Report of the Australian National Polio Reference Laboratory
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Margery Kennett, Kerri Anne Brussen, Vicki Stambos, Ann Turnbull, Aishah Ibrahim and Heath Kelly
The Australian National Polio Reference Laboratory, Locked Bag 815, Carlton South, Melbourne, Victoria 3053.

Abstract

The Australian National Polio Reference Laboratory was established at the Victorian Infectious Diseases Reference Laboratory (VIDRL) in late 1994 to carry out virological confirmation of the eradication of poliomyelitis in Australia. The laboratory is responsible for transporting samples from all Australian patients with acute flaccid paralysis (AFP) to VIDRL for poliovirus culture, identification and intratypic differentiation. The laboratory also performs polio serology on selected serum samples from AFP patients when faecal samples are not available. In 1998, faecal specimens were received from 11 patients with AFP. Adenovirus type 2 was isolated from 1 patient and an untypable non-polio enterovirus from another. No viruses were isolated from the other 9 patients. Since 1995, over 820 isolates have been transported to VIDRL from laboratories in five Australian states for testing. Three hundred and seventy three (45%) were confirmed as Sabin vaccine-like polioviruses, 416 (51%) were non-polio enteroviruses and 24 (3%) yielded no virus or viruses other than enteroviruses. Eight polioviruses are still uncharacterised. *Commun Dis Intell* 1999;23:124-128.

Introduction

In 1988, the World Health Assembly passed a resolution which committed the World Health Organization (WHO) to the global eradication of poliomyelitis by the year 2000. The eradication strategy is three-fold: routine and supplementary immunisation, surveillance and, where required, outbreak response.[1,2]

Almost all countries of the six WHO regions are committed to polio eradication. Each country is required to verify the absence of wild poliovirus circulation in the presence of high quality surveillance. Acute flaccid paralysis (AFP) has been proven to be a sensitive indicator for detecting wild poliovirus and was used successfully in the Americas prior to certification of that region as being free of wild poliovirus.[3] In a country where polio is not endemic, there is normally a background incidence of at least one AFP case for every 100,000 children under 15 years of age per year. Surveillance of cases of AFP by most recently endemic and some non-endemic countries of the Western Pacific has resulted in the documentation of AFP rates of at least one per 100,000.[4] Based on Australia's population

distribution, there are likely to be approximately 40 children with AFP each year.[5]

The laboratory plays a crucial role in surveillance as AFP may have many aetiologies. Wild poliovirus infection can only be confirmed by virological investigation. Faecal samples collected within 14 days of onset of paralysis are transported to a WHO approved laboratory for enterovirus isolation and identification. If poliovirus is isolated, the strain is characterised as Sabin or wild type.

This report describes the functions of the polio reference laboratory and its activities during 1998.

Terms of reference of the Australian National Polio Reference Laboratory

The Australian National Reference Laboratory at the Victorian Infectious Diseases Reference Laboratory (VIDRL) was established in late 1994. It is one of three WHO Western Pacific regional reference laboratories, the other two being in Tokyo and Beijing. Faecal samples from all reported AFP cases in Australia are transported to VIDRL and cultured for entero and poliovirus. Poliovirus strains isolated from these samples, and those isolated from non-AFP patients by virus laboratories throughout Australia are then referred to VIDRL, and identified and characterised as wild or Sabin vaccine-like. Polio neutralisation antibody tests are carried out on selected serum samples and for serosurveys.

Implementation of the terms of reference

Acute Flaccid Paralysis surveillance was commenced in Australia in March 1995 through the Australian Paediatric Surveillance Unit (APSU), the National Centre for Disease Control (NCDC) and the Australian National Polio Reference Laboratory at VIDRL. Prior to May 1996, AFP faecal samples were cultured in virology laboratories in Australian states and, if polio strains were isolated, these were referred to VIDRL. Since May 1996 all AFP faecal samples have been processed at VIDRL which is accredited by WHO as both a regional and national polio reference laboratory. The process of accrediting all state virology laboratories was not feasible considering the small numbers of specimens expected.

When VIDRL is notified of an AFP case under investigation or if enterovirus isolates are ready for shipment, the appropriate instructions for packing and, if necessary, containers and documentation are provided to

Table 1. Samples received for enterovirus culture from Australian AFP cases in 1998

