Epidemiol. Infect. (1992), **108**, 529–536
Printed in Great Britain

Enteroviruses in recreational waters of Northern Ireland

M. S. HUGHES[1]*, P. V. COYLE[2] AND J. H. CONNOLLY[2]

[1] *Department of Microbiology and Immunobiology, Queen's University, Belfast*
[2] *Regional Virus Laboratory, Royal Victoria Hospital, Belfast*

(*Accepted 19 December 1991*)

SUMMARY

Virus surveillance of Northern Ireland recreational waters, between April 1986 and May 1989 demonstrated widespread enteroviral contamination of coastal and inland waters. In 1986, enteroviruses were detected in 4 of 46 (8·7%) water samples, collected from 6 coastal bathing waters. In 1987, 49 of 107 (45·8%) samples, from 16 coastal bathing waters, yielded enteroviruses; 33 of the enterovirus positive samples passed one or both of the coliform standards outlined by the European Economic Community (EEC) bathing water directive (76/160/EEC). Enteroviruses were also detected in 33 of 39 (84·6%) samples tested from 3 inland recreational waters.

INTRODUCTION

In the early 1960s, no information was available concerning the presence of viruses in any surface water in the United Kingdom [1]. In 1965 viruses were first isolated from the River Thames, London [2]. In Northern Ireland, the viral status of recreational waters was virtually unknown until 1986 when a 4-year water virology programme, funded by the Department of the Environment for Northern Ireland (DoE, NI), was initiated. The primary aim of this study was to determine the existence and extent of viral contamination of Northern Ireland recreational waters, with a particular emphasis on coastal waters.

In 1987, 16 Northern Ireland coastal bathing waters were formally designated as bathing waters. These waters were subsequently monitored, between May and September 1987, for the microbiological parameters outlined in the EEC bathing water directive (76/160/EEC): total and faecal coliforms, faecal streptococci, salmonella and enteroviruses. The monitoring of EEC-designated waters for the presence of faecal streptococci, salmonella and enteroviruses is not obligatory but, when tested, the directive stipulates that enteroviruses should be absent in a ten-litre sample. The EEC bathing water directive (76/160/EEC) is also applicable to inland bathing waters.

The results of enteroviral analyses of Northern Ireland coastal and inland recreational waters are presented in this report.

* Author for correspondence and reprints: Dr M. S. Hughes, Bacteriology Department, Veterinary Sciences Division, Stoney Road, Stormont, Belfast BT4 3SD, N. Ireland.

