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Echovirus type 11 infection in Melbourne – 1953 to 1980

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SUMMARY

Echovirus type 11 (echo 11) has been isolated at the virus laboratory of Fairfield Hospital, Melbourne, Australia, in 20 of the 28 years since the laboratory was established. During this time two major epidemics have occurred; the first, in 1971–2 involved 90 patients with aseptic meningitis or respiratory illness. The second began in June 1979 and lasted for 11 months, during which echo 11 was isolated from 174 patients admitted to Fairfield Hospital, other Victorian and Tasmanian hospitals and a children's reception centre. The patients' illnesses included viral meningitis (66%), fever (10%), respiratory infections (7%) and gastroenteritis (2%). One baby died.

Echo 11 was recovered from nasopharyngeal swabs or aspirates, cerebrospinal fluid and faecal specimens and was isolated most frequently in the Borrie cell line. Isolates were readily identified by immune electron microscopy and/or neutralization tests.

INTRODUCTION

The prototype strain of echovirus type 11 (echo 11) was isolated from the faeces of a healthy child in Cincinatti, Ohio, U.S.A., in 1954 (Ramos-Alvarez & Sabin, 1956) during a study of poliovirus infection in the United States of America and Mexico. Since then, echo 11 has been associated with a wide range of illnesses, including meningitis, paralysis, exanthem, respiratory infections and myocarditis (Melnick, Wenner & Phillips, 1979; Drew, 1973).

Since late 1953 the Fairfield Hospital virology laboratory has been isolating enteroviruses from patients with viral illnesses and has conducted studies to determine the frequency of virus excretion from healthy children at a day creche and from children with minor illnesses in a residential institution.

The first local strain of echo 11 was isolated in 1954 but was not identified until antisera became available several years later. Apart from the outbreak in 1971 and 1972, only sporadic isolations of the virus were made prior to June 1979 when a major outbreak began.

The pattern of echo 11 infection observed at Fairfield Hospital over the past 27 years is described with emphasis on clinical, virological and epidemiological features of the recent epidemic.

MATERIALS AND METHODS

Specimens

Specimens were collected from patients admitted to Fairfield Hospital, other Hospitals in Victoria and Tasmania, a children's reception centre in Melbourne, and from 1958 to 1964, a day creche in an inner Melbourne suburb.

Initially only faecal samples were cultured but later throat swabs or combined nose and throat swabs were collected. Since 1976 the most common specimens have been either nose and throat swabs (NTS), nasopharyngeal aspirates (NPA), saliva, cerebrospinal fluid (CSF), faeces, skin, conjunctival or corneal specimens, and occasional biopsy and autopsy material has been received.

Throat swabs and faecal specimens were collected each month from children attending the creche. Most of the children were aged less than 5 years. The methods of collecting and processing these specimens were as previously described (Donaldson *et al.* 1978; Kennett *et al.* 1972; Kennett *et al.* 1974).

Since 1976 specimens from patients with aseptic meningitis have been inoculated routinely into primary cynomolgus monkey kidney (MK), heteroploid monkey embryonic kidney (MEK), rhinovirus-sensitive HeLa cells and Borrie heteroploid human epithelial cells (Bo). Specimens from patients with respiratory illness and eye disease have been inoculated into MK, HeLa and diploid human embryonic fibroblasts (HEL) and specimens from cases of febrile illness or gastroenteritis into MK, HeLa, Bo and HEL cultures. The methods used for the isolation of viruses were outlined in an earlier report (Kennett *et al.* 1974).

Identification of echo 11 *strains*

Virus isolates were identified either by tube neutralization (Kennett *et al.* 1972), microneutralization (Irving & Smith, 1981) or immune electron microscopy (IEM). The antisera used were produced in rabbits using prototype viruses or were obtained through the World Health Organization from the Research Resources Branch of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Maryland, U.S.A.

Tests were performed on each isolate, usually with antisera against echo 11 and another enterovirus which was prevalent in the community at the same time. The antisera were used at a concentration of 20 antibody units per 0·1 ml for tube neutralization and IEM, and 10 antibody units per 0·1 ml for microneutralization.

