Infectious gastroenteritis in Norfolk Island and recovery of viruses from drinking water

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SUMMARY

A high incidence of gastroenteritis in both islanders and tourists has been recorded in recent years on Norfolk Island – a popular tourist resort for Australians and New Zealanders. No bacterial cause has been found. However, electron microscopic examination of 28 faecal specimens revealed viruses associated with gastroenteritis in 21 (75%). No viruses were isolated in cell cultures. Bore water is used for drinking purposes on the island and 32 samples from 15 bores were examined for viruses by electron microscopy and culture as well as for bacterial contamination. Seven polioviruses (all type 1 vaccine strain) and adenoviruses 1 and 5 were isolated in cell cultures. In addition one rotavirus, one adenovirus and two small round viruses were detected by electron microscopy. Six of 21 samples tested showed unacceptably high levels of bacteria for drinking water. The deep ground water has apparently become contaminated with sewage effluent and is almost certainly the main cause of the high level of gastroenteritis on the island.

INTRODUCTION

Norfolk Island is situated in the South Pacific, 1676 km ENE of Sydney, Australia; 1063 km from Auckland, New Zealand and 772 km from Noumea, New Caledonia. The island is volcanic in origin, 8 km long and 5 km wide with a land area of 3455 ha. The average elevation is 100 m with two peaks rising to over 305 m.

The residential population is approximately 1800 and about one third of these, known as Islanders, are descendants of the *Bounty* mutineers' families who moved from Pitcairn Island in 1856 following two previous penal settlements on the island. Later settlers from Australia, New Zealand, the United Kingdom and other countries are referred to as Mainlanders. Tourism is the basis of the island's economy with the tourist intake varying from 200 per day in winter to 1200 per day in summer. During the year 1979/80 aproximately 23000 tourists visited the island mainly from Australia, New Zealand and Noumea.

Since the Pitcairn settlement of Norfolk Island, gastroenteritis, known locally

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as 'the sick and the vomits', has occurred both endemically and epidemically. Because it is accepted as a 'fact of life' many Islanders would not bother to seek medical attention, so that the true incidence of this disease is difficult to estimate. However, in recent years there has been an increasing number of cases of vomiting and diarrhoea on the island and in 1978/79 the incidence was 384/1000 for older children and adults and 225/1000 for infants and young children. (Sexton, Grohmann & Murphy, 1980). Since 1975 attempts to isolate any causative agents have failed except for a single case of giardiasis.

Repeat attacks may occur in the same individual suggesting the initial attack does not confer long lasting immunity or, more likely, that a number of different organisms are involved. Both tourists and local inhabitants are affected which supports the view that there is a constant introduction of different organisms, rather than a single uncommon organism which tourists have not previously encountered. The signs and symptoms are typical of acute gastroenteritis, consisting of an acute attack of nausea, vomiting, abdominal cramps and diarrhoea lasting usually 24–36 h and sometimes up to 96 h. Children up to two years of age are mostly febrile, with diarrhoea the most common symptom, sometimes preceded by or accompanied by vomiting. Clinical recovery usually appears to vary between 36 and 72 h.

