

Australia's last reported case of wild poliovirus infection

Margery L Kennett,¹ Kerri Anne Brussen,² David J Wood,³ Harrie G van der Avoort,⁴
Albert Ras,⁴ Heath A Kelly⁵

Abstract

In 1986 tests on faeces collected from a 22 year old Australian born man who had symptoms consistent with poliomyelitis yielded poliovirus type 3. In a neutralisation test using a panel of monoclonal antibodies the isolate was identified as wild poliovirus type 3 at that time. After further classification using microneutralisation, nucleic acid probe hybridisation, immunoassay and sequencing carried out in three laboratories between 1994 and 1997, the isolate has been reclassified as 'Sabin-like' with 'wild type' characteristics. This case has been quoted in the literature as Australia's last case of locally acquired wild poliovirus. Efforts are now being made to identify the true last case in Australia. This article describes the isolation, identification and further characterisation of this virus. *Comm Dis Intell* 1999;23:77-79

Introduction

In May 1988, the 41st World Health Assembly committed the member states of the World Health Organization (WHO) to the global eradication of poliomyelitis by the year 2000. The strategy to achieve this aim involves high routine immunisation coverage, supplementary immunisation in the form of National Immunisation Days or mass campaigns, effective surveillance and, in the final stages, 'mopping up' in areas where wild polio virus is present.¹

Committees have been established to certify that Australia is free of wild poliovirus by assessing national acute flaccid paralysis (AFP) surveillance and immunisation. In order for a country to be declared polio-free there must be no evidence of indigenous wild poliovirus transmission for a period of at least three consecutive years of quality surveillance.²

The last case of poliomyelitis caused by wild poliovirus acquired in Australia has been believed to have occurred in a 22 year old male.³ A virus was isolated from the case's faeces and identified as poliovirus type 3 in the Entero-Respiratory Laboratory at Fairfield Hospital (renamed The Victorian Infectious Diseases Reference Laboratory, VIDRL in 1992) in September 1986. It was characterised, in a neutralisation test using monoclonal antibodies as 'wild type', at the National Institute for Biological Standardisation and Control (NIBSC) in the United Kingdom (UK) in early 1987. Subsequent testing from 1994 to 1997 at the Victorian Infectious Diseases Reference Laboratory (VIDRL), at NIBSC UK, and at the National Institute for Public Health and the Environment (RIVM), the Netherlands has led to the reclassification of this virus as a 'drifted Sabin-like' virus. This virus, for over

10 years, had been recognised as the last indigenous wild poliovirus in Australia.

Methods

Patient

A man aged 22 years was admitted to a teaching hospital in September 1986 suffering from lower back pain, slight weakness of his left leg and difficulty in initiating micturition. The physical and CSF findings at the time were consistent with a diagnosis of poliomyelitis (Dr L. Irving, former Medical Microbiologist, Fairfield Hospital, personal communication).

Isolation and Identification (1986)

Faeces were collected from the patient, placed in a sterile container and transported to the Fairfield Hospital virus laboratory for virus culture. The methods used for the collection and treatment of the faeces in cell culture for the identification of enteroviruses were as previously described.⁴ Virus isolates which developed rapid cytopathic effects in primary monkey kidney epithelial cells (PMK), continuous monkey embryonic kidney epithelial cells (MEK), HeLa cells (Rhinovirus sensitive), Borrie epithelial cells and human embryonic lung fibroblast cells were provisionally considered to be polioviruses. These were identified in microneutralisation tests using reference horse antisera prepared against prototype polioviruses supplied by the National Institutes of Health, Maryland, USA.⁵

Intratypic differentiation (1986/7)

In the United Kingdom laboratory (NIBSC), microneutralisation tests were performed using pools of monoclonal antibodies prepared against the prototype poliovirus type 3 (Mab-N).⁶

1. Director, Australian National Polio Reference Laboratory (ANPRL) and Victorian Infectious Diseases Reference Laboratory (VIDRL).
2. Australian National Polio Reference Laboratory, VIDRL.
3. World Health Organization Specialised Reference Laboratory. National Institute for Biological Standards and Control (NIBSC), Potters Bar, Hertfordshire, United Kingdom.
4. World Health Organization Specialised Reference Laboratory. National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.
5. Head Epidemiology and Public Health Division, VIDRL.