State/City/District	Epid No.	Date received	Result
AFP			
Papua New Guinea/TSI	Aus001/98	Anal25-02-98	Negative
		TS 25/2/98	Negative
		F 25/2/98	Negative
		F 25/2/98	Negative
		F 25/2/98	Negative
NSW/ Hunter Valley	Aus002/98	F 3/4/98	Adeno 2
		F 3/4/98	Negative
Vic/Belgrave	Aus003/98	F 30/4/98	Negative
ACT/Canberra	Aus004/98	F 7/5/98	Negative
		F 7.5.98	Negative
Qld/Brisbane	Aus005/98	F 21/5/98	Negative
Qld/Cairns	Aus006/98	F 25/8/98	NPEV
NSW/Tweed Heads	Aus007/98	F 25/8/98	Negative
		F 25/8/99	Negative
Qld/Tugun	Aus008/98	F 23/9/98	Negative
		F 23/9/99	Negative
SA/Adelaide	Aus/009/98	F 02/10/98	Negative
SA/Adelaide	Aus/10/98	F 02/10/99	Negative
		F 13/10/98	Negative
Qld/Townsville	Aus/011/98	F 21/10/98	Negative
NON AFP			
Vic/Melbourne		F26/8/98	Negative

the requesting laboratory by VIDRL. The laboratory is instructed to pack the samples and then contact the laboratory's preferred carrier who collects these containers and ships them overnight to VIDRL for testing.

Faecal samples are processed and inoculated into the WHO-supplied cell lines, HEp2, RD and L20B. The use of the L20B cell line, which is a mouse cell line with receptors for human poliovirus, facilitates the preliminary separation of polio and most non-polio enteroviruses which have been isolated from AFP specimens or submitted from laboratories in all Australian states. The L20B positive strains are identified using type-specific poliovirus antisera and, if polioviruses, are characterised as Sabin vaccine or wild type using nucleic acid probe hybridisation (NAPH). The L20B negative virus isolates from all AFP samples are fully identified while those submitted from other laboratories are identified on request.

Polio antibody testing is performed only on samples from patients with paralysis when faecal samples have not been collected within 14 days of onset of paralysis. The interpretation of results may be difficult as type-specific antibodies are often raised at the time of onset of symptoms. In addition, the test does not distinguish between antibodies against wild and vaccine virus. Serosurveys are carried out in collaboration with researchers usually as part of immunisation studies.[6]

During 1997, a Polio site on the internet was established (*www.vidrl.org.au*). The information available includes global, regional and Australian updates on poliomyelitis eradication; AFP surveillance in Australia; the polio laboratory network; instructions for collection and transportation of stool samples and results of testing on enteroviruses referred to VIDRL for intratypic differentiation. The homepage has links to other related global sites and is up-dated annually and when new information is available.

The annual *Australian Polio Network Newsletter*, which contains similar material to that found on the homepage, is posted to Australian laboratories involved either with enteroviruses or which are likely to transport faecal samples and isolates to VIDRL for polio testing. The newsletter is also available to interested public health and other medical personnel (see below for subscription address).

Table 2. Cumulative summary of identification of enteroviruses and intratypic differentiation of polioviruses from Australian laboratories tested at VIDRL from 1995 to 1998

State	Year	Polio Sabin Like	Polio Pending ITD	Reovirus	Non-Polio Enterovirus	Herpes Simplex/ Negative	Total
Victoria	1995	9					9
	1996	16	1				17
	1997	5	1				6
	1998	7					7
Queensland	1995	41			5	8	54
	1996	98	1		4	9	112
	1997	40	1				41
	1998	8			15	2	25
Western Australia	1995-6	125	1	5	359		490
	1997	9			33		42
	1998						0*
Tasmania	1995	1					1
	1996	3					3
	1997	3	1				4
	1998	3	1				4
NSW	1995-8						0**
South Australia	1997	3					3
	1998	2	1				3
Total	1995-1998	373	8	5	416	19	821

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

** Polioviruses stored for shipment in 1999

Activities in 1998

During 1998, 19 faecal samples, one anal swab and one throat swab were received from 11 patients with AFP (Table 1). A faecal sample was also cultured to detect viral shedding in a Victorian transplant patient who had been given Sabin vaccine. Four AFP patients were from Queensland, two each from New South Wales (NSW) and South Australia and one each from the Torres Strait Islands, Victoria and Canberra. Duplicate samples were collected from only seven of the eleven patients. No samples yielded polioviruses and 20 of the 22 samples did not yield any virus. Adenovirus type 2 was isolated from the initial sample from one patient in NSW (Aus002/98). An enterovirus, isolated from a patient in Cairns in August, (Aus006/98) could not be identified with available antisera and will be further investigated using molecular techniques.

The first case (Aus001/98) was one of three young men from the same village on the south coast of Papua New Guinea who often travelled in the Torres Strait and visited islands there. All young men developed AFP in late 1997 or early 1998 and were admitted to hospitals in Port Moresby and Thursday Island. No samples were available from the first patient. Serum samples were collected from the second patient 5 days after admission and 31 and 91 days later. Rising antibody levels were detected between days 5 and 31 to all three polioviruses. The interpretation of these findings is uncertain. It suggests that this patient received polio vaccine after the first bleed was taken. However it is known that this did not occur as he was in the Thursday Island Hospital during this time (personal communication, Dr Jeffrey Hanna, Tropical Public Health Unit, Cairns). It appears that there may have been a mixup with the first sample which was transported via several pathology departments to VIDRL. Four stool samples and a throat and anal swab from the third patient were negative for enteroviruses. Serum samples collected from this patient on admission and after 3, 25 and 69 days showed elevated but stationary antibody levels to types 1, 2 and 3, suggestive of past immunisation or infection.

