Outbreak of echovirus 30 meningitis in Wingecarribee Shire, New South Wales

Iain Gosbell,[1,2] David Robinson,[1] Kerry Chant,[3,4] Stephen Crone[3]

Abstract

An outbreak of aseptic meningitis due to echovirus 30 occurred in the Wingecarribee Shire, NSW, during October to November 1994, with 30 cases fitting the clinical case definition. Cases were ascertained from attendees of the local hospital. Medical files were reviewed and a standard questionnaire administered. Viral cultures were performed on CSF, throat swabs and stool specimens. The clinical presentation and laboratory findings were typical of viral meningitis. Cases were aged 8 months to 51 years; 26 were admitted to hospital. Headache was present in 93%, photophobia in 86%, vomiting in 69%, fever in 72%, and neck stiffness in 62%. In spite of temporal clustering, the mode(s) of transmission in this outbreak remain speculative. Although the route of transmission was not established, general hygiene measures to stop transmission were implemented when a common water source was excluded on epidemiological grounds. *Commun Dis Intell* 2000;24:121-124.

Keywords: echovirus, meningitis, disease outbreak

Introduction

Echoviruses (enteric cytopathogenic human orphan viruses) are a type of enterovirus, small RNA viruses responsible for many diseases including aseptic meningitis.[1,2] Transmission of enteroviruses is generally regarded as faecal-oral, and humans are the only known reservoir of human enteroviruses.[2,3] Close contact appears to be the primary avenue of spread, through faecal contamination of fingers, table utensils and food.[3] Young children are the usual reservoir, and abundant circulation of enteroviruses amongst them has been demonstrated.[2] It is possible that respiratory spread occurs but this seems less likely given that faecal shedding lasts longer than oropharyngeal shedding.[2] Transmission via insect vectors such as flies may also occur.[3]

Transmission has occurred in families, nurseries and institutions.[3] Waterborne outbreaks of enterovirus infection have occurred and the source is often difficult to establish, although contamination with sewage is considered likely.[2,3] Enteroviruses are hardy in fresh and salt water, particularly in the presence of organic matter, survive sewage treatment and chlorination, and can travel many miles downstream from the source.[2,3]

There are several reports of large outbreaks of aseptic meningitis due to echovirus 30,[4] including one involving 47 cases in Western Australia.[5] An average of 4 cases of viral meningitis are seen per year at Bowral Hospital (Figure 1). Nine cases presented between 2 November and 10 November 1994, prompting notification of an outbreak of aseptic meningitis to the South Western Sydney Public Health Unit (SWSPHU). This report describes the clinical and public health investigation of this outbreak in the Southern Tablelands of New South Wales. Echovirus 30 was subsequently shown to have caused the outbreak.

Materials and Methods

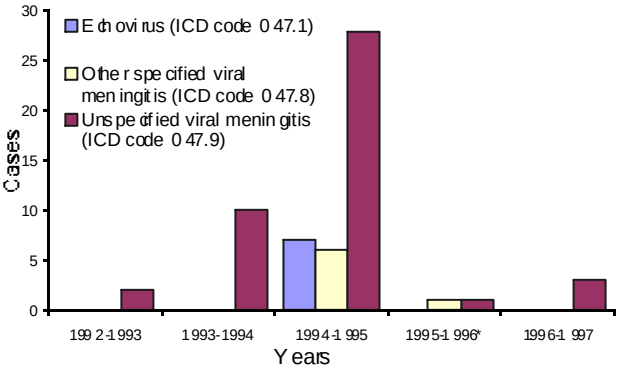
Case ascertainment

The medical superintendent of Bowral Hospital notified SWSPHU of persons who presented to the hospital's emergency department and were diagnosed as having viral/aseptic meningitis during the period 4 October to 28 November 1994, the dates of the first and last notifications.

Case classification

The medical records of the above patients were reviewed by staff of the SWSPHU . The diagnosis of aseptic meningitis was accepted where the person had a headache associated with fever, photophobia and/or neck stiffness, the

Figure 1. Separations from Bowral Hospital due to viral meningitis, 1992-1997



* PCR for enterovirus introduced

1. Department of Microbiology and Infectious Diseases, South Western Area Pathology Service, Liverpool, New South Wales.
2. School of Pathology, Faculty of Medicine, University of New South Wales.
3. Public Health Unit, South Western Sydney Area Health Service, New South Wales.
4. School of Community Medicine, Faculty of Medicine, University of New South Wales.

cerebrospinal fluid (CSF) microscopy and culture were negative for bacteria (when performed) and where no other cause for the headache was evident.