For IEM 0·5 ml of supernatant fluid from cells showing at least 50 % cytopathic effect (CPE) was incubated with 0·03 ml (1 drop) of antiserum for 1 h at 37 °C and examined immediately or after storage at 4 °C for not more than 24 h. One drop of the mixture was placed on a Formvar-carbon coated grid, negatively stained with 3 % phosphotungstic acid (pH 7) and examined using a screen magnification of × 40000 with a Phillips 301 electron microscope. All specimens were processed and read under code and reported positive only if no complexes were seen in a control preparation and at least two immune complexes, each with a minimum of four virus particles, recognized in the experimental preparation. Plate 1 shows typical echo 11 immune complexes.

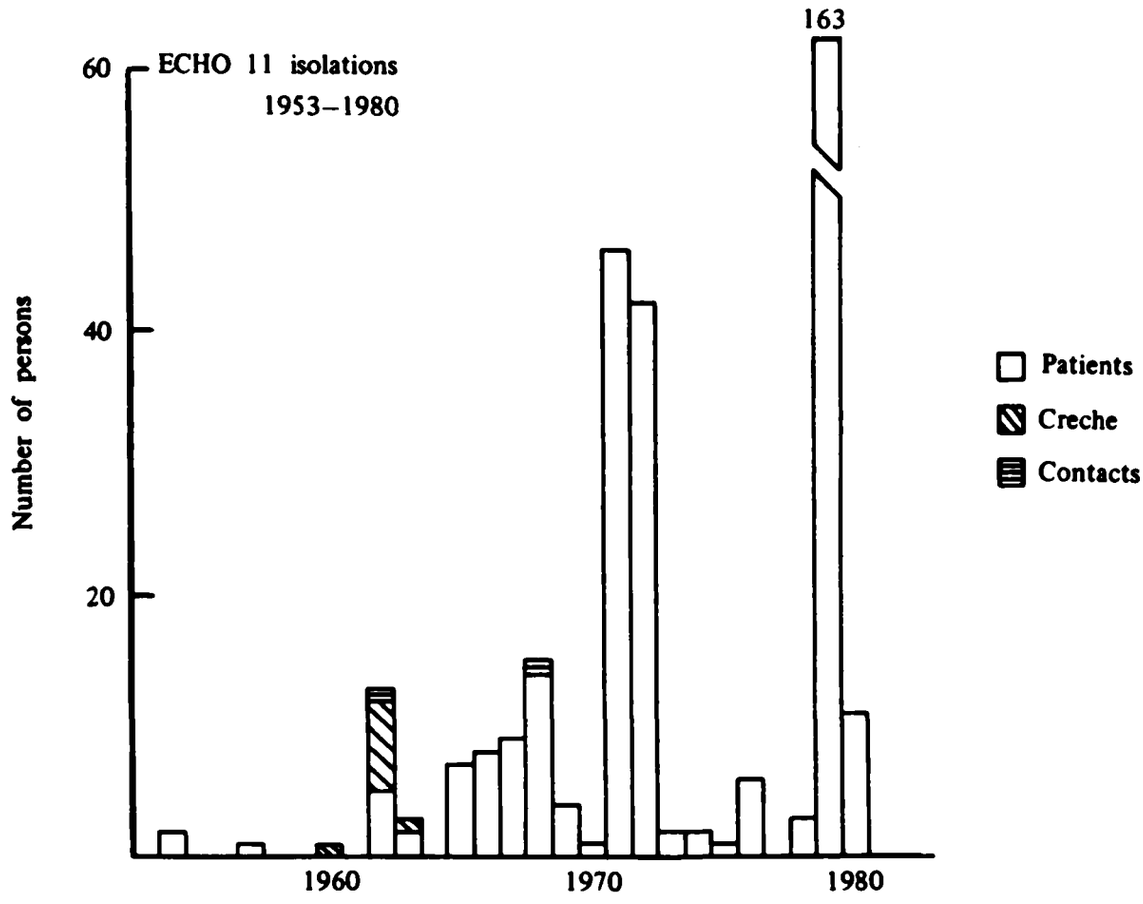

Fig. 1. Yearly isolations of echo 11 — 1953 to 1980. □, Patients admitted to hospital. ▨, Healthy children in a creche. ▤, Contacts of patients with poliomyelitis.