Fewer polio and enterovirus isolates were submitted for identification and characterisation in 1998 than in 1996 and 1997. The State laboratories in Western Australia and Queensland have introduced molecular methods for enterovirus detection and no longer culture samples for enterovirus isolation. The State laboratory in NSW has stored all polioviruses identified since 1995 and plans to ship them to VIDRL in early 1999. During 1998, of 39 'enteroviruses' submitted, 22 were polioviruses (Table 2). Twenty poliovirus isolates were confirmed as poliovirus Sabin-like and intratypic differentiation results are still pending for two. Echovirus types 6 (two isolates) and 19 (11 isolates) were cultured at the Queensland Health Scientific Services Laboratory in Brisbane from healthy children who lived in the same village in Papua New Guinea as the three young men with AFP. Coxsackievirus type A9 was isolated from two samples from another Queensland patient and two did not yield virus.

Further intratypic differentiation and enterovirus identification has been performed on isolates submitted in 1996 and 1997 (Table 2). Overall since early 1995, there were 373 (45%) Australian polioviruses characterised as Sabin-like, 416 (51%) were non-polio enteroviruses and 19 (3%) were enterovirus negative or herpes simplex virus.

Five isolates submitted from Western Australia in 1995-96 which produced a non-enterovirus cytopathic effect in L20B cells were confirmed by electron microscopy as reoviruses. There is some evidence that the eight polioviruses still uncharacterised are Sabin vaccine-like but further testing is necessary as titres were low or mixtures were detected.

Neutralisation tests to detect poliovirus antibodies were performed on serum samples of three Australian patients with suspected paralysis. Only single bleeds were collected. All patients had elevated antibody levels to poliovirus type 1, 2 or 3 which may suggest past immunisation or infection.

Discussion

Certification of non-endemic industrialised countries was discussed at the second meeting of the Global Commission for the Certification of the Eradication of Poliomyelitis in Geneva 1997.[7] The commission reaffirmed that the absence of wild poliovirus in the presence of high quality routine AFP surveillance among children aged less than 15 years should be regarded by all countries, regardless of their endemic status, as the gold standard of polio eradication. The WHO has set standard performance indicators for AFP surveillance. Reports must be timely and reporting sites must represent the geography and demography of the country. The surveillance system should be sensitive with all AFP cases being investigated soon after onset of symptoms and there should be a follow-up examination after 60 days. At least 80% of AFP cases should have two adequate stool specimens collected and tested in an accredited laboratory.[8]

Although some non-endemic countries have decided that it is impracticable to establish routine AFP surveillance, Australia has chosen to do so.[9] Since the last case of poliomyelitis was most likely to have occurred in the 1970s,[10] it is unlikely that indigenous wild poliovirus transmission will be detected. However there should still be a background of about 40 cases of AFP in children, mainly due to Guillain-Barre syndrome and transverse myelitis. Since AFP surveillance commenced in Australia in 1995, reporting of AFP cases and stool sample collection has not reached the WHO targets. Efforts are being made to address this problem.[9]

Under-reporting of AFP cases has been demonstrated in a recent Victorian study. The two-source capture-recapture method was used to estimate ascertainment of AFP cases in Victoria. The APSU study was used as the primary source and children admitted to two major teaching hospitals as the secondary source.[11] Only 27% of patients with symptoms of AFP had been reported to APSU in Victoria between 1995 and 1997.

There has been a positive response from staff in laboratories in hospitals around Australia who have been contacted by letter and telephone to advise them of the program. On some occasions, these scientists have been responsible for alerting clinical staff of the need to report AFP cases to the APSU and the NCDC.

The state virology laboratories have also supported VIDRL in its task to characterise all polioviruses isolated since 1995. It is fortunate that the program for characterisation of isolates was well established in 1995 since with the

introduction of molecular methods for enterovirus detection, there would be fewer isolates available in future years. To date, nearly 400 poliovirus isolates have been proven to be Sabin vaccine-like. Once the large number of isolates from NSW has been screened, over 950 enteroviruses will have been tested to exclude wild poliovirus.

With the expected global eradication of wild poliovirus by the end of the year 2000 or 2001, the next task for laboratories will be to either transfer wild poliovirus strains to designated repositories or destroy them. The continued cooperation of virologists in Australia will be sought to carry out this task.

Australian Polio Network Newsletter
Subscriptions: Locked Bag 815
Carlton South, Melbourne, Victoria, 3053

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