Report of the Australian National Polio Reference Laboratory

1 July to 31 December 1999

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Abstract

Since 1994, as part of the global eradication of poliomyelitis, the Australian National Polio Reference Laboratory (NPRL) at the Victorian Infectious Diseases Reference Laboratory (VIDRL) has been responsible for virological testing to confirm the absence of poliomyelitis in Australia. Samples from patients with acute flaccid paralysis are transported to VIDRL for viral culture. Polio and enteroviruses are referred for intratypic differentiation as wild or Sabin (vaccine) strains. A total of 23 faecal specimens from 17 patients were processed for ente rovirus culture in the period 1 July to 31 December 1999. Since 1995, 1,078 enterovirus isolates from six states have been tested for the presence of wild poliovirus. To date, 562 strains were confirmed as Sabin vaccine-like, one non Sabin-like strain was identical with a laboratory control virus and the other strains were non-polio enteroviruses or other viruses. A World Health Organization (WHO) workshop in diagnostic polio polymerase chain reaction techniques was held at VIDRL in November 1999. The laboratory was reaccredited as a regional polio reference laboratory for the WHO Western Pacific region and a national laboratory for Australia, the Pacific Island countries and Brunei Darussalam. Planning is proceeding for the polio-free certification and containment of laboratory stocks of wild poliovirus infectious materials in Australia. *Commun Dis Intell* 2000;24:118-121.

Keywords: poliovirus, surveillance, acute flaccid paralysis, enterovirus

Introduction

This is the third report of the activities of the National Polio Reference Laboratory (NPRL). Earlier reports for the year 1998[1] and the first half of 1999[2] summarised information on acute flaccid paralysis surveillance, the terms of reference of the laboratory and their implementation. Ongoing activities of the NPRL include the culture of faecal samples from patients with acute flaccid paralysis referred from all Australian states and the characterisation of polioviruses and enteroviruses. The NPRL is also attempting to locate and test all the polioviruses and untyped enteroviruses reported to the Serology and Virology Surveillance Scheme (LabVISE) since 1997. A workshop for selected national polio laboratories in the region served by NPRL was held at the Victorian Infectious Diseases Reference Laboratory (VIDRL) in November 1999 to train staff from these laboratories in polymerase chain reaction (PCR) techniques for the identification and characterisation of polioviruses. NPRL was also successfully reaccredited at that time as a World Health Organization (WHO) regional polio reference laboratory, for a further year. The task of the containment of wild poliovirus infectious materials in Australia was contracted to VIDRL and an inventory of laboratories which may contain these materials, and a national plan to contain the materials are being developed. Further detail on each of these activities is presented in this report.

Methods

Collection and culture of samples from patients with acute flaccid paralysis

Adequate specimens are defined as 2 stool samples collected 24 to 48 hours apart within 14 days of onset of paralysis arriving at the laboratory with ice present. It is recommended that they are transported to the laboratory within three days of collection.

Neutralisation tests to detect poliovirus antibodies were performed on single serum samples from 3 patients with suspected paralysis.

Polymerase chain reaction workshop for selected national laboratories in the region

Prior to September 1999, all poliovirus isolates regardless of their source, were referred from national laboratories for characterisation in an accredited regional reference laboratory. In November 1999, a workshop was organised to provide training in diagnostic PCR techniques for the identification and intratypic differentiation of entero and polioviruses for selected national laboratories in the region. Three NPRL polio laboratory staff members and one each from the national laboratories in Singapore and Hong Kong, and the regional reference laboratory in China (Beijing) participated.

Two staff members from the WHO specialised polio reference laboratory at the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia, USA, facilitated the workshop.

A proficiency test of PCR testing will be conducted by CDC in June 2000 to confirm that NPRL has the capability to correctly identify the panel viruses. If the laboratory is successful the diagnostic PCR may then be used routinely to test referred samples from the region and within Australia. At present, the laboratory is accredited to perform nucleic acid probe hybridisation and enzyme immunoassay for the intratypic differentiation of polioviruses.