Corresponding author: Mrs M. Kennett, Victorian Infectious Diseases Reference Laboratory, 10 Wreckyn Street, North Melbourne, Victoria 3051

Intratypic differentiation (1994-1997)

Nucleic acid probe hybridisation (NAPH) tests, using DIG (digoxigenin) labelled probes that recognise the VP1/2A region (either of Sabin poliovirus type 3 or of all polioviruses from all three types), were performed using a dot blot technique⁷ at VIDRL in 1994.

As in 1986/7, Mab-N tests were performed at NIBSC in 1995, but using different monoclonals.⁸

Enzyme immunoassay tests were performed in 1994 at VIDRL using cross absorbed polyclonal rabbit antisera prepared against Sabin and wild poliovirus type 3.⁹

RNA sequence analysis of the VP1/2A region using the method of Mulders et al¹⁰ and neutralisation tests using cross absorbed polyclonal antisera prepared against Sabin polio 3 and wild polio 3¹¹ were carried out at RIVM in the Netherlands in 1997.

Results

Isolation and identification (1986)

Poliovirus type 3 was isolated from the faecal specimen and was identified using a microneutralisation test at Fairfield Hospital.

Intratypic differentiation (1986/7)

Microneutralisation tests using type-specific monoclonal antibodies (Mab-N) were performed in the UK laboratory (NIBSC). Initial tests on the isolate in 1986/7 using a panel of 7 monoclonal antibodies identified it as a 'normal type 3 wild' virus with no relationship to the 'Sabin type 3' virus.

Intratypic differentiation (1994-1997)

In a nucleic acid probe hybridisation test performed at VIDRL, the isolate was clearly identified as 'Sabin-virus polio type 3'.

In an enzyme immunoassay at VIDRL and a serum neutralisation test at RIVM, the isolate reacted equally to cross absorbed polyclonal antisera, to both Sabin and wild poliovirus type 3.

When testing was repeated in 1995 at NIBSC on the isolate by Mab-N using the current monoclonal antibody panel, it was found to contain a type 3 vaccine-like isolate. At high virus challenge doses the Sabin specific monoclonal was unable to completely neutralise a type 3 virus.

RNA sequence analysis of the VP1/2A region at RIVM showed 100% identity with the 'Leon type 3', the wild-type progenitor of the Sabin poliovirus type 3 used in oral polio vaccine.

Discussion

After OPV was introduced in Australia in 1966,³ both wild and vaccine polioviruses were co-circulating for several years. In most situations, vaccine virus isolation was incidental to patients' illnesses (for example, from a throat swab from a child with acute respiratory infection) and could be linked to OPV immunisation several days earlier. Several methods for differentiation of wild and vaccine polioviruses which were employed in the 1960s and 1970s were found to be unreliable once more specific serological and molecular methods were developed.¹² As the newer

methods were not established at that time, polioviruses isolated at the Fairfield Hospital Virus Laboratory from suspected paralytic poliomyelitis cases in 1977 and 1986 were referred to international laboratories for characterisation. In 1986, the type 3 isolate from the 22 year old man was characterised as 'wild' at the UK reference laboratory. The finding was reported to the State and Australian Health Departments. It was then believed to be the last confirmed wild poliovirus infection contracted within Australia in a person who had not travelled outside Australia and who had not been a contact of any person recently returned from a polio-endemic country.

Currently, intratypic differentiation (ITD) of polioviruses may be carried out by at least three serological methods. Enzyme immunoassay (EIA) using cross-absorbed polyclonal antisera, or neutralisation tests using either cross-absorbed polyclonal antibodies or monoclonal antibodies (Mab-N), are those most commonly used. Most poliovirus strains are clearly identified as either 'wild' or 'Sabin vaccine-like'. However, some may react with both anti-wild and anti-Sabin antisera or with other than expected monoclonal antibodies.

