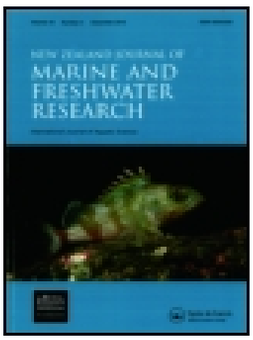

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Human enteroviruses in marine sediments near a sewage outfall on the Otago Coast

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Abstract Sediment and beach sand samples from around a sewer outfall were collected and tested for the presence of enteroviruses and faecal coliforms. Enteroviruses were recovered from 5 of 14 samples collected on three different occasions. Enteroviruses were recovered from two samples in which faecal coliform levels were low. These results suggest that the faecal coliform test may not be a reliable indicator of viral pollution. Virus types recovered were Poliovirus 2, Coxsackievirus B4 and B5, and a number of isolates unable to be identified using the Lim Benyesh-Melnick pooled antisera.

Keywords enterovirus; marine sediments; faecal coliforms; virus detection

INTRODUCTION

Disposal of sewage effluents and sludges into the sea is a common practice both in New Zealand and overseas (OECD 1981). In 1982, 48% of sewage treatment plants in New Zealand, involving 60% of the country's sewage, were discharging effluents into marine waters (New Zealand Institute of Engineers Technical Group on Water 1982).

To ensure that waters which receive sewage effluents are safe for other users and for fisheries, they must be monitored for possible disease-causing organisms. The faecal coliform test for detecting bacteria is currently used for such monitoring in many countries (IAWPRC 1983).

Enteroviruses are found in sewage in large numbers, and because they are relatively resistant to inactivation by treatment processes, they are discharged with sewage effluents into receiving waters (WHO 1979). The health hazard presented by human enteroviruses entering the environment in this way has been established, with outbreaks of viral hepatitis A and gastroenteritis being traced to the consumption of contaminated shellfish (Gerba & Goyal 1978; Murphy et al. 1979).

Attempts to isolate viruses from marine waters have met with only moderate success except in heavily polluted areas (Feachem et al. 1981). Attention has now been focused on the possibility that viruses may also be present in sediments as well as in the overlying water, as De Flora et al. (1975) showed that enteroviruses could be recovered from marine sediments. Other researchers have supported this finding and have demonstrated that enteroviruses can be isolated from marine sediments even when they are not detectable in the overlying water (Gerba et al. 1977; Bitton et al. 1982). A similar observation has been made for faecal coliforms (Gerba & McLeod 1976; Loutit & Lewis 1985).

The purpose of this study was to see if human enteroviruses could be detected in marine sediments around an ocean sewer outfall.

MATERIALS AND METHODS

Site

All sampling sites were in an area of c. 35 km^2 and included 7 km of coastline adjacent to Dunedin City and an offshore area (Fig. 1). The predominant current in this area flows towards the north-east along the coast (Brodie 1960; Robertson 1980). Weather conditions have some influence on the water currents with the predominant winds in the area being from the south-west. On occasion however, strong northerlies may be experienced for several days at a time. The Dunedin City sewer outfall is at Lawyers Head and discharges c. 1.1×10^7 *l* day^{-1} of sewage effluent. This study was carried out in the summer of 1982–83 prior to full commissioning of the waste water treatment plant.