Norfolk Island is wholly volcanic and is made up of lava flows of a basaltic type with some beds of tuff which vary in thickness. The eruptive cycle responsible has been dated to the late pliocene era. There are some 290 groundwater extraction points which can be used to tap ground water almost exclusively in the weathered profile (Abel, 1976). Hydrothermal activity is absent so that all water present on the island is derived from rainfall which is approximately 1100 mm per annum. There is no water reticulation on the island and stored rainwater is used for most domestic purposes. However, due to limited storage facilities this source is insufficient, particularly in dry weather, to supply the needs of the island without recourse to bore water which may be obtained from two different sources; (a) depressions in the eroded surface of the uppermost basalt layer which in some areas is only 3–4 m below the surface. These vary in shape and size and are readily filled with rainwater; and (b) porous beds of agglomerate, tuff and ash below the basalt layer which form the deep saturated zone and contain reservoirs of water. Both sources are readily tapped by bores. The former areas are small producers of water compared to the latter (Duvall, R. G., personal communication). There are at present 18 main bores on the island and the total volume of water delivered to households from these sources was 528000 gallons in 1977 and 419000 gallons in 1978. Bore water is delivered by water-cart and most of the deliveries occur from October to April each year. The major hotels and motels rely largely on their own rainwater collection, with some bore water supplementation when necessary usually obtained from their own particular bores, located in nearby valleys. Sewage and waste water from these hotels is, in most cases, disposed of by small sewage plants with the effluent being absorbed into the surrounding soil. Some effluent is treated with chlorine. Private homes use pit and trench disposal methods for sewage and effluent disposal. Studies using tracer dyes revealed contamination of bore water sources by effluents and, in addition, detergent levels have been shown to be steadily increasing since 1973. Detergent levels of water samples from a
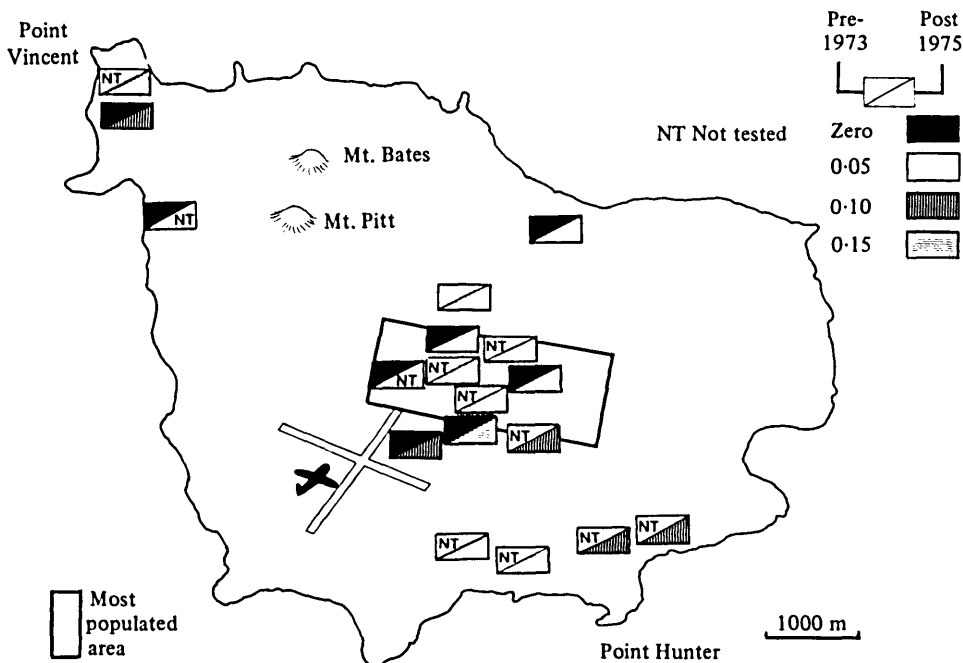
Fig. 1. Map of Norfolk Island showing increasing detergent levels (mg/l) in water samples as measured pre-1973 and post-1975, taken from 18 bore-water sites.

number of sites as measured pre-1973 and post-1975 are shown in Fig. 1 (unpublished data).

The increasing incidence of infectious gastroenteritis on Norfolk Island appeared to parallel increasing contamination of these ground water sources, and with all attempts to culture bacterial agents having failed it was therefore decided to collect faecal specimens and bore water samples for viral studies, including electron microscopy, in an attempt to identify the aetiological agent(s).

