Report of the Australian National Polio Reference Laboratory

1 January 1999 to 30 June 1999

Towards WHO certification as wild poliovirus-free: laboratory surveillance in Australia

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Abstract

Since 1994 the Australian National Polio Reference Laboratory at the Victorian Infectious Diseases Reference Laboratory has been responsible for virological confirmation of the eradication of poliomyelitis in Australia. The laboratory is also a World Health Organization Western Pacific regional polio reference laboratory and the national polio laboratory for the Pacific Island countries and for Brunei Darussalam. It is now over two years since the last case of poliomyelitis was detected in the Western Pacific region of the World Health Organization. The co-operation of staff in all laboratories where polioviruses are handled and where samples from acute flaccid paralysis (AFP) patients are submitted is required until Australia and the region can be declared wild poliovirus-free. The characterisation of all polioviruses isolated in Australia in this reporting period led to the apparent detection of a non Sabin vaccine-like poliovirus in an environmental sample. The virus was found to be identical to a laboratory control isolate by sequencing. The environmental virus is therefore characterised as a contaminant, not a wild isolate. The investigation is outlined in this article, as well as the results of investigation and characterisation of all referred polioviruses. *Commun Dis Intell* 1999;23:324-327.

Introduction

Australia is working towards being certified as wild poliovirus-free. This requires that Australia meet criteria set by the World Health Organization (WHO) Regional Commission for the Certification of Poliomyelitis Eradication in the Western Pacific. Specifically, there must be no cases of poliomyelitis detected through a high quality acute flaccid paralysis (AFP) surveillance scheme.

Acute flaccid paralysis surveillance includes the collection and testing of two faecal samples from at least 80% of AFP cases at least 24 hours apart and within 14 days of onset.[1] The samples must be cultured for enteroviruses in a WHO accredited laboratory; the National Polio Reference Laboratory (NPRL) at the Victorian Infectious Diseases Reference Laboratory (VIDRL). In addition, staff in all laboratories that handle enteroviruses must transport all polio and untyped enteroviruses to the WHO accredited laboratory at VIDRL for identification and characterisation as wild or Sabin vaccine-like polioviruses. These processes have been in place since 1996.

This report describes the laboratory results from AFP surveillance and the identification and characterisation of polioviruses referred to VIDRL in the period from 1 January to 30 June 1999. Regular reports from the NPRL commenced in *CDI* in May 1999.[2] This is the second report from the National Polio Reference Laboratory. Further investigation of a non Sabin-like poliovirus is also described.

Methods and Results

AFP surveillance: samples from patients with acute flaccid paralysis

During the first 6 months in 1999, specimens were received from 10 patients with acute flaccid paralysis (AFP). There were 4 patients in Western Australia, 2 in Victoria, 2 in Queensland and 1 each in New South Wales and South Australia. Eighteen samples were received from the 10 patients. This represents twice as many specimens as received for the same period in 1998. The date of onset was available for only 5 patients. Specimens were collected from 3 of these patients within the recommended 14 days while those from the other 2 patients for whom date of onset was available were collected and referred 1 month and 3 months after onset. No samples yielded polioviruses. Two faecal specimens from 1 patient in Western Australia were positive for enterovirus type 71 (EV71), and 15 samples did not yield any virus (Table 1). There were duplicate stool samples from 4 children with AFP in Western Australia between March and June 1999 but only those from 2 patients were collected within 14 days of onset. An outbreak of EV71 occurred in Western Australia from March to June 1999.[3]

Neutralisation tests to detect poliovirus antibodies were performed on a serum sample from a 2 year old patient from Thursday Island with suspected paralysis. Elevated antibody levels to poliovirus type 1, 2 and 3 suggestive of past immunisation were detected.

Identification and characterisation of referred and stored entero/polio viruses

One poliovirus isolate from South Australia and one from Victoria were received for characterisation. Both were confirmed as Sabin-like by nucleic acid probe hybridisation (NAPH).

Table 1. Enterovirus culture results from 10 Australian acute flaccid paralysis cases, 1 January to 30 June 1999, by district

State	District/City	Specimen type and date	Result
Qld	Miami	F* 16-12-98	Negative
SA	Adelaide	F 04-03-99	Negative
		F 05-03-99	
NSW	Sydney	F 22-03-99	Negative
Qld	Cairns	F 24-03-99	Negative
WA	Perth	F 1 8-04-99	Negative
		F 19-04-99	Negative
WA	Perth	F 16-04-99	Enterovirus 71
		F 19-04-99	Enterovirus 71
		PNA# 21-04-99	Negative
Vic	Melbourne	F 12-05-99	Negative
		F 15-05-99	Negative
Vic	Melbourne	F 27/8-05-1999	Negative
		F 29-05-99	Negative
WA	Hamilton Hill	F 08-06-99	Negative
		F 10-06-99	Negative
WA	Yangebup	F 16-06-99	Negative
		F 17-06-99	Negative

* F = faeces
\# PNA = post nasal aspirate

All stored polioviruses isolated in Australia since January 1995 are required to be tested at the NPRL to enable WHO certification. In March 1999, the NPRL received 137 retrospective poliovirus isolates that had been identified and stored at the State reference laboratory in New South Wales between late 1994 and early 1999. Of these 131 were Sabin vaccine-like, 5 were non–polio enteroviruses (no cytopathic effects seen in polio selective L20B cells) and 1 was non Sabin-like poliovirus type 2.