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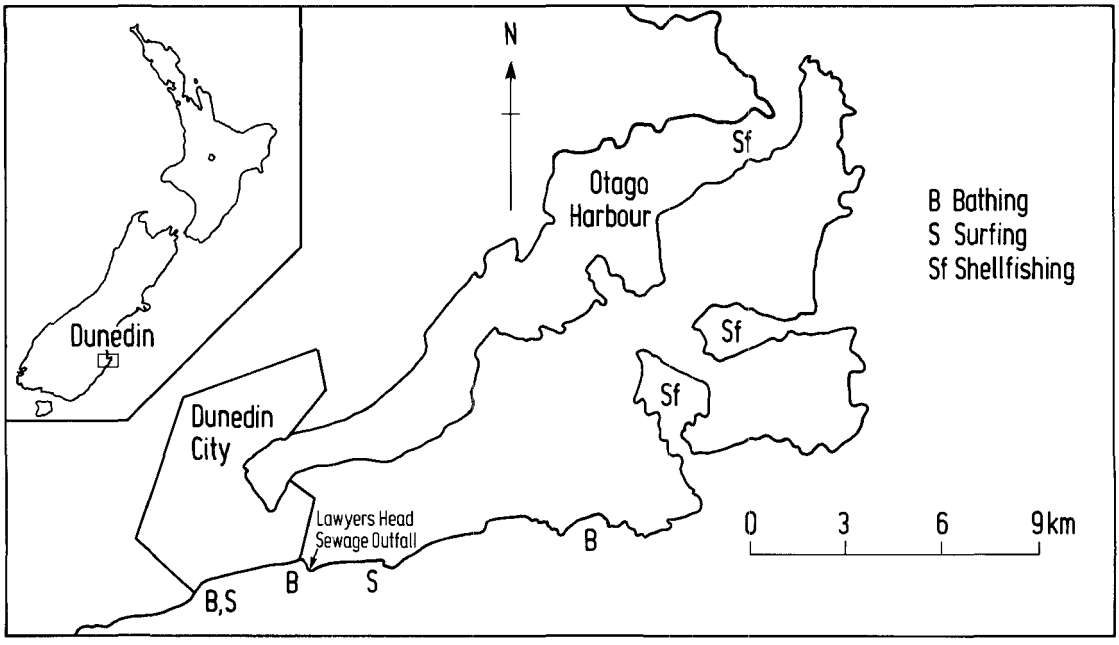

Fig. 1 Map of the Otago Peninsula showing the location of the Lawyers Head sewage outfall in relation to recreational beaches and major shellfishing areas.

Samples

Samples from the sites indicated in Fig. 2 were collected from the beaches at the low tide mark and off-shore sediment samples were obtained by anchor dredge from the University of Otago research vessel, *Munida*. Where possible only the top 5 cm of sand or sediment was collected. Samples could not be collected at all sites on each sampling date because of access difficulties. All samples were collected into sterile plastic bags and transferred directly to the laboratory where they were processed within 12 h.

Bacterial estimates

The methods used for establishing the most probable number (MPN) of presumptive coliforms (PC) and faecal coliforms (FC) were those recommended by the New Zealand Microbiological Society's Committee on Coliform Bacteria (1976). Oxoid minerals modified glutamate medium was used in the five tube method to estimate PC. Difco lauryl sulphate tryptose broth inoculated with material from positive tubes from the PC test and incubated at 44.5 $\pm$ 0.5°C was used to determine MPN of FC and to confirm PC. Inocula from positive FC tubes were streaked on to Levine's eosin methylene blue agar. Colonies showing a green metallic sheen were deemed positive and used to establish the MPN of confirmed FC.

Virus concentration

Viral recovery from sediments was carried out using a method developed in this laboratory (Lewis et al. in press). Briefly the technique is as follows. Sediment samples (c. 100 g) were weighed and mixed with three times the amount (w/v) of 6% beef extract at pH 9.0, shaken for 1 h at 4°C and then centrifuged at 10 000 g for 20 min. Enteroviruses were concentrated from the supernatant by mixing with polyethylene glycol 6000 (BDH Chemicals) to a final concentration of 8%, for 1 h at 4°C followed by centrifugation for a further 20 min at 10 000 g. The pellet was resuspended in BGM tissue culture medium containing 5% calf serum, and the whole frozen at −70°C until assay.