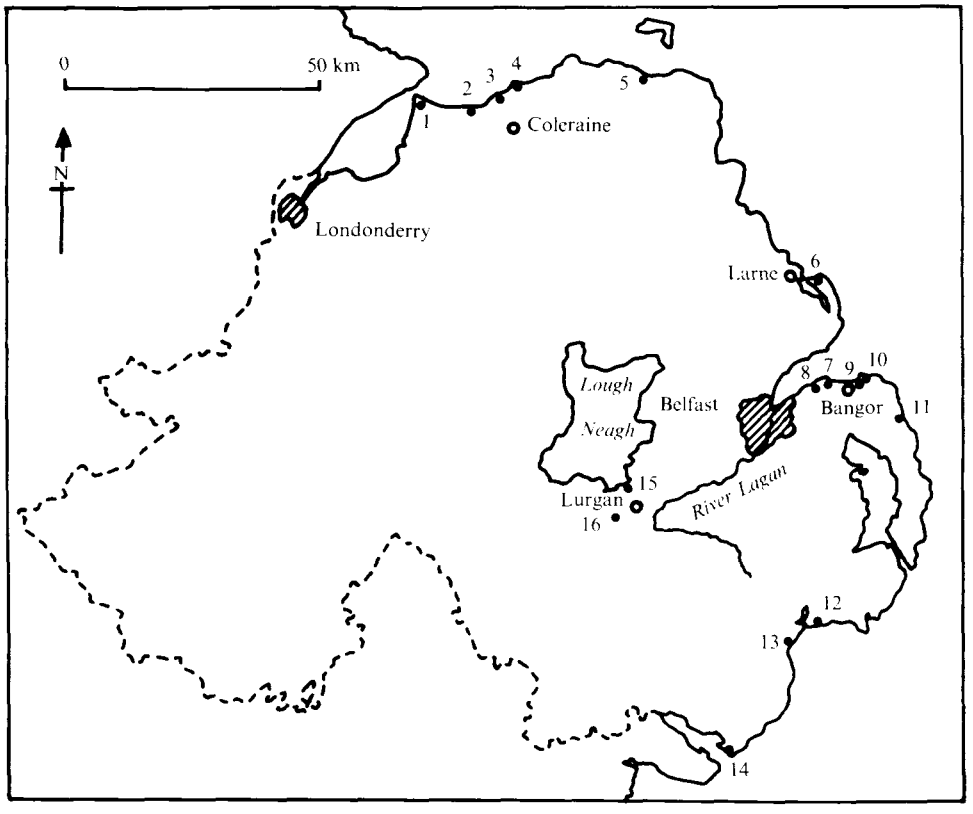

Fig. 1. Locations of the sampling sites and relevant major population centres of Northern Ireland. Sampling sites include: 1. Magilligan. 2. Castlerock. 3. Portstewart. 4. Portrush. 5. Ballycastle. 6. Brown's Bay. 7. Helen's Bay. 8. Crawfordsburn. 9. Ballyholme Bay. 10. Groomsport. 11. Millisle. 12. Tyrella. 13. Newcastle. 14. Cranfield. 15. Kinnego Bay (Lough Neagh). 16. Craigavon Balancing Lakes and the River Lagan.

METHODS

Sample processing

Sampling sites. Water samples from six Northern Ireland coastal waters, Ballyholme Bay, Groomsport, Newcastle, Portrush (Mill Strand and Curran Strand) and Portstewart, were collected during the bathing season (May–September), in 1986. In 1987, a further ten coastal bathing waters, Ballycastle, Brown's Bay, Castlerock, Cranfield Bay, Crawfordsburn, Helen's Bay, Magilligan, Millisle, Nicholson's Strand at Cranfield and Tyrella were monitored, in addition to the previous six tested in 1986. Major coastal towns are situated at Ballycastle, Ballyholme Bay, Newcastle, Portrush and Portstewart with Newcastle and Portrush being Northern Ireland's leading tourist resorts. Water samples from three inland recreational waters, Kinnego Bay (Lough Neagh), the Balancing Lakes (Craigavon) and the River Lagan, were also collected for enteroviral analysis. Figure 1 shows the locations of these sites and other major centres of population which may have contributed to microbial contamination of the aforementioned recreational waters.

Sample collection. Water samples for enteroviral analysis were ten-litre volumes

https://doi.org/10.1017/S0950268800050020 Published online by Cambridge University Press

Table 1. *Viral analyses of coastal bathing water samples May–Sept. 1986–7*

Bathing water	Site no.	No. of sampling stations	Sampling incidence	Samples positive for enteroviruses	Range of P.F.U. values in samples analysed
Ballycastle	5	3	4	8/10	1–1826
Ballyholme Bay	*9	4	4	0/10	0
	9	5	5	6/13	4–611
Brown's Bay	6	2	3	1/4	2
Castlerock	2	1	6	3/6	11–32
Cranfield	14	5	5	4/10	2–13
Crawfordsburn	8	2	1	1/2	8
Groomsport	*10	2	3	1/4	1
	10	2	8	4/8	2–14
Helen's Bay	7	1	1	0/1	0
Magilligan	1	3	3	1/8	4
Millisle	11	2	3	4/6	2–20
Nicholson's Strand	14	5	5	0/5	0
Newcastle	*13	8	4	2/11	3–15
	13	3	4	9/11	13–700
Portrush: Curran Strand	*4	2	1	0/2	0
	4	3	3	1/5	3
Portrush: Mill Strand	*4	4	4	1/13	4
	4	3	5	6/11	2–143
Portstewart	*3	3	2	0/6	0
	3	2	1	1/2	2
Tyrella	12	3	3	0/5	0

The 1986 results are prefixed with *. Every station was not always sampled when each site was monitored.

in accordance with the EEC bathing water derivative (76/160/EEC). These were collected in sterile polyethylene containers at an approximate depth of 30 cm, where permissible, by direct immersion of the container. The coastal water samples were collected by the Industrial Science Division (ISD), Department of Economic Development, Northern Ireland, from specific sampling stations between 10.00 h and 11.00 h on pre-selected days thus covering a range of tidal states. With few exceptions, notably Helen's Bay, Crawfordsburn and Portstewart, each coastal bathing water was sampled on at least three occasions, at two or more sampling stations, during the bathing seasons of 1986 and 1987 (Table 1). Bacterial parameters were measured by the ISD.

Sample concentration. Sample concentration was carried out by an adsorption–elution flocculation method [3]. The pH of the water sample was adjusted to 3·5, using 2 M-HCl, and the sample was subsequently filtered through a 293 mm diameter cellulose nitrate filter, 0·45 μm pore size (Sartorius SM 11306). A 293 mm diameter Metrigard glass fibre filter (Gelman Sciences Inc) was also employed if the sample contained a large amount of particulate matter. The nitrocellulose filter was rinsed with 100 ml of sterile distilled water, pH 3·0 (this step was introduced for seawater samples only dating 23 June 1986 and onwards) before elution of adsorbed viruses with 400 ml of 3% w/v beef broth at pH 9·5. Three types of beef broth were used for sample concentration: 'Lab-Lemco' powder L29 (Oxoid Ltd, lot no. 1724585) from April to June 1986, 'Lab-Lemco' broth CM15

https://doi.org/10.1017/S0950268800050020 Published online by Cambridge University Press