RESULTS

Epidemiology 1953–78

Between 1953 and 1978, echo 11 was isolated from 156 hospital patients, two contacts of patients with poliomyelitis and nine healthy children in a day creche (Fig. 1). The number of people with echo 11 infections each year during this period varied widely. None were detected in some years, while there was a moderate epidemic extending over 20 months from December 1970 to July 1972. Virus was isolated more commonly during the summer and autumn, December to April.

The 156 patients presented with a range of clinical syndromes. Eighty-one had viral meningitis, 39 respiratory infections, 14 gastroenteritis and the remainder had fever, rashes or miscellaneous illnesses. Nearly half the patients were aged less than 5 years, especially those with respiratory infections, gastroenteritis and rashes. The patients with meningitis were mostly aged less than 30 years; 34 (42%) were less than 10 years of age and six younger than 1 year.

Six of the eight people with echo 11 during 1966 and one in 1971 were children at the reception centre who had bronchitis and/or gastroenteritis.

The children at the creche were sampled monthly between 1958 and 1964 and, although many respiratory, entero and reoviruses were isolated over this period, echo 11 was only found in November 1960, May to July 1962, and March 1963. The children were assumed to be well when specimens were taken.

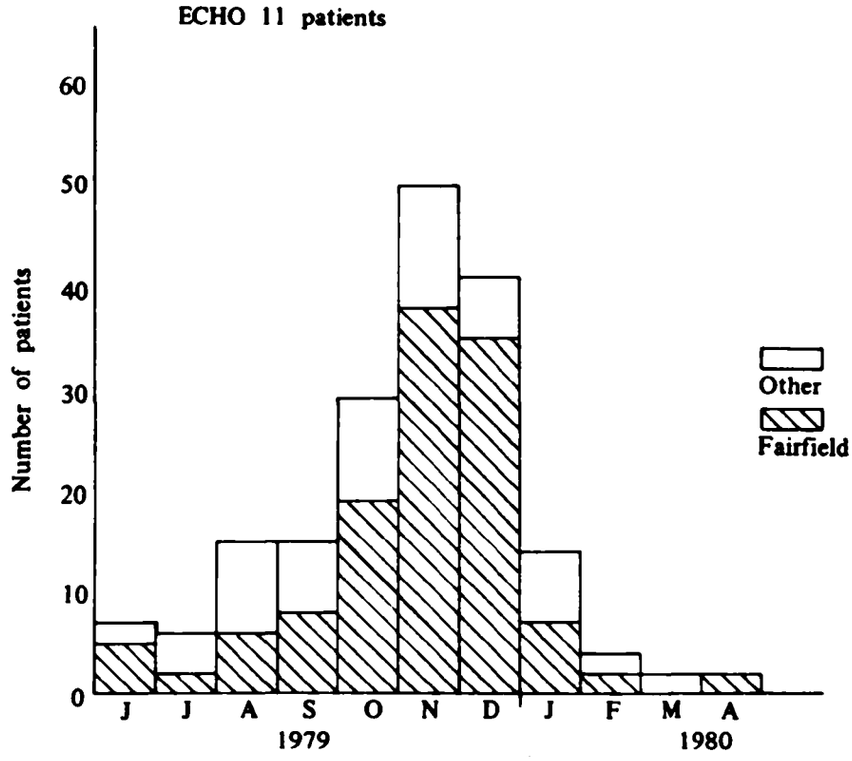

Fig. 2. Monthly isolations of echo 11 – 1979/80. ▨, Patients admitted to Fairfield Hospital. □, Patients admitted to other hospitals in Victoria and Tasmania.