The Centers for Disease Control, Atlanta, case definition for aseptic meningitis is a syndrome characterised by the acute onset of meningeal symptoms, fever, and cerebrospinal fluid pleocytosis, in the presence of bacteriologically sterile cultures.[6] The SWSPHU modified this to the following:

Definite case: Isolation of echovirus 30 from the CSF by culture;

Probable case: Clinical diagnosis of viral meningitis and isolation of echovirus 30 from throat swabs or stool culture;

Possible case: Clinical diagnosis of viral meningitis without virological confirmation.

Polymerase chain reaction (PCR) only became available after the outbreak had occurred and was performed retrospectively. Unfortunately, this meant that it was performed on available stored CSF supernatants, compromising its sensitivity. Accordingly, PCR was not included in the above definitions.

Epidemiological investigation

The cases were interviewed by telephone using a structured questionnaire. Clinical histories were confirmed, and epidemiological links were sought. Patients were asked about their recent travels within Wingecarribee Shire, contacts with others with symptoms of viral illness, especially with prominent headaches, attendance at institutions (schools or child care centres), the source of their drinking water, and if and where they had been swimming.

A chart review was performed and the following data were obtained: name, medical record number, age, sex, underlying illness, date of onset of symptoms (fever, headache, photophobia, neck stiffness, drowsiness, focal neurological signs, gastrointestinal, cardiovascular or respiratory symptoms), signs (temperature, photophobia, neck stiffness, Kernig's sign, focal neurological signs) and laboratory data (full blood count, differential white cell count, biochemistry including liver function tests).

Microbiological investigations

CSF microscopy, bacterial culture and biochemical analysis were performed by the microbiology laboratory at Bowral District Hospital. Protein and glucose were determined. Cell counts and Gram stains were performed. CSF was inoculated onto horse blood and chocolate agar and examined daily for one week.

CSF was initially sent to Westmead Hospital for viral culture, but was subsequently processed by the Virology Laboratory at the South Western Area Pathology Service. CSF was inoculated into viral culture tubes containing primary monkey kidney (Edward Keller, Australia) or MRC-5 (Edward Keller, Australia) cells. The tubes were examined periodically for cytopathic effect (CPE) by inverted microscopy.

Throat gargles and faecal specimens were obtained and sent to the Virology Laboratory at South Western Area Pathology Service. Throat gargles were incubated in a viral transport medium containing penicillin, gentamicin and amphotericin B for 30 minutes prior to inoculation. Stool specimens were prepared by washing in phosphate buffered saline and centrifuging at 2,800g for 10 minutes. The supernatants were incubated in a solution of penicillin, gentamicin and amphotericin B for 30 minutes prior to incubation. Prepared throat gargles and stools were inoculated into primary monkey kidney and MRC-5 cell lines.

Cultures were observed for a total of three weeks. Those cultures which exhibited CPE suggestive of enterovirus were referred to the reference laboratory (Institute of Clinical Pathology and Medical Research, Westmead, Sydney, Australia) for typing by neutralisation using pooled antisera.

The Amplicor Polymerase Chain Reaction Kit (Roche Molecular Systems, Basel, Switzerland) was used to retrospectively perform PCR on 11 CSF specimens which had been stored at -80^{o}C. Kit insert instructions were followed, as published previously.[7]

Blood cultures were performed with the BACTEC NR 960 system.

Results

Clinical details

A total of 30 cases were detected; 7 definite, 7 probable and 16 possible. Of the 7 definite cases, 6 were the result of infection with echovirus 30 and one with echovirus 3. Twenty-six patients were admitted to hospital for an average of 2.8 days (range 1-9 days). Their ages ranged from 8 months to 51 years (median 25 years). The male:female ratio was 2:3. Headache occurred in 26/28 cases (93%), photophobia in 24/28 (86%), vomiting in 20/29 (69%), temperature >37.4^{o} C in 21/29 (72%), neck stiffness in 18/29 (62%), upper respiratory symptoms on presentation in 7/27 (26%), and gastrointestinal symptoms on presentation in 5/28 (18%). No patient had a rash.