The polio type 2 isolate was originally from an environmental specimen and was further investigated. It did not react in an NAPH test with Sabin-specific probes on three occasions, which suggested it was non Sabin-like. Enzyme immunoassay (EIA) tests at VIDRL and sequencing of the viral protein 1 (VP1) region at the Centers for Disease Control and Prevention, Atlanta, USA (CDC) also confirmed that the virus was non Sabin-like (Table 2). As wild poliovirus has not been isolated in Australia for over 20 years, it was considered that the isolate of non Sabin-like type 2 might have been a laboratory contaminant. Control viruses used at both the environmental and New South Wales State reference laboratories were therefore referred to the NPRL for characterisation.

The results of testing at NPRL and later at CDC are summarised in Table 2. The State reference laboratory control strains were Sabin vaccine-like. The two attenuated control polioviruses types 1 and 2 from the environmental laboratory were non Sabin vaccine-like (by NAPH and EIA at NPRL and sequencing at CDC). The environmental isolate of poliovirus type 2 was identical to the laboratory's control attenuated type 2 when the sequences of their VP1 regions were compared. It is therefore characterised as a contaminant.

Polioviruses isolated in the Fairfield Hospital Virology Laboratory from the early 1950s to 1991 are being recovered from storage and tested by NAPH. Forty strains

Table 2. Intratypic differentiation testing on isolates and control viruses referred to NPRL for characterisation by State reference and environmental laboratories in New South Wales

Isolate/control	Source laboratory	Test	Poliovirus type	Testing laboratory
Polio 2 NSW "isolate"	EL	NAPH,	Non Sabin 2,	
		EIA	Polio 2 indeterminate	VIDRL
		Sequence VP1	Non Sabin 2♦	CDC
Polio 2 "Atten" control	EL	NAPH,	Non Sabin 2,	
		EIA	Polio 2 indeterminate	VIDRL
		Sequence VP1	Non Sabin 2♦	CDC
Polio 1 Sabin control	VIDRL ex WHO	NAPH#, EIA	Sabin1	VIDRL
Polio 1 Wild control	VIDRL ex WHO	NAPH, EIA	Non Sabin 1	VIDRL
Polio 2 Sabin control	VIDRL ex WHO	NAPH, EIA	Sabin2	VIDRL
Polio 2 Wild control	VIDRL ex WHO	NAPH, EIA	Non Sabin 2	VIDRL
Polio 1 "Sabin" control	RL*	NAPH, EIA	Sabin1	VIDRL
Polio 2 "Sabin" control	RL	NAPH, EIA	Sabin2	VIDRL
Polio 1 "Atten" control	EL Ψ	NAPH, EIA	Non Sabin 1	VIDRL
		Sequence VP1θ	Non Sabin 1	CDC

"Atten" attenuated
*Reference Laboratory
Ψ EL Environmental Laboratory
♦ Indentical sequence
NAPH Nucleic acid probe hybridisation
EIA Enzyme Immuno Assay
θ VP1 Viral Protein 1

Table 3. Chronological summary of identification of enteroviruses and intratypic differentiation of polioviruses from Australian laboratories, 1995 to 30 June 1999 , by State

State	Year	Polio Sabin-like	Polio non Sabin-like	Non Polio Enterovirus	Non Enterovirus/ Negative	Total
Vic	1995	9				9
	1996	17				17
	1997	5				6
	1998	7				7
	1999	1				1
Qld	1995	41		5	8	54
	1996	99		4	9	112
	1997	41				41
	1998	8		15	2	25
WA	1995/ 6	126		359	5	490
	1997	9		33		42
	1998					0*
Tas	1995	1				1
	1996	3				3
	1997	4				4
	1998	4				4
NSW	1994	4				4
	1995	74		5		79
	1996	24				24
	1997	10				10
	1998	19	1[#]			20
SA	1997	3				3
	1998	3				3
	1999	1				1
Total	1995-June 1999	514		421	24	960

*PCR has replaced culture for enteroviruses, so isolates are no longer available.

of poliovirus type 1 have been tested to date. The stored viruses isolated prior to 1963 have been identified as non Sabin-like. All those recovered after this date have been identified as Sabin-like.

The chronological results of testing on entero and polioviruses submitted from all States are summarised in Table 3. Since 1994, 960 virus isolates have been transported to VIDRL from laboratories in five Australian States. Five hundred and fourteen (53%) were confirmed as Sabin vaccine-like polioviruses, 421 (44%) were non polio enteroviruses and 24 (3%) yielded no virus or viruses other than an enterovirus and one was non Sabin-like poliovirus type 2.