Virus assay and isolation

All extracts were assayed on BGM cells (Barron et al. 1970) obtained from Dr L. Irving, Fairfield Hospital, Melbourne, Australia. Extracts from sediment samples were assayed using an agar cell suspension technique as described by Simmonds et al. (1982). Viruses to be identified were isolated directly from these plates.

Virus identification

Enteroviruses from individual plaques in agar cell suspensions were identified by neutralisation tests on BGM cells grown on flat bottom micro-titre trays

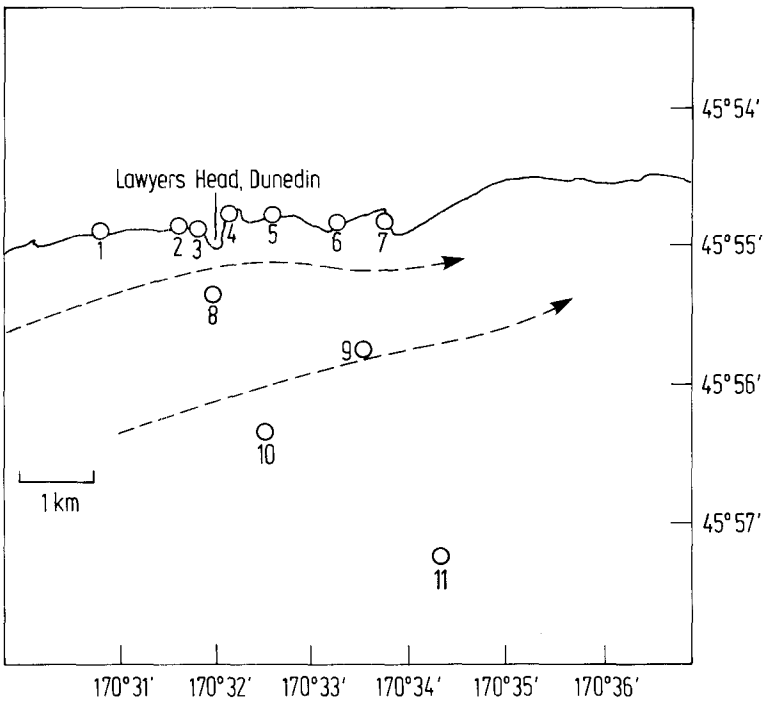

Fig. 2 Position of sampling sites in relation to the Lawyers Head outfall.

(Simmonds et al. 1982) using the Lim Benyesh-Melnick pools of antisera obtained from the United States National Institute of Health, Bethesda, Maryland. These antisera pools allow for the identification of a range of enteroviruses of the poliovirus, coxsackievirus, and echovirus groups.

RESULTS AND DISCUSSION

Samples were collected over a period of three months, from the sites shown in Fig. 2 and the results of estimations of viral numbers and faecal coliform concentrations are shown in Table 1.

The possibility that human enteroviruses may be present in sediments has been considered only recently and little is as yet known of their survival capabilities or distribution (Feachem et al. 1981). Recently the relationship between enteroviruses in the environment and standard indicators, for example faecal coliforms, has been questioned (Berg et al. 1978; LaBelle et al. 1981). In this study, the first to be carried out in New Zealand, an attempt has been made to establish the importance and extent of enteroviral contamination in a local marine environment. The study was intended to provide some insight into whether enteroviruses in sewage effluents survived in sediments and if faecal coliforms were a satisfactory indicator for detecting viruses in at least one area in New Zealand.

From the results, it can be seen that human enteroviruses occur in marine sediments near the sewer outfall. They were detected at sites 1, 3, 7, and 8 (Fig. 2).

The recovery of viruses from the sand at site 1 is of interest as this area is part of one of Dunedin's most popular bathing beaches. The sample was collected in midsummer and it must be a matter of speculation as to whether this isolate came from the sewer outfall or directly from a bather.

The recovery of virus from sites 3 and 8 could be predicted, as these areas are close to the outfall and solid debris from the outfall could be found at these points.

