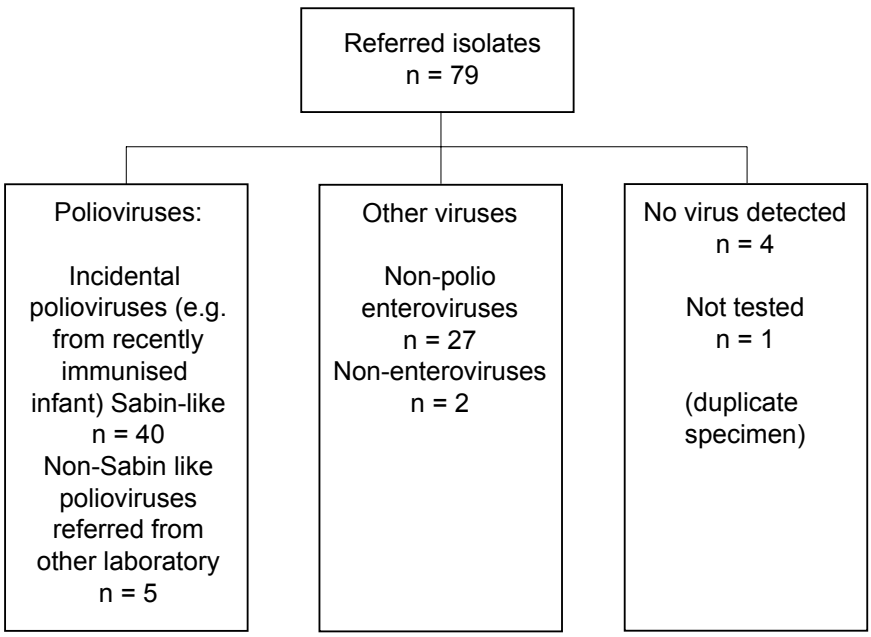


Table 4. Results of National Poliovirus Reference Laboratory testing performed, Australia, 2001

Isolation result	Isolates from AFP cases		Referred enterovirus isolates*	Total isolates
	< 15 years	≥ 15 years		
Poliovirus Sabin-like type 1†	2	0	14	**16**
Poliovirus Sabin-like type 2†	2	0	10	**12**
Poliovirus Sabin-like type 3†	2	0	16	**18**
Poliovirus non-Sabin-like type 3‡	0	0	5	**5**
Adenovirus type 5	0	0	1	**1**
Adenovirus (untyped)	1	0	0	**1**
Coxsackie A9	0	0	1	**1**
Coxsackie A24	1	0	0	**1**
Echovirus 6	0	0	6	**6**
Echovirus 9	0	0	2	**2**
Echovirus 11	2	0	0	**2**
Echovirus 13	0	0	2	**2**
Enterovirus 71§	3	0	2	**5**
Non-polio enterovirus	0	0	14	**14**
Rhinovirus	0	0	1	**1**
No virus detected after 14 days culture‖	61	7	4	**72**
Not tested¶	2	0	1	**3**
Total	**76**	**7**	**79**	**162**

* Includes polioviruses isolated incidentally from recently immunised infants.

† Three poliovirus serotypes in each of 2 specimens.

‡ Isolates referred as part of laboratory containment of poliovirus.

§ EV71 isolated from 2 cases with onset in late 2000 and one with onset in 2001.

‖ Includes specimens from patients with onset in 2000 but tested in 2001.

¶ Inappropriate specimens (e.g. urine).

Table 5. Summary of enterovirus testing at the National Poliovirus Reference Laboratory, Australia, 1995 to 2001

Year	Poliovirus		Non-polio enterovirus	Non-enterovirus detected or no virus detected	Total isolates 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**

* From 2000, Australian laboratories have been referring untyped enteroviruses and undifferentiated poliovirus as part of the laboratory containment of poliovirus.

Quality assurance, accreditation and training

The National Poliovirus Reference Laboratory at VIDRL retained its full accreditation status after a review by a representative of the WHO's Vaccine Assessment and Monitoring Team in August 2001. Proficiency panels for the techniques of poliovirus isolation and serotyping from faecal samples, nucleic acid probe hybridisation, ELISA and diagnostic PCR were also successfully completed in 2001. During November 2001, in collaboration with the polio reference laboratory from the Netherlands, VIDRL hosted an ELISA workshop with participants from the poliovirus laboratories of China, Hong Kong, Japan, New Zealand, the Philippines and Singapore.