Table 1. *Age and illness of* 174 *patients infected with echo* 11 – 1979/80

Age (Years)	Viral menin-gitis	Head-ache and fever	Febrile illness	Cardio myopathy	Respir-atory in-fection	Gastro-enter-itis	Miscel-laneous	Total
< 1	11	—	7	1*	3	1	6	29
1–4	8	1	1	—	6	2	1	19
5–9	23	1	2	—	—	—	—	26
10–14	3	1	—	—	1	—	—	5
15–19	8	1	2	—	—	—	1	12
20–24	14	—	—	—	2	—	—	16
25–29	19	1	1	—	1	—	1	23
30–34	16	1	2	—	—	—	—	19
35–39	9	1	—	—	—	—	1	11
⩾ 40	4	1	2	—	—	—	6	13
Not stated	—	—	—	—	—	—	1	1
Total	115	8	17	1	13	3	17	174

* Patient died.

The 1979–80 *epidemic – Epidemiology*

The outbreak extended over 11 months beginning in June 1979, peaking in November and ending in April 1980. During this period, echo 11 was isolated from 174 patients, of whom 72% were inpatients of Fairfield Hospital. The number of patients from whom echo 11 was isolated each month is shown in Fig. 2.

The ages of the patients from whom the virus was isolated and the major clinical syndromes encountered are shown in Table 1. The ages ranged from 4 days to 78 years, with a mean of 18·2 years, 43% being less than 10 years and 17% aged under 1 year. Overall the male to female ratio was 1·3 to 1.

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A total of 115 patients (66%) had aseptic meningitis confirmed by lumbar puncture. A further eight patients had headache and fever but no leucocytes were detected in the cerebrospinal fluid. Meningitis was most common in children and young adults.

Some patients, especially children, presented with fever or acute respiratory infection. Four children at the reception centre, all aged less than 5 years, had lower respiratory infections and two were subsequently admitted to Fairfield Hospital for treatment. Other children presented to hospital with tonsillitis or otitis media.

Of the 29 children aged less than 1 year, nine were in neonatal nurseries in Melbourne and Launceston. Five had a febrile illness and three had hydrocephalus, convulsions and myocarditis respectively, not thought to be related to echo 11 infection.

Echo 11 was isolated from swabs taken at post-mortem from the lungs of another neonate. Following a normal pregnancy and delivery in a country hospital, a 4-day-old baby girl collapsed suddenly and aspirated thick mucus. Attempts to resuscitate the child were unsuccessful. At autopsy the only abnormalities found were pathological changes in the myocardium consistent with a diagnosis of early viral myocarditis and pulmonary involvement which histologically showed a small amount of congestion and collapse. No other specimens were collected for virus isolation.

Other patients had a miscellany of illnesses such as glandular fever, urinary tract infections, non-A non-B hepatitis and conjunctivitis, in which the aetiological role of echo 11 was uncertain.

The 1979–80 *epidemic – Virus isolation*

Analysis of all the specimens received from the 174 patients in whom echo 11 infection was confirmed, showed that faeces was the most reliable source of virus, with 69 of 71 (97%) specimens positive. The virus was also isolated from 96 of 118 (81%) CSF's and 97 of 140 (69%) nasopharyngeal specimens. Echo 11 was also isolated from the eye swabs of a patient with conjunctivitis and the post-mortem lung swab of a 4-day-old girl who died suddenly.

Of the five cell types in which echo 11 was recovered, the Borrie cell line was most sensitive, with 219 of 252 (86%) isolation attempts positive. The isolation rate in MEK-3 cells was 71% in HEL 61%, MK 47% and in HeLa 29%.

Identification of epidemic strains

All strains were readily typable by one of the methods described. No further tests to determine the physico-chemical properties of the isolates were attempted.

DISCUSSION

Both of the major Melbourne epidemics of echo 11 infection in the last 28 years have followed outbreaks in other countries. In the northern summer of 1971 in Japan, over 1100 echo 11 strains were obtained from patients in most areas of the country (Tagaya & Moritsugu, 1973). In the following 18 months, 90 Fairfield Hospital patients, most of whom had aseptic meningitis or respiratory symptoms, were identified as having echo 11 infections. Drew (1973, 1974) reported a cluster

of echo 11 infections in a Melbourne neonatal nursery in 1972 and noted a possible association with myocarditis and hepatitis.