Table 1. Cumulative summary of identification of enteroviruses and intratypic differentiation of polioviruses from Australian laboratories from 1995 to 30 June 1999

State	Year	Polio Sabin-like	Non-polio enterovirus	Non-enterovirus/ negative	Total
NSW	1994	4			4
	1995	74	5		79
	1996	24			24
	1997	10			10
	1998	19			20#
	1999				^
Qld	1995	41	5	8	54
	1996	99	4	9	112
	1997	41			41
	1998	8	15	2	25
	1999	2			2
SA	1997	3			3
	1998	3			3
	1999	1			1
Tas	1995	1			1
	1996	3			3
	1997	4			4
	1998	4			4
	1999	4			4
Vic	1995	9			9
	1996	17			17
	1997	5			5
	1998	7			7
	1999	16			16
WA	1995/6	133	384	5	522
	1997	30	76		106
	1998				0*
	1999		2		2
Total	1995-99	562	491	24	1,078

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

\# Includes one non-Sabin poliovirus type 2.

^ A batch of polioviruses isolated in NSW and ACT from 1994 to 1999 were received at VIDRL in April 2000.

Laboratory accreditation

It is a requirement for each country's certification as 'polio-free' that all AFP samples and intratypic differentiation be performed in a WHO-accredited laboratory. An on-site inspection and review of work carried out in the previous year was conducted by a representative from WHO and verified that the laboratory had fulfilled all the criteria for accreditation as a national and regional polio reference laboratory.

Containment of wild poliovirus

The Western Pacific region of the World Health Organization has included containment of wild poliovirus infectious or potentially infectious materials as one of the criteria for each nation's certification as 'polio-free'. Currently, a regional pilot project is underway to facilitate the implementation of a draft plan for poliovirus containment. In order that Australia may meet this criterion the Australian National Certification Committee has appointed a National Coordinator of Poliovirus Containment who is located at VIDRL and approved the development of a national plan for containment of wild polioviruses. As part of this national plan a list of laboratories with stored wild poliovirus infectious materials will be prepared as a part of a national inventory to be submitted to the WHO regional office and included with the national certification documents.

Results

Characterisation of referred entero/polioviruses

One hundred and nineteen polio or untyped enteroviruses were referred to the laboratory between July and December 1999. Ninety-eight of these were viruses isolated in Western Australia between 1996 and 1999. The remaining isolates were from Victoria, Tasmania and Queensland. Forty-nine (41%) of the original 119 referred were recovered in L20B cells (cell selective for polioviruses) and were identified as Sabin vaccine-like polioviruses. Thirty-five (29%) were recovered in rhabdomyo sarcoma (RD) but not in L20B cells, so were non-polio enteroviruses. Thirty-five (29%) not recovered in RD or L20B cells were non-polio enteroviruses or were no longer viable.

The cumulative results of testing on entero and polioviruses submitted from all States and Territories are summarised in Table 1. Since 1995, 1,078 virus isolates have been transported to NPRL from laboratories in five Australian States. Five hundred and sixty two (52%) have been confirmed as Sabin vaccine-like polioviruses, 491 (46%) were non-polio enteroviruses and 24 yielded no virus or viruses other than enteroviruses. One poliovirus characterised in March 1999 as non Sabin-like was described in an earlier report.[2]

Acute Flaccid Paralysis

During the second half of 1999, 23 specimens were received from 17 patients with acute flaccid paralysis (AFP) (Table 2). Samples were received from 7 patients in Queensland, 4 in Victoria, 3 in Western Australia, 2 in New South Wales and one in Tasmania.

Onset dates were only available for 2 patients, both of whom had faeces collected within 14 days of onset of symptoms. Samples from 5 patients were dispatched to NPRL within three days of collection, while 4, 6 and 2 were sent after three to seven, eight to 14 and greater than 14 days respectively. No information was given on storage of the samples before transport. No enteroviruses were isolated from samples from any AFP patient in this reporting period.

One Sabin-like poliovirus type 1 was isolated from faecal samples from a 59 year-old woman, who had received Sabin (oral polio vaccine) prior to travel to Indonesia and developed fever and rigors, possibly vaccine-related, three days later.