Laboratory findings

The mean peripheral white cell count was $11x10^{9}$/L (range $4.4\text{-}20.2x10^{9}$/L, reference range $4\text{-}11x10^{9}$/L). The mean CSF parameters were: white cell count $239x10^{6}$/L (range $0\text{-}1160x10^{6}$/L, reference range $<2x10^{6}$/L), neutrophils $202x10^{6}$/L (range $0\text{-}1136x10^{6}$/L, reference range $<1x10^{6}$/L) and protein 0.32g/L (range 0.21-0.47g/L, reference range 0.15-0.45g/L). Virology results are presented in Table 1.

Epidemiology

The results of the epidemiological investigation are given in Table 2. Wingecarribee Shire experienced a crude attack rate (AR) of 0.8 per 1,000 with the highest AR recorded in the Mittagong postal area (1.5 per 1,000). The Mittagong postal area reported 21 of the 29 cases of acute viral meningitis attributed to the outbreak. (One case did not have the postcode recorded). Drinking water was not sampled for viral studies as the multiple, separate sources ruled out drinking water as a source.

Discussion

An outbreak of aseptic meningitis due to echovirus 30 occurred in the Wingecarribee Shire during October to November 1994, with 30 cases fitting the clinical case definition. Several outbreaks of meningitis due to echovirus 30 have been reported, including one in Australia.[5]

Table 1. Virology results for 18 patients from whom CSF and/or stool was submitted for viral culture

Case No.	CSF viral culture	CSF enterovirus PCR	Stool viral culture
9	No growth	Negative	No growth
18	No growth	Negative	Not tested
20	No growth	Negative	Not tested
24	No growth	Positive	Not tested
13	Echo 30	Positive	No growth
23	Echo 30	Positive	Echo 30
3	Echo 30	Positive	Not tested
4	Echo 30	Positive	Not tested
15	Echo 30	Negative	Not tested
22	Echo 30	Negative	Not tested
27	Echo 3	Negative	Not tested
7	Nottested	Nottested	No growth
10	Nottested	Nottested	No growth
11	Nottested	Nottested	No growth
14	Nottested	Nottested	No growth
12	Nottested	Nottested	Echo 30
16	Nottested	Nottested	Negative
19	Nottested	Nottested	Echo 30

The clinical presentations were fairly typical of viral meningitis.[1] Most patients had fever, headache and meningism. The onset was often short (a few hours), and patients were often debilitated, requiring admission to hospital. Other systemic features of myalgia and arthralgia were also typical.[1]

The laboratory findings were also consistent with viral meningitis. CSF neutrophil pleocytosis is well recognised in viral meningitis, especially in the first 1-2 days,[1] and has been documented with echovirus 30.[5,8-12] CSF proteins were within the reference ranges, but may be slightly elevated,[5,8,11-13] and CSF glucose may be slightly depressed as occurred in some cases.[12,13] Peripheral leukocytosis may occur.[8] Unfortunately, PCR for enterovirus became available two months after the outbreak finished, and was performed on CSF supernatant stored at –80°C. Not surprisingly, as the viral RNA is mainly found in the cells and the supernatant is largely acellular, the PCR positivity rate was low.[14]

The mode(s) of transmission in this outbreak remain speculative. There was no obvious geographical clustering. Cases were distributed throughout Wingecarribee Shire although the majority lived in the Mittagong postal area. However, this area is large and isolated, and has multiple sources of drinking water, including four reticulated town water supplies drawing water from different dams, and several independent rainwater tanks. Under the circumstances water samples were not submitted for virological testing. Only one patient swam in a public pool or other body of water during September to November 1994.

Enteroviruses are usually transmitted by the faecal-oral route, except for respiratory tract infections where transmission may be by respiratory secretions, and conjunctivitis where person to person spread can occur.[1,2,3] The occurrence of some person to person transmission was suggested by the 3 clinical cases in one family (one confirmed by culture) and a health care worker who became ill after nursing a case. However, the other cases were not epidemiologically linked to a confirmed case suggesting other means of transmission.

The echovirus 3 case is probably a sporadic case that occurred in the midst of the echovirus 30 outbreak. We cannot determine if the outbreak involved more than one type of echovirus.

Although the route of transmission was not established, general measures to stop transmission were implemented when a common water source was excluded on epidemiological grounds. These measures included information on personal, food and domestic hygiene publicised through preschools, schools and the local media. Education of patients and doctors was instituted early. Nonetheless, the collection of the appropriate specimens was suboptimal. Throat swabs and stool specimens (or rectal swabs) are easily obtained, and were the only positive specimens in some of our cases.