In 1978, an extensive outbreak of echo 11 infection was reported from Great Britain (WHO Yearly Virus Report, 1978) during the winter and spring. A few isolations were made in Melbourne in late 1978, but it was not until June 1979 that strains were detected in both patients' specimens and samples of sewage (L. Irving, personal communication). Echo 11 was first detected amongst children with lower respiratory infections at the reception centre where it had previously been found in 1966 and again in 1971. As respiratory syncytial and adenoviruses were also circulating there at the time, the aetiological role of echo 11 was unclear. The virus was subsequently isolated from patients throughout the Melbourne metropolitan area and in a number of country centres.

In addition to the 174 isolations made at the Fairfield laboratory, a further 69 strains were identified from patients admitted to the Royal Children's Hospital, Melbourne (E. Uren *et al.*, personal communication). In both centres the most frequent disease association was aseptic meningitis, while fever, respiratory and gastrointestinal illnesses were also common. In most of these patients the disease was not severe and usually of short duration, with no sequelae. Epidemic myalgia (Bornholm Disease) which was reported in the U.K. epidemic was not seen in Melbourne, but the predominance of meningitis, respiratory symptoms and gastroenteritis was similar to that encountered in the Japanese outbreak (Tagaya & Moritsugu, 1973; WHO Yearly Viral Diseases Report, 1978).

Seventeen of the 174 patients were admitted to hospital with illnesses such as hepatitis, appendix abscess, urinary tract infection and glandular fever, none of which were considered to be caused by echo 11.

No studies on healthy people were being carried out in 1979–80 so the extent of echo 11 infection in the community could not be determined. However, the presence of echo 11 in the above 17 patients and also in sewage samples suggested that there may have been a high level of echo 11 excretion during that time.

There are occasional reports of echo 11 infections causing severe illness or death, particularly in neonates, pregnant women and immunocompromised patients (WHO Yearly Virus Report, 1978; Australian Communicable Diseases Intelligence 79/9; and Drew, 1973).

Neonatal infections were detected in nine patients in this series and a further 11 at the Royal Children's Hospital, Melbourne. These children had meningitis (1), fever (5), no symptoms at all (10) or symptoms unrelated to echo 11 infection (3). One child died at 4 days of age and was found to have evidence of myocarditis at autopsy. The role of echo 11 in the illness was unknown as no specimen of myocardium was available for virus studies.

The severe fulminating viraemia described by Nagington *et al.* (1978) and Modlin (1980) was not seen in Melbourne. However, severe neonatal infections including two deaths occurred in Perth, Western Australia (G. Cullity and M. Bucens, personal communications). It would be expected that if such illnesses had been seen in neonates in either Victoria or Tasmania, specimens for virus studies would have been sent either to the virus laboratory at Fairfield or the Royal Children's Hospital. A wide range of symptoms has been shown to occur even in the same nursery (Nagington *et al.* 1978) so it may have been fortuitous that the more severe

manifestations did not occur in Victoria and Tasmania. Perhaps host factors play a role and the timing of the mother's or child's illness also appears important (Modlin, 1980).

Several local strains have been sent to Professor Hans Eggers in West Germany to compare with antigenic variants isolated from an outbreak in a maternity ward in Cologne, in which one child died and six others had viral meningitis (Hager, Mertens & Eggers, 1980). Results of these comparisons have not yet been received but there is a possibility that the potentially fatal strains may be different from those associated with milder illness.

Relatively high isolation rates were obtained from faeces, nasopharyngeal specimens and CSF's. These findings contrast with previous enterovirus outbreaks, notably the echo 18 epidemic in Melbourne in 1968–9 (Kennett *et al.* 1972), when the virus was readily isolated only from faeces, and the entero 71 outbreak in 1972–3 (Kennett *et al.* 1974) when no isolations were made from CSF.

The Borrie heteroploid human cell line which was established at Fairfield Hospital in 1970 (Ellis *et al.* 1974) has been routinely used since then for the isolation of enteroviruses. Although enzyme studies carried out at the Institute of Medical and Veterinary Science, Adelaide, South Australia, suggest that Borrie cells are probably a cloned line of HeLa cells (B. Marmion, personal communication), the sensitivity of the two lines to various enterovirus types is very different. Echo 11 CPE can be seen as early as 3 days after inoculation of Borrie cells.