MATERIALS AND METHODS

Specimens and samples

Twenty eight faecal specimens from individuals with gastroenteritis were collected within 24 h of the onset of illness. Thirty two bore-water samples and one effluent sample were also collected at various times between September 1980 and November 1981. These samples were collected in sterile plastic containers at the bore head and were representative of the water being transported throughout the island. The samples were transported by air to Sydney (a 5 h journey) and then by road to the laboratory where they were stored at 4 °C for no more than 12 h before laboratory examination. In most cases specimen preparation was immediately initiated.

Preparation of faecal specimens for electron microscopy (EM)

Faecal specimens were prepared by making a 20 % extract in phosphate-buffered saline (PBS) and shaking with glass beads for 15 min. The suspension was clarified

by centrifuging at 2000 g for 15 min in an MSE bench centrifuge. The supernatant was added to an equal volume of 'Arklone' (trichlorotrifluoethane) and mixed thoroughly. The Arklone was removed by centrifugation for 10 min at 1000 g. The supernatant was then ultracentrifuged at 180000 g for 2 h in an MSE superspeed 75 using a 10 × 10 ml angle rotor. The resulting pellet was resuspended in a few drops of PBS for examination by electron microscopy.

Preparation of bore-water samples for EM

Water samples (5 l) were concentrated to approximately 50 ml using an Amicon model CH4 hollow fibre concentration/dialysis system. A 10 ml aliquot was removed for cell culture studies and the remaining concentrate was ultracentrifuged in an MSE superspeed ·75 ultracentrifuge at 138000 g average for 4 h at 4 °C in an 8 × 50 ml angle rotor. The resulting pellet was resuspended in PBS for examination by EM. The CH4 concentrator was sterilized between each sample with a 5 % formalin solution and the prefilter was also replaced in between each test sample. The one effluent sample was also examined in this way. Samples (25 l) were reduced to 1–2 l with a DC10 Amicon hollow fibre concentrator and then treated as for the 5 l samples.

The pre-filter used for each sample was retained, placed into 4 ml of sterile cell culture medium and held at 4 °C for at least 18 h. The fluid was then clarified and inoculated into cell cultures.

EM

All specimens for EM were placed on 400-mesh parlodion carbon-coated grids for 1 min, stained with phosphotungstic acid (pH 7·0) for 30 s and examined at 80 kV with a Philips EM 400 G electron microscope at a plate magnification of 48000. At least five intact grid squares were examined per grid and for the water/effluent preparations at least 10 grid squares were examined.

Bacteriology

Samples of the 28 faecal specimens were immediately forwarded to the Bacteriology Department, Institute of Clinical Pathology and Medical Research, Westmead Centre, Sydney, New South Wales, where they were examined for bacteria associated with gastroenteritis, namely salmonella, shigella, thermophilic campylobacter, as well as ova, cysts and parasites. Isolation of salmonella/shigella was attempted using salmonella-shigella agar, desoxycholate citrate agar, xylose lysine desoxycholate agar and selenite F and tetrathionate enrichment broths. Selective campylobacter agar (Skirrow's formulation) was used for the isolation of campylobacter at 42·5 °C under microaerophilic conditions.

Twenty-one aliquots (1200 ml) of water samples were forwarded to the Division of Analytical Laboratories, Lidcombe, New South Wales for bacteriological and chemical examination.

The samples were examined for the standard aerobic plate count, *Escherichia coli*/faecal coliforms, coliform organisms and salmonella using methods prescribed by the Standards Association of Australia (AS1095.4.1). Chemical examination of the samples included tests for nitrites, nitrates, total hardness, alkalinity, calcium hardness, chlorides, colour, turbidity, specific conductance, pH, iron,

Table 1. *Results of electron microscopy on 28 faecal specimens*

	Rotavirus	Astrovirus	Adenovirus	Calicivirus	SRV*	No viruses	Total
Adults (17–75 years)	3	1	1	1	5	3	14
Children (1–8 years)	3	4	—	2	1	4	14
Total	6	5	1	3	6	7	28

* Small round viruses 22–25 nm in size.

detergent, sodium and potassium, using methods described by the American Public Health Association (Standard Methods for the Examination of Water and Wastewater, 1980).