The well-established molecular methods for intratypic differentiation (ITD) in use are nucleic acid probe hybridisation (NAPH), polymerase chain reaction (PCR) or restriction fragment length polymorphism (RFLP). These methods use different primers and probes specifically reacting with coding sequences from the VP1/2A region. As all Sabin strains are similar, these can be readily differentiated from wild viruses whose sequences vary over this region.¹³

From the findings of a multi-centre study of five methods for intratypic differentiation of polioviruses initiated by WHO, two methods, NAPH and EIA, were selected for introduction into the Polio Laboratory Network. The combination of serological and molecular tests diminishes the number of possible discrepancies.¹⁴

In optimising NAPH and EIA testing at VIDRL in 1994, several stored polioviruses, including the poliovirus type 3 isolate, which had previously been characterised as 'wild' in international laboratories, were retested. However, when the NAPH test result interpretation was Sabin-like and that of the EIA test was 'double reactive' for this isolate, its characterisation as a 'wild poliovirus type 3' was in doubt.

The staff at the United Kingdom reference laboratory were briefed on these findings in 1995. Using their current panel of monoclonal antibodies for differentiating poliovirus type 3 isolates, they carried out further monoclonal work on the strain which had been stored in their laboratory. The panel of monoclonals used for the 1995 analysis was different from that used in 1987. Although the isolate contained a type 3 vaccine-like isolate, a minor population most likely to be a vaccine variant, with changes in the antigenic site recognised by the Sabin-specific monoclonal, was not neutralised by this monoclonal at high challenge doses.

The virus was also despatched to RIVM in 1996 for further analysis. This included microneutralisation using cross-absorbed polyclonal antisera prepared against wild and vaccine poliovirus type 3, and sequencing of the VP1/2A region of the virus. The double reactivity of the virus in the EIA test performed at VIDRL can be interpreted in two ways. Either the virus is a mixture of a wild and a vaccine strain of poliovirus type 3, or is a single

strain with antigenic properties of both parent strains as a result of antigenic drift or a recombination event. As the virus was neutralised equally by both the wild virus-specific and the vaccine-specific cross-absorbed antiserum, a mixture of two viruses could be excluded.

Sequence analysis over the VP1/2A region outside of major antigenic sites provides definitive proof that the virus was indeed a Sabin poliovirus type 3. The aberrant antigenic features indicated the drifted nature of the Sabin strain. The patient's diagnosis is now reclassified as vaccine-associated paralytic poliomyelitis.

When OPV is administered for the first time, mutations of the Sabin poliovirus types 1,2 and/or 3 may appear in the gut of the recipient. Some, particularly those of types 2 and 3, may revert to virulent viruses which may be excreted in the faeces. However, the recipient should have developed antibodies by this time and is unlikely to become symptomatic. A reverted virulent virus in the faeces of a vaccine recipient may infect an unimmunised individual who may develop symptoms of polio infection.¹⁵

In Victoria, the last confirmed wild poliovirus was a type 1 isolated from a baby girl who had been born in Australia in 1977 but taken to Turkey soon after birth. On return to Australia in October 1977 at age 3 months she was admitted to the Royal Children's Hospital, Melbourne and later to Fairfield Hospital with a diagnosis of paralytic poliomyelitis. Because it was likely that the child became infected in Turkey, her isolate has been considered to be an imported strain.

With the imminent global eradication of polio, laboratories will become the only remaining reservoirs of polioviruses. One year after the last wild poliovirus has been detected, it is planned that all institutes in which wild polioviruses or clinical material which may contain wild polioviruses are stored will be required to transfer them to WHO designated repositories or destroy them.

It would be of interest to identify Australia's last locally acquired wild poliovirus. Over the next few months, the Polio Reference Laboratory at VIDRL plans to characterise local poliovirus isolates stored since 1954 and offers intratypic differentiation testing of strains stored in other Australian laboratories.