Table 2. Specimens processed from Australian patients with AFP 1 July to 31 December 1999

State	District/city	Specimen date	Result
Qld	Brisbane	1-05-99	No virus isolated
Qld	Brisbane	23-06-99	No virus isolated
Qld	Brisbane	29-06-99	No virus isolated
Qld	Brisbane	3-07-1999	No virus isolated
NSW	Jerrabomberra	23-07-1999	No virus isolated
		24-07-1999	No virus isolated
Vic	Omeo	10-08-1999	No virus isolated
Vic	Dandenong	15-08-1999	No virus isolated
Vic	Dandenong	18-08-1999	No virus isolated
Vic	Dandenong	23-08-1999	No virus isolated
		23-08-1999	No virus isolated
Qld	Middlemount	3-09-1999	No virus isolated
Qld	Toombul	12-09-1999	No virus isolated
Qld	Samford	9-10-1999	No virus isolated
		13-10-1999	No virus isolated
WA	Girrawheen	14-10-1999	No virus isolated
		15-10-1999	No virus isolated
NSW	Wallsend	3-11-1999	No virus isolated
		4-11-1999	No virus isolated
		8-11-1999	No virus isolated
WA	Glen Forest	4-11-1999	No virus isolated
		TS 4/11/99	No virus isolated
WA	Kalgoorlie	15-11-1999	No virus isolated
		16-11-1999	No virus isolated
Tas	Gravelly Beach	24-12-1999	No virus isolated
		25-12-1999	No virus isolated

Two patients had elevated antibody levels to poliovirus types 1, 2 and 3, suggestive of past immunisation. The third patient had antibodies to poliovirus types 2 and 3 only, suggestive of a failed type 1 response to vaccination.

Discussion

Samples from AFP patients

Although still below the target of 78 samples from 39 children in Australia aged less than 15 years (one per 100,000), there was a further improvement in the numbers of samples referred to NPRL from patients with acute flaccid paralysis. During 1999, 41 faecal and 1 respiratory sample were processed from 27 patients. In 1997 and 1998, samples were referred from 4 and 11 patients with AFP respectively.[1]

Most of these patients with AFP were also reported to the AFP study group of the Australian Paediatric Surveillance Unit[3] (APSU) and reviewed by the Polio Expert Committee. The absence of wild polioviruses in these samples was used to classify these patients as non-poliomyelitis.[4] There were several patients from whom faecal samples were received but details of their histories have not yet been forwarded to APSU by the clinicians involved in their management. More information is being sought on these patients so they may be included in the final analysis of AFP cases in Australia in 1999.

Characterisation of polioviruses

As a requirement for Australia's certification as a polio-free country, all polioviruses isolated regardless of source must be characterised as Sabin (vaccine) or wild types. The *Communicable Diseases Intelligence (CDI)* publishes virology and serology laboratory reports received through the LabVISE programme. Over 60 uncharacterised polioviruses and nearly 800 untyped enteroviruses were reported in 1999. To date, only 23 polioviruses and two non-polio enteroviruses isolated in 1999 have been referred and tested at NPRL. The other strains received in 1999 were isolated from 1996 to 1998.

Western Pacific Regional certification

The last case of locally acquired poliomyelitis due to wild poliovirus in the Western Pacific occurred in Cambodia in March 1997. In November 1999, a strain of wild poliovirus type 1 was isolated in China. However, the case was epidemiologically and virologically linked to a virus possibly imported from the Indian sub-continent. There has been no evidence of re-established indigenous transmission in China.[5]

The Americas were certified in 1994, three years after their last case was detected in Peru. The Western Pacific Regional Certification Commission is meeting in July 2000 to examine evidence to prepare a case for certification. If the decision is favourable, the Western Pacific Region will be the second of the six WHO regions to be certified polio-free.

Containment of wild poliovirus

As a part of certification each country is required to provide evidence of three years of high immunisation rates, quality AFP surveillance, no wild polioviruses isolated from any source and a plan developed in the event that wild poliovirus is imported.[1] The last indigenous wild poliovirus in Australia was most likely in the mid-1960s.[2]

For certification in the Western Pacific Region, an additional criterion has recently been added. Since circulation of wild poliovirus has ceased, the only sources of wild polioviruses or wild poliovirus infectious materials are from importations from countries where endemic poliomyelitis still occurs or in laboratories. The region has developed an action plan, which includes a national search of all medical/biological laboratories which may have wild poliovirus infectious or potentially infectious materials, and the preparation of a national inventory system for laboratories which contain such materials.[6] Once containment issues have been addressed in the Western Pacific region, a detailed plan will be available which may be adapted in other regions. A more comprehensive report on laboratory containment of wild polioviruses is being prepared.

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