Virus isolation in cell cultures

A 0·2 ml aliquot of supernatant from the clarified 20 % suspensions of faecal specimens was inoculated into a monkey kidney, Vero, Hep2 and human embryonic fibroblast culture. Effluent and water concentrates were inoculated into the same cell culture types using 0·5 ml volumes. The inocula were allowed to absorb to the cell sheet for 2 h at 37 °C, then removed and replaced with cell culture medium. All cell cultures were examined regularly for cytopathic effect. Poliovirus isolates were identified as vaccine strains or otherwise according to their thermo-sensitivity at 40 °C (Lwoff & Lwoff, 1960).

In addition, six specimens positive for small round viruses (entero/parvo-like particles) by electron microscopy were inoculated into suckling mice which were observed for 10 days for signs of paralysis.

RESULTS

Examination of faecal specimens

Twenty-eight specimens were examined by electron microscopy and cell culture. No viruses were isolated in cell cultures or suckling mice but viruses were observed in 21 (75 %) specimens by electron microscopy (Table 1), representing the highest percentage of positive results obtained from sporadic cases of gastroenteritis since this type of work commenced at this Institute in 1974. No bacterial pathogens or parasites were identified.

Examination of bore-water samples

Thirty-two samples collected from 15 sites on six separate occasions were examined and the results are summarized in Table 2. Six samples (29 %) showed unacceptable bacteriological levels (i.e. $\geqslant 1$ *E. coli*/100 ml and $\geqslant 10$ coliform organisms per 100 ml), one sample showed an excessive chloride level (583 mg/l) and one sample showed an excessive level of iron (0·39 mg/l). Seven isolates of poliovirus type 1, all vaccine strains, and two adenovirus isolates (type 1 and type 5) were obtained. Rotavirus, adenovirus and small round viruses (22–25 nm) were detected by electron microscopy in four separate samples and bacteriophages were frequently observed in most samples. Also, from the septic tank effluent,

Table 2. *Results of virological examination of* 32 *bore-water samples from* 15 *sites on Norfolk Island*

Date collected	Number of samples	Volume collected	Viruses identified (number) by		Unacceptable bacteriology*
			EM	Cell culture	
Sept. 80	5	5 l	Rotavirus (1) SRV (2)	—	Not tested
Nov. 80	9	5 l	—	Poliovirus (2)	2
Dec. 80	4	25 l	Adenovirus (1)	Poliovirus (2)	1
Jan. 81	6	25 l	—	Poliovirus (2)	1
Mar. 81	2	25 l	—	Poliovirus (1)	2
Nov. 81	6	25 l	—	Adenovirus (2)	Not tested

* See text.

A septic tank effluent sample (4·5 l) was also examined and rotavirus, poliovirus type 1 were detected. Four bore sites were sampled twice. Three of these sites yielded viruses on each sampling. One bore site was sampled three times and viruses recovered on two occasions.

rotavirus and poliovirus type 1 were detected. Of interest was the detection of poliovirus from three water samples which had met an acceptable bacteriological standard.

The finding of rotaviruses in one bore-water sample was both surprising and significant as only 4·5 l of water was collected on this occasion. The adenoviruses were detected in 25 l samples. In samples positive by EM only a few virus particles per 10 grid squares were observed. Nearly all the virus particles were either damaged or 'empty' but viruses showing definite rotavirus and adenovirus structure were seen. These are depicted in Plate 1 together with astrovirus, calicivirus and 22–25 nm small round viruses which were detected in faecal specimens.

DISCUSSION

This study indicates the majority of gastroenteritis on Norfolk Island in 1980 was viral in origin, since viral particles were detected in 75 % of faecal samples examined by electron microscopy. Failure to propagate any viruses in cell culture is in accordance with present-day technology, as most gastroenteritis viruses cannot be cultivated in the laboratory. It is also reasonable to assume that most of the unexplained aetiology in previous years was probably of viral origin.