Acknowledgments

We wish to thank the staff of the Entero-Respiratory Laboratory, Fairfield Hospital, 1986 for isolation and identification of the isolate.

We would like to acknowledge the work of the late Dr David Magrath of NIBSC who performed the original monoclonal antibody neutralisation tests in 1986/7.

We also wish to thank Dr Louise Irving former Medical Microbiologist, Fairfield Hospital, for advice on the case history, subsequent public health information and critical review of the manuscript.

References

1. Hull HF, Birmingham ME, Melgaard B, Lee JW. Progress toward Global Polio Eradication. *J Infect Dis* 1997;175:(Suppl 1) S4-9.
2. Herceg A, Kennett M, Antony J, Longbottom H. Acute flaccid paralysis surveillance in Australia: The First Year. *Comm Diseases Intell* 1996;20:403-405.
3. Hall R. Notifiable Diseases Surveillance, 1917 to 1991. *Comm Diseases Intell* 1993;17:226-236.
4. Kennett ML, Donaldson A, Marshall JA, Williamson HG. Echovirus type 11 infection in Melbourne - 1953 to 1980. *J Hyg Camb* 1981;87:305-312.
5. Irving LG, Smith FA. One Year Survey of Enteroviruses, Adenoviruses and Reoviruses Isolated from Effluent at an Activated Sludge Purification Plant. *Applied and Environmental Microbiology* 1981;41:51-59.
6. Ferguson M, Minor P, Spitz M, Qi Y, Magrath D, Schild G. Monoclonal Antibodies Specific for the Sabin Vaccine Strain of Poliovirus 3. *The Lancet*: 1982 ii;122-4.
7. De L, Nottay CF, Yang BP, Holloway MJC, Pallansch M, Kew OM. Identification of Vaccine-related Polioviruses by Hybridization with Specific RNA Probes. *J Clin Microbiol*. 1995;33: 561-571.
8. Wood DJ. Polioviruses: Concurrent serotyping and intratypic differentiation of polioviruses. in Stephenson JR, Warnes A, editors. *Diagnostic Virology Protocols: Humana Press Inc; New Jersey, USA 1998.p189-197.*
9. Osterhaus AD, van Wezel A, Hazendonk T, Uytehaag F, van Asten J, van Steenis B. Monoclonal Antibodies to Polioviruses. Comparison of Intratypic Strain Differentiation of Poliovirus type 1 using Monoclonal Antibodies versus Cross-Absorbed Antisera. *Intervirology* 1983;20:129-136.
10. Mulders MN, Lipskaya GY, van der Avoort HGAM, Koopmans MPG, Kew OM, van Loon AM. Molecular epidemiology of wild poliovirus type 1 in Europe, the Middle East and the Indian subcontinent. *J Infect Dis* 1995;171:1399-1405.
11. van Wezel AL, AG Hazendonk. Intratypic differentiation of poliomyelitis virus strains by strain-specific antisera. *Intervirology* 1979;11:2-8.
12. van Loon AM, Ras A, Poelstra P, Mulders M, van der Avoort H. Intratypic Differentiation of Polioviruses. In: Edwouard Kurstak, editor. *Measles and Poliomyelitis; Springer Verlag 1993;p.359-369.*
13. Kew OM, Mulders MN, Lipskaya GY, de Silva E, Pallansch MA. Molecular Epidemiology of Polioviruses. *Seminars in Virology* 1995;6:401-414.
14. van der Avoort H, Hull BP, Hovi T, Pallansch MA, Kew OM, Crainic R, Wood DJ, Mulders MN, van Loon AM. Comparative Study of Five Methods for Intratypic differentiation of Polioviruses. *J Clin. Microbiol* 1995;33:2562-2566.
15. Ogra PL, Faden HS, Abraham R, Duffy LC, Sun M, Minor PD. Effect of Prior Immunity on the Shedding of Virulent Revertant Virus in Feces after Oral Immunization with Live Attenuated Poliovirus Vaccines. *J Infect Dis* 1991;164:191-194.