Containment of wild poliovirus

A letter and flyer containing information about the containment of wild polioviruses and potentially infectious materials was distributed by the Department of Health and Aged Care to over 600 staff in Australian hospitals and research institutions. The flyer was included, with an article on polio eradication, in the May issue of *Microbiology Australia*.[4] One year after the last case of poliomyelitis is detected in the world, all such viruses and materials must be destroyed or transported to a designated 'Physical Containment Level 3' laboratory.[5] The NPRL welcomes queries concerning the status of stored materials and appropriate action to be taken.

Other activities

The NPRL provided support services to other laboratories. Cell cultures of RD and L20B cells were supplied to laboratories in New South Wales and Western Australia to assist in the identification of polio and non polio enteroviruses. An aliquot of rabbit anti-enterovirus type 71 serum was supplied to a Western Australian laboratory for the identification of enterovirus isolates during the outbreak in early 1999.

The laboratory was designated as one of three WHO Western Pacific regional polio reference laboratories in 1990 and serves as the national polio laboratory for the Pacific Island countries and for Brunei Darussalam. Activities include intratypic differentiation of polioviruses referred from national laboratories, administration of the quality assurance program for the region, accreditation of laboratories, provision of assistance to the regional office, training of personnel in the region and the banking and distribution of cell cultures and of reagents. Virus isolation is performed on samples from AFP cases for countries without virus laboratories.

Discussion

Surveillance and investigation of acute flaccid paralysis

None of the samples from 10 patients with AFP in the first 6 months of 1999 yielded poliovirus. However, the number of patients who had samples for testing in this period was inadequate to meet WHO requirements. The target number for this period was 20 (there should be at least 40 children less than 15 years of age diagnosed with AFP in Australia each year[6]). Nonetheless, compared with 1998 there has been an improvement in the number of patients reported. Awareness of the need for surveillance and testing has been raised by the AFP Study Group of the Australian Paediatric Surveillance Unit, communications in *CDI*[6] and the Australian Poliovirus Laboratory Newsletter.[2]

Identification and characterisation of referred enteroviruses

Apart from the role of determining the vaccine-like status of polio isolates, the value of this AFP surveillance and the reference laboratory was illustrated in the first 6 months of 1999 by the identification of enterovirus type 71 associated with acute flaccid paralysis in an outbreak in Western Australia. As well as the four AFP patients, children were diagnosed with aseptic meningitis and hand-foot and mouth disease.[3] This virus has also been associated with fatal central nervous system disease in Japan, Taiwan, Malaysia and Hong Kong in the last 2 years.[7] As wild poliovirus is eradicated from the world, surveillance leads to the recognition of non-poliovirus causes of acute flaccid paralysis.

Characterisation of polioviruses

One isolate that had been stored in New South Wales and was originally identified in an environmental sample was characterised as not vaccine-like (non Sabin). This isolate is considered to be a laboratory contaminant. The detection of this non Sabin-like poliovirus type 2 highlights the necessity for State laboratories to transport all polioviruses to VIDRL for characterisation in a timely manner. The non Sabin-like virus was originally detected in July 1998, transported to NPRL at VIDRL in March 1999 and first sequenced in May 1999. As part of its certification process, Australia is committed to carry out a rapid outbreak response if wild poliovirus is detected. Had this been a confirmed wild poliovirus isolated from a patient or environmental sample a rapid outbreak investigation would not have been possible given the reported timeline.

The last wild poliovirus in Australia

In early 1998, stored polioviruses isolated from Victorian and some interstate patients and contacts between 1954 and 1966 were transported to CDC. All were presumed to be wild polioviruses as Sabin vaccine was first administered to Australians in 1966. The VP1 region of these viruses was sequenced and compared with viruses from other countries and periods. There is some evidence that circulation of wild poliovirus type 1 may have ceased in Victoria by 1963 (personal communication, Olen Kew CDC). These confirmed wild polioviruses are now archived in the global strain bank located at CDC. NPRL is now recovering and characterising other stored polioviruses with the view to identifying the last wild poliovirus detected in Australia.[8] This testing is offered to all Australian laboratories.

Containment of wild poliovirus

WHO recommends that laboratories prepare inventories of their historic collections of wild poliovirus isolates (including prototypes) with a view to destruction or transfer to a designated laboratory. Since Victoria's historically important polioviruses have now been transferred to the global strain bank at CDC, it is planned that NPRC will destroy all its remaining stored wild polioviruses one year after the last case is detected globally.

There are still reservoirs of polio endemicity in the Indian subcontinent and sub-Saharan Africa. However the strategies to achieve and maintain high routine vaccine coverage, national immunisation days, improved surveillance systems and mopping-up vaccination activities have been accelerated in most of the major reservoir countries to achieve the year 2000 goal.[9]

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