Many human enteric viruses, including picornaviruses, rotaviruses, adenoviruses, hepatitis A and Norwalk virus have been linked to outbreaks of illness in which water has been shown to be the vehicle of transmission. However, the actual detection of viruses in water has proved to be a difficult problem because, when present, they are usually in such low concentrations that large quantities of water need to be concentrated to recover a sufficient number of virus particles for identification. A variety of techniques have been reported for the recovery of viruses from drinking water, waste water, sewage effluent and sea water with varying recovery rates (Shuval & Katzenelson, 1972). Membrane adsorption and elution techniques have been most extensively used but recently ultrafiltration was

reported as a suitable system for the recovery of adenovirus, reoviruses and enteroviruses from sewage effluent, with a recovery rate of over 80 % (Ratnamohan, Garretson & Irving, 1980). In earlier preliminary studies (unpublished data) we found that the ultrafiltration system (Model CH4) would consistently recover 80 % of enteroviruses and 72 % of adenoviruses from 5 l of seeded distilled water. It was also found that virus was recoverable from the prefilter disk (a coarse filter to stop particulate matter from occluding the hollow fibres). In testing the samples from Norfolk Island, poliovirus was isolated from the prefilter disks as well as the concentrates. Virus presumably adsorbs onto the filter disk and this is a disadvantage in the system unless the pre-filter is also tested for virus.

Little work has been carried out using electron microscopy to detect viral paticles in water and effluents and most workers have relied solely on culture techniques. However, as already mentioned, most gastroenteritis viruses cannot be cultured in the laboratory and if they are to be found in water samples, electron microscopy is the only presently available technique which will detect them. The low concentration of viral particles likely to be present in drinking water has, perhaps, led most workers to consider electron microscopy an exercise of doubtful value – yet in this study rotaviruses were found in the concentrate of a 4·5 l sample of hotel drinking water. The combination of ultrafiltration, ultracentrifugation and electron microscopy has been successful in the detection of viral particles in water and, added to the normal culture techniques used, has markedly increased the number of positive samples in this study.

The very high incidence of gastroenteritis on Norfolk Island is consistent with the transmission of viruses by such a commonly used vehicle as water, and the finding of a rotavirus, three adenoviruses, seven polioviruses and two small round viruses in bore-water samples is indicative of sewage effluent pollution of the deep ground water. This is particularly so in areas where several large hotels are grouped together. Water and sewage effluents seep down to nearby valleys where water bores are situated and in some instances it can be fairly said that diluted sewage is being recycled with minimal intermediate cleansing. This is a serious problem for the island as it is likely to become worse with increasing tourism. Should outbreaks of cholera, typhoid fever or hepatitis ever occur, great economic hardship for the island would result because of the inevitable decline of tourism – the basis of the island's economy. A sharp decline in the number of tourists occurred recently as the result of an outbreak of suspected dengue fever which was subsequently shown to be due to echovirus type 17 (King *et al.* 1982).

Clearly, the natural capabilities of the island to cope with pollution have been over-extended and some public health measures are urgently required in the near future. Ideally, a fully piped sewage disposal system, and a piped, clean, non-polluted water supply should be installed. The latter requires a drilling programme to locate a non-polluted water supply followed by the installation of a modern filtration and chlorination plant. Failing this, a sewage system for the most populated area – the hotel/motel and shopping district together with the installation of ultraviolet sterilization plants for drinking water in all major hotels, should markedly reduce the incidence of gastroenteritis.

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EXPLANATION OF PLATE

Electron micrographs of rotaviruses (A) and adenoviruses (B) detected in samples of bore water and astroviruses (C), caliciviruses (D) and 22–25 nm, small round viruses (E) (× 120000) detected in faecal specimens.
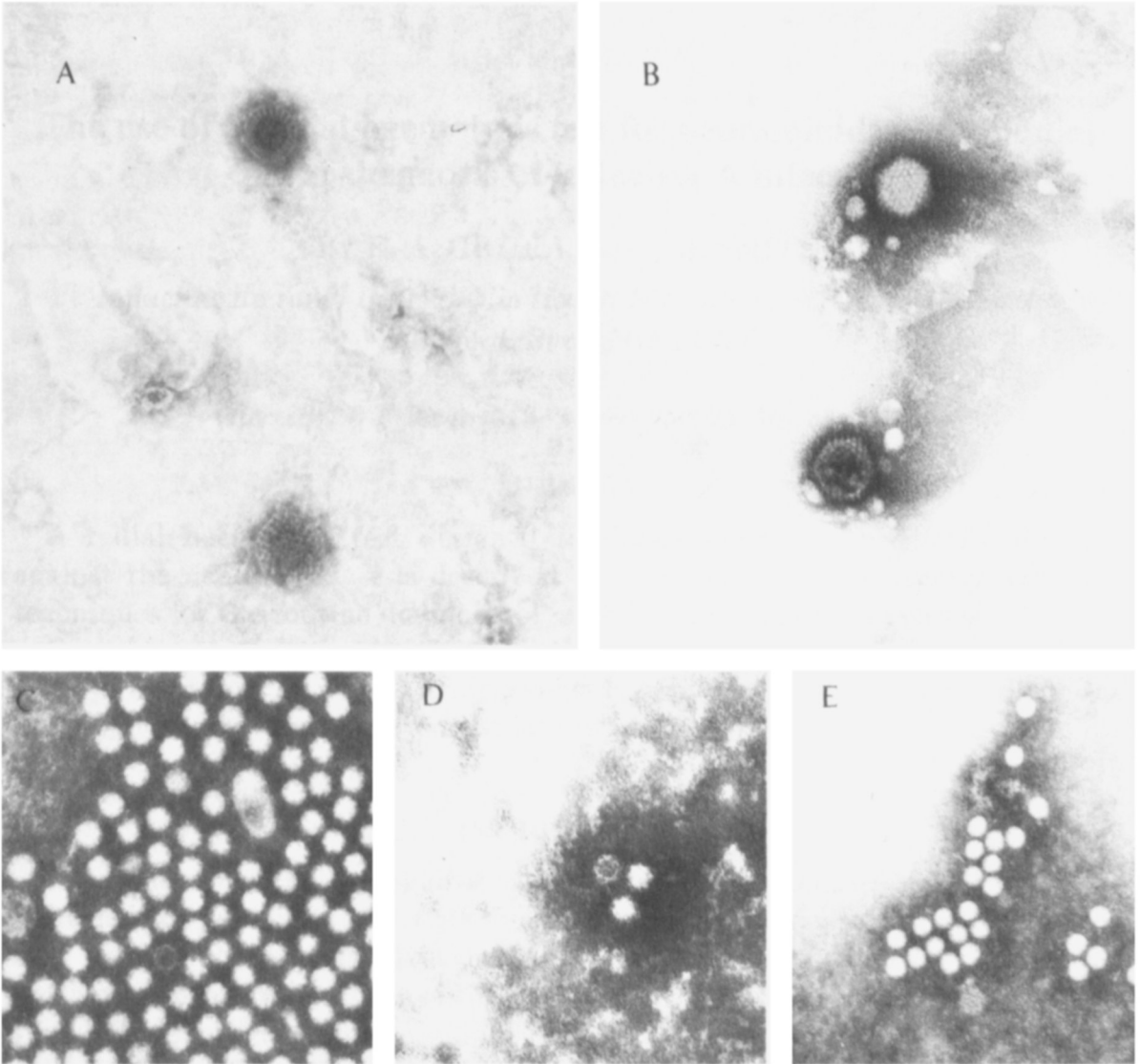
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