(Oxoid Ltd, lot no. 19638244) from June 1986 to September 1988 and Beta-lab beef extract powder (Difco Laboratories, batch no. D1045) from September 1988 onwards. The pH of the eluent was decreased to 3·5 and a flocculation promoter ($FeCl_3$) was added when required. The addition of $FeCl_3$, recommended by Payment and colleagues [4], was introduced on 3 July 1986 following problems in flocculating 'Lab-Lemco' broth CM15. The acidified eluent was left at 4 °C for 30 min (samples collected prior to October 1986 were gently agitated at 25 °C) and the resulting floc pelleted by centrifugation at 3500 rev. min^{-1} for 15 min at 4 °C in a MSE Mistral 4L centrifuge (MSE Scientific Instruments). The pellet was resuspended in 5–10 ml of 0·15 M-Na_2HPO_4 and the concentrate stored at −70 °C until it was assayed.

Sample detoxification. Concentrates of samples collected on 7 July 1986 and onwards were detoxified with an equal volume of 0·005 M solution of diphenyl thiocarbazone (Sigma Chemical Company) in chloroform before assaying.

Virus assay

Cell line. Buffalo Green Monkey Kidney (BGM), a continuous African green monkey (*Cereopithecus aethiops*) kidney cell line [5], was cultured [6] and used for virus assays.

Plaque assay. Concentrates were analysed for the presence of enteroviruses by a suspended-cell plaque assay developed by Cooper [6] and adapted by the Metropolitan Water Board, London in 1974 for analyses of water concentrates [7].

Confirmation of viruses and identification. Plaques were picked and inoculated on to BGM preformed monolayers to confirm their viral origin. The cultured viruses were subsequently identified by neutralization tests using type-specific antisera supplied by the Division of Microbiological Reagents and Quality Control, Central Public Health Laboratory, London.

Efficiency of virus recovery

The efficiency of poliovirus type 1 recovery from four dechlorinated tap water samples seeded with $4·4 \times 10^6$, 7×10^6, $10·75 \times 10^6$ and $10·75 \times 10^6$ p.f.u. respectively, ranged from 1·5 to 7·4%. The efficiency of recovery of the same virus type from 11 beef broth samples seeded with 620, 1333, 4800, $1·3 \times 10^4$ and 7×10^6 p.f.u. ranged from 0 to 40%.

RESULTS

Coastal recreational waters

Enteroviruses were detected in 4 of 46 (8·7%) water samples collected from 6 coastal bathing waters in 1986 (Table 1). Two bathing waters, Newcastle and Groomsport, failed to comply with the viral standard stated in the EEC bathing water directive (76/160/EEC). Enteroviruses were also recovered from a river crossing the foreshore of the beach at Mill Strand, Portrush. Such rivers, however, were not officially recognized as coastal bathing waters.

In 1987, enteroviruses were recovered from 49 of 107 (45·8%) water samples from the 16 designated coastal bathing waters (Table 1). Six of 7 (85·7%) water samples from rivers associated with coastal bathing waters were positive for

Table 2. *Viral analyses of inland recreational waters*

Location	Site no.	Sampling dates	Samples positive for enteroviruses	Range of P.F.U./10 l sample
Kinnego Bay; Lough Neagh	15	Oct. 1986–Apr. 1987	9/9	2–143
Balancing Lakes: Craigavon	16	Jan. 1987–May 1988	12/18	0–43
River Lagan		Apr. 1986–May 1989	12/12	5–593

enteroviruses in comparison to 43 of 100 (43%) seawater samples yielding enteroviruses. Four bathing waters, Crawfordsburn, Helen's Bay, Nicholson's Strand (Cranfield) and Tyrella were accepted by the DoE, NI as fulfilling the viral standard stipulated by the EEC directive (76/160/EEC), although enteroviruses were detected in a river crossing the foreshore at Crawfordsburn.

A comparison of the viral and bacterial counts of 103 coastal water samples, taken in 1987, revealed that 33 of the samples found to be enterovirus positive passed one or both of the coliform standards outlined in the EEC directive (76/160/EEC).

Inland recreational waters

Nine water samples collected from Kinnego Bay between October 1986 and April 1987, and 12 water samples taken from the River Lagan between April 1986 and May 1989 were all found to contain enteroviruses. Enteroviruses were also detected in 12 of 18 (66·7%) water samples collected from the Balancing Lakes between January and May 1987 (Table 2). Virus yields were generally higher for River Lagan samples, ranging from 5 to 593 plaque-forming units (p.f.u.) per 10 l, than for samples from Kinnego Bay (2–143 p.f.u./10 l) and the Balancing Lakes (0–43 p.f.u./10 l).

Virus identification

Five hundred and thirty-four virus isolates