Regional surveillance conducted by the National Poliovirus Reference Laboratory

More than 400 enteroviruses, including 285 polioviruses, were characterised in the laboratory's role as a regional reference laboratory. The laboratory was involved in the isolation and characterisation of 3 cVDPV isolates from the Philippines. All cVDPV isolates demonstrated a non-Sabin-like reaction in the poliovirus ELISA. Nucleotide sequencing of the isolates revealed they were derived from the Sabin oral polio vaccine, with more than 3 per cent nucleotide sequence variation in the VP1 gene and up to one per cent variation between the isolates. A recombination event had occurred within the non-capsid region with a non-polio enterovirus in all 3 viruses.

Discussion

AFP surveillance in Australia

Australia was certified free of circulating endemic wild poliovirus in 2000, although the last case of poliomyelitis in Australia due to an endemic infection was in the early 1970s.[11] Australia achieved the WHO target for notification of AFP cases (prospectively) for the first time in 2000.[12] Retrospective reviews of hospital records had to be undertaken in order for Australia to reach the target necessary for certification.[13,14] In 2001, Australia was again able to achieve the AFP surveillance target (prospectively). The AFP surveillance system has improved steadily since 1996.

Guillain-Barré syndrome remains the major cause of AFP in countries that are not poliovirus endemic. However, other viruses such as enterovirus 71 can cause AFP. Enterovirus 71 is a common cause of hand, foot and mouth disease but has also been associated with neurological disease including encephalomyelitis. Some cases of hand, foot and mouth disease with neurological complications have had a similar clinical presentation to poliomyelitis. Large outbreaks, which included fatal cases as a result of enterovirus 71 infection with severe neurological disease, have recently occurred in Taiwan[15] Malaysia,[16] Singapore[17] and Western Australia.[18,19]

AFP surveillance has also highlighted the occurrence of infant botulism in Australia, with 3 cases identified in 2000 and a further 2 cases in 2001. Poliovirus was isolated incidentally from the faeces of three of these 5 cases. Since infant botulism occurs during the first 12 months of life, this may be around the time of the administration of the oral polio vaccine. The similarity of presenting symptoms makes it imperative to differentiate infant botulism from VAPP.

Uncharacterised polioviruses identified as non-Sabin-like

Five non-Sabin-like poliovirus isolates were identified amongst a collection of uncharacterised polioviruses referred to the reference laboratory in 2001. This highlights the importance for all laboratories to undertake a thorough examination of the contents of their freezers to identify any material (biological or environmental) that potentially contain poliovirus. The WHO strategy for the post-eradication phase of poliomyelitis is for the global containment of all polioviruses in specified laboratories prior to cessation of poliovirus immunisation. The finding of previously unsuspected non-Sabin like polioviruses in one Australian laboratory highlights the importance of following WHO guidelines for the containment of poliovirus. Any laboratory that has material no longer required but potentially containing poliovirus or untyped enteroviruses should destroy the material by incineration or refer the samples to the National Poliovirus Reference Laboratory. If the material is still required, an aliquot should be referred to the reference laboratory for testing.

Implications for Australia of AFP surveillance in other Western Pacific Region countries

The circulating vaccine-derived serotype 1 polioviruses isolated from the Philippines were determined to have more than 3 per cent nucleotide substitutions within the VP1 gene compared to the parental Sabin sequence and to have undergone a recombination event with a non-polio enterovirus in the non-capsid region.[3] These genomic modifications were comparable to those of the cVDPVs isolated from the island of Hispaniola in 2000.[4,5] The two recent cVDPV outbreaks occurred in countries that are part of

WHO administrative regions, the Americas and the Western Pacific, that had been declared free of circulating wild poliovirus. Widespread vaccination programs have interrupted both outbreaks with ongoing surveillance to monitor potential VDPV circulation. However, the experience in these countries underlines the need for maintaining a high coverage of polio vaccination, even where the circulation of wild poliovirus has been eliminated. High quality clinical and laboratory surveillance is important to detect cVDPV, cases of VAPP, and cases of AFP due to imported wild poliovirus. It is for these reasons that Australia must continue surveillance for some years after the world has been certified as free of circulating wild poliovirus.

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