The large number of viruses recoverable at site 7 on the 16/10/82 is of particular interest as this area is 2.5 km from the outfall. Viral contamination of mussels at this point has also been reported (Lewis et al. 1982). Although no virus was isolated at site 7 on 12/1/83 the faecal coliform numbers were significantly higher than at points closer to the outfall on that date. These observations may indicate that either there is an unknown source of contamination near site 7, or that sewage associated organisms are being concentrated by some means,

Table 1 Enterovirus and faecal coliform in Otago Coast sediments. pfu = Plaque forming units, CB4 = Coxsackievirus B4, P2 = Poliovirus 2, CB5 = Coxsackievirus B5, and UI = Unidentified virus.

Sample point	Date	Faecal coliforms $100\ g^{-1}$	Enteroviruses pfu $100\ g^{-1}$	Enterovirus identification
1	12/1/83	50	1.2	CB4*
2	12/1/83	50	0	-
3	16/10/82	2000	300	P2**
	12/1/83	20	1.8	UI***
4	12/1/83	20	0	-
5	12/1/83	20	0	-
6	12/1/83	0	0	-
7	16/10/82	130 000	2400	P2, CB5, UI****
	12/1/83	250	0	-
8	5/11/82	1800+	0	-
	17/11/82	140	2.5	UI***
9	17/11/82	350	0	-
10	17/11/82	20	0	-
11	17/11/82	20	0	-

*One plaque occurred which was identified as CB4.
**Eleven viruses were isolated (26% of plaques occurring) of which 6 were P2 and 5 could not be identified.
***One plaque occurred but it could not be identified.
****Twelve viruses were isolated (40% of plaques occurring) of which 8 were P2, 3 were CB5, and 1 could not be identified.

such as an observed backwash between the headland and an island and sand bar between sites 6 and 7 which may bring sewage solids and any associated viruses into the area. The uneven distribution of viruses in the sediments and the result obtained for site 7 suggests that currents and coastal topography play a part in distributing viruses. The viral distribution around the sewer outfall varies considerably over the time frame of the study with no clear indication of the areas which are likely to be contaminated with enteroviruses. Even if viruses at sites 1 and 7 came from some source other than the sewage outfall it would be difficult to support the contention that viruses at sites 3 and 8 came from a source other than sewage.

A number of viral types were isolated and those identified were Poliovirus 2 and Coxsackieviruses B4 and B5. A number of the viral isolates could not be identified using the Lim Benyesh-Melnick pools of antisera.

It is also evident that there is no apparent correlation between FC and virus numbers in the sediments at the same site on the same day. Virus concentrations were not always high when FC were high (site 8, 5/11/82), although at site 7 on 16/10/82 both were extremely high. Similarly viruses may be present even when FC numbers are relatively low (site 8, 17/11/82). The results of this study imply that FC cannot be used as reliable indicators of the presence of viruses. This observation is supported by the findings of others who have demonstrated a lack of correlation between FC and enteroviruses (Berg et al. 1978; LaBelle et al. 1981).

The enteroviruses found in the sediments in this study should be regarded as minimal numbers only. All the enteric viruses present would not be detected for a number of reasons. The BGM cell line used in the assay system would not allow the multiplication of some virus types. Further the detection method itself would not recover all of the viruses that may be present. The viruses of particular concern, such as hepatitis A, Norwalk agent, and rotavirus cannot, as yet, be cultured easily and would not be detected by the methods used. The detection of any enteric virus is the most useful indicator yet established of such important viral pathogens. It should also be pointed out that use of the Lim Benyesh-Melnick pools of antisera only allows identification of isolates from within the enterovirus genus.