For several years, immune electron microscopy has been used to confirm the identity of many enterovirus isolates. Until recently, at least one isolate from each person was typed by either tube or microneutralization and further isolates from the patient confirmed by IEM. As no anomalous results had been detected and the technique was rapid and simple, IEM alone was used as the epidemic progressed. This enabled identification time to be reduced from between 3 and 7 days to about 1½ h after viral CPE was detected.

The use of an extremely sensitive cell line and a rapid method of identification can confirm enteroviral infection within 3 days of specimen inoculation.

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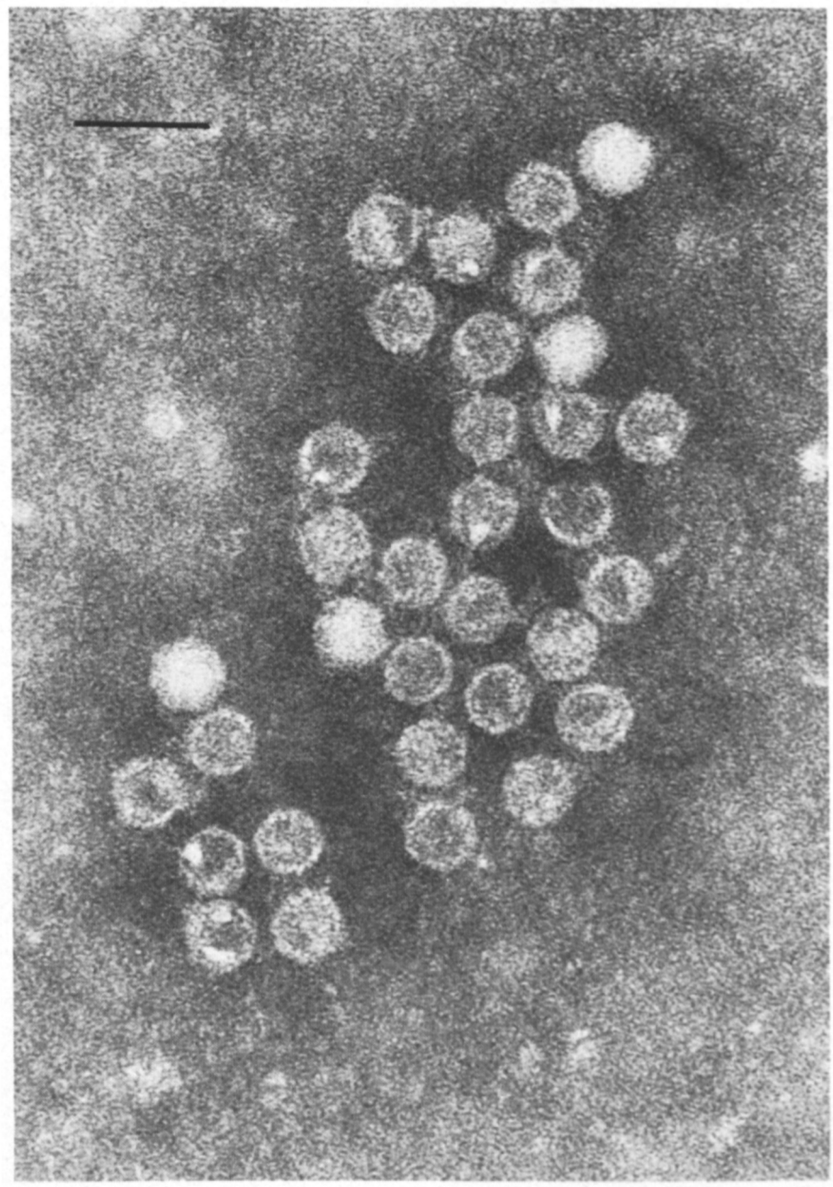
WORLD HEALTH ORGANIZATION YEARLY VIRUS REPORT (1978). Prepared by the WHO Virus Diseases Unit (used with permission).

EXPLANATION OF PLATE

PLATE 1

Typical echo 11 immune complexes. The bar represents 50 nm.



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