This is the second outbreak of enterovirus-related aseptic meningitis reported in Australia.[5] Although recognised early

Table 2. Epidemiological investigations

Investigation	Comment
Time period	4 October to 21 November 1994
Geographical clustering	Wingecarribee Shire; 21 of 29 cases where the postcode was recorded, resided in Mittagong postal area.
Attack rates	Wingecarribee Shire = 0.8 per 1,000 population.
	Mittagong postal district = 1.5 per 1,000population.
	OR 1.94, 95% CI 1.07-3.51
	Expected 4 cases per year in the Wingecarribee Shire ie 0.01 per 1,000 in a similar 48 day period.
Source of drinking water	Four reticulated town water supplies drawing water from different dams.
Swimming	Only 1 case swam in a public pool during September to November 1994.
Day care centres	No clustering of cases was observed.
Person-to-person transmission	One family had 3 members fall ill (one laboratory confirmed) several days apart, suggesting transmission within the family rather than co-primary infection.
	A nurse caring for a case fell ill 5 days later.
	The other cases were not epidemiologically linked.

because most of the cases presented to one hospital, bacterial meningitis was difficult to exclude initially as many of the patients had a neutrophil pleocytosis both peripherally and in the CSF. In addition, the source of the outbreak remains unknown despite prompt and thorough investigation.

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References

1. Modlin JF. Coxsackieviruses, echoviruses, and newer enteroviruses. Mandell GL, Bennett JE, Dolin R, eds. *Mandell, Douglas and Bennett's Principles and Practice of Infectious Diseases*, 4th ed. Churchill Livingstone, Melbourne, 1995.
2. Minor PD, Bell EJ. Picornaviridae (excluding Rhinovirus). Parker MT, Collier LH, eds. *Topley and Wilson's Principles of Bacteriology, Virology and Immunity*, 8th ed. Edward Arnold, Melbourne, 1994.
3. Melnick J. Enteroviruses: Polioviruses, coxsackieviruses, echoviruses, and newer enteroviruses. Fields BN, Knipe DM, eds. *Fields Virology*, 2nd ed. Raven Press, New York, 1990.
4. Beckett G, Gensheimer KF, Silva J, et. al. Aseptic meningitis in New York. *MMWR* 1991;40:773-775.
5. Mackay-Scollay EM, Hobday JD, Harnett GB, Masters PL. Echovirus type 30 infection: clinical and virological observations on an epidemic in Western Australia. *Med J Aust* 1973;2:417-421.
6. Anonymous. CDC. Case definitions for public health surveillance. MMWR - Morbidity & Mortality Weekly Report 1990;39 RR-13:6.
7. Rotbart HA, Sawyer MH, Fast S, et al. Diagnosis of enteroviral meningitis by using PCR with a colorimetric microwell detection assay. *J Clin Microbiol* 1994;32:2590-2.
8. Hall CE, Cooney MK, Fox JP. The Seattle virus watch program. I. Infection and illness experience of virus watch families during a community wide epidemic of echovirus type 30 aseptic meningitis. *Am J Public Health Nations Health* 1970;60:1456-1465.
9. Likosky WH, Emmons RW, Davis LE, Thompson RS. U.S. cases in 1968: epidemiology of echovirus 30 aseptic meningitis. *Health Serv Rep*1972;87:638-642.
10. Gravelle CR, Noble GR, Feltz ET, Saslow AR, Clark PS. An epidemic of echovirus type 30 meningitis in an arctic community. *Am J Epidemiol* 1974;99:368-374.
11. Kaplan GJ, Clark PS, Bender TR, Feltz ET, List-Young B. Echovirus type 30 meningitis and related febrile illness: epidemiologic study of an outbreak in an Eskimo community. *Am J Epidemiol* 1970;92:257-265.
12. Leonardi GP, Greenberg AJ, Costello P, Szabo K. Echovirus type 30 infection associated with aseptic meningitis in Nassau County. New York. USA. *Intervirology* 1993;36:53-56.
13. Wang DM, Zhao GC, Zhuang SM, Zhang YC. An epidemic of encephalitis and meningoencephalitis in children caused by echovirus type 30 in Shanghai. *Chin Med J* 1993;106:767-769.
14. Yerly S, Gervaix A, Simonet V, et al. Rapid and sensitive detection of enteroviruses in specimens from patients with aseptic meningitis. *J Clin Microbiol* 1996;34:199-201.