Human enteroviruses have been found to adsorb very rapidly to suspended particles, especially in marine environments (Bitton et al. 1982; Lewis unpublished data). The settling of particles with attached virus may result in elevated levels of enteroviruses in the upper sediment layers. It must be remembered that adsorption of enteroviruses to solids does not decrease their infectivity and enteroviruses adsorbed to clay particles have been shown to be capable of producing infections in both cell

cultures and experimental animals (Moore et al. 1975; Schaub & Sagik 1975). Enhancement of viral survival has also been demonstrated following deposition in sediments (Smith et al. 1978; LaBelle & Gerba 1980). Adsorption occurs not only to inorganic particles but also to organic matter such as sewage solids, bacteria, and algae (Bitton 1980a) all of which may be deposited eventually into sediments. Subsequently any factor which disturbs contaminated sediments, for example, currents, tides, weather, and human activities, may result in reintroduction of potentially infective viruses into the water column (Bitton 1980b). The presence of enteric viruses in the water column, especially those associated with organic material, represents a source of contamination for filter-feeding shellfish in the area. These organisms have been shown to be capable of acquiring and concentrating viruses from the water column on numerous occasions (Gerba & Goyal 1978) and are the largest recorded cause of food-borne viral disease (Cliver et al. 1981).

The health implications of these phenomena must be considered. Recreational contact with sediments is common and often unintentional, and may be a means by which people are exposed to potential pathogens. Theoretically, the amount of virus required to produce infection in tissue culture assays is sufficient to infect a human being, therefore low levels of virus have the potential to cause disease (Melnick et al. 1978). However, no instance of viral disease has as yet been linked to contaminated sediments, although shellfish have been implicated extensively (Gerba & Goyal 1978; Murphy et al. 1979; Cliver et al. 1981). It is likely that any clinical cases stemming from contaminated sediments would be sporadic and difficult to relate to this source.

The observations reported in this paper indicate that enteroviral contamination of sediments near a sewer outfall does occur and that viruses are detectable at considerable distances from the outfall. It has also been shown that the faecal coliform test does not appear to be adequate as an indicator for enteroviruses.

At present no standards have been set in New Zealand with regard to viral levels in the environment and there is no requirement for routine surveillance of any area for such contamination. If public health and a rapidly expanding shellfish export industry are to be protected, consideration must be given to this problem in the near future.

Investigations are continuing in this laboratory to allow further insight into the occurrence of human enteric viruses in the environment including studies on freshwater and marine sediment and shellfish. A number of common wastewater treatment methods are also being evaluated to gauge their effectiveness for removing viruses.

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REFERENCES

Barron, A. L.; Alshersky, C.; Cohen, M. M. 1970: Characteristics of the BGM line of cells from African green monkey kidney. *Archiv fur die gesamte virusforshung 32*: 389–392.

Berg, G.; Dahling, D. R.; Brown, G. A.; Berman, D. 1978: Validity of faecal coliforms, total coliforms and faecal streptococci as indicators of viruses in chlorinated primary sewage effluents. *Applied and environmental microbiology 36(6)*: 880–884.

Bitton, G. 1980a: Adsorption of viruses to surfaces: Technological and ecological implications. *In*: Adsorption of micro-organisms to surfaces. Bitton, G.; Marshall, K. C. *ed.* New York, Interscience Publishers. Chapter 10, 331–374.

———— 1980b: Fate of viruses in aquatic sediments. *In*: Introduction to environmental virology. New York, Wiley Interscience, John Wiley and Sons. Chapter 4, 67–90.

Bitton, G; Chow, Y. J.; Farrah, S. R. 1982: Techniques for virus detection in aquatic sediments. *Journal of virological methods 4*: 1–8.

Brodie, J. W. 1960: Coastal currents around New Zealand. *New Zealand journal of geophysics 3*: 235–252.

Cliver, D. O.; Ellender, R. D.; Sobsey, M. D. 1981: Methods for detecting viruses in foods: Background and general principles. *Journal of food protection 46*: 248–259.

De Flora, S.; De Renzi, G. P.; Badolati, G. 1975: Detection of animal virus in coastal seawater and sediments. *Applied and environmental microbiology 30*: 472–475.

Feachem, R.; Garelick, H.; Slade, J. 1981: Enteroviruses in the environment. *Tropical diseases bulletin 78(3)*: 185–230.

Gerba, C. P.; McLoed, J. S. 1976: Effect of sediments on the survival of *Escherichia coli* in marine waters. *Applied and environmental microbiology 32*: 114–120.

Gerba, C. P.; Smith, E. M.; Melnick, J. L. 1977: Development of a quantitative method for detecting enteroviruses in estuarine sediments. *Applied and environmental microbiology 34*: 158–163.

Gerba, C. P.; Goyal, S. M. 1978: Detection and occurrence of enteric viruses in shellfish — a review. *Journal of food protection 41(9)*: 743–754.

IAWPRC Study Group on Water Virology 1983: Health significance of viruses in water. *Water research 17*: 121–132.

LaBelle, R. L.; Gerba, C. P. 1980: Influence of estuarine sediment on virus survival under field conditions. *Applied and environmental microbiology 39*: 749–755.

LaBelle, R. L.; Gerba, C. P.; Goyal, S. M.; Melnick, J. L.; Cech, I.; Bogdan, G. F. 1981: Relationship between environmental factors, bacterial indicators, and the occurrence of enteric viruses in estuarine sediments. *Applied and environmental microbiology 39*: 588–596.

Lewis, G. D.; Austin, F. J.; Loutit, M. W. 1982: A method for the detection and identification of human enteroviruses in the green-lipped mussel *Perna canaliculus*. *New Zealand journal of science 25*: 367–370.

———— 1985: Method for detecting human enteroviruses in aquatic sediments. *Journal of virological methods* (in press).

Loutit, M. W.; Lewis, G. D. 1985: Faecal bacteria from sewage effluents in sediments around an ocean outfall. *New Zealand journal of marine and freshwater research 19*:

Melnick, J. L. 1978: Viruses in water. *Bulletin WHO 56*: 499–508.

Moore, B. E.; Sagik, B. P.; Malina, J. F. Jr. 1975: Viral association with suspended solids. *Water resources 9*: 197–203.

Murphy, A. M.; Grohmann, G. S.; Christopher, P. J.; Lopez, W. A.; Davey, G. R.; Millson, R. H. 1979: An Australian-wide outbreak of gastroenteritis from oysters caused by Norwalk Agent. *Medical journal of Australia 1979(2)*: 329–333.

New Zealand Institute of Engineers Technical Group on Water 1982: Waste water treatment and disposal practices. New Zealand Water Conference 1982.

OECD 1981: Environmental policies in New Zealand. Paris. 78p.

Report of recommendations of the Microbiological Society's Committee on Coliform Bacteria 1976. *New Zealand journal of science 19*: 215–219.

Robertson, D. A. 1980: Hydrology and quantitative distrubution of planktonic eggs of some marine fishes off the Otago Coast, south-east New Zealand. *Ministry of Agriculture and Fisheries, New Zealand, Fisheries research bulletin 21*: 16–27.

Schaub, S. A.; Sagik, B. P. 1975: Association of enteroviruses with natural and artificially introduced colloidal solids in water and infectivity of solids-associated virions. *Applied and environmental microbiology 30*: 212–222.

Simmonds, R. S.; Lewis, G. D.; Loutit, M. W., Austin, F. J. 1982: A method for detecting and identifying enteroviruses in effluents. *New Zealand journal of science 25*: 57–60.

Smith, E. M.; Gerba, G. P.; Melnick, J. L. 1978: The role of sediment in the persistence of enteroviruses in the estuarine environment. *Applied and environmental microbiology 35*: 685–689.

WHO Scientific Group on human viruses in water, waste water and soil 1979: Human viruses in water, waste water and soil. *WHO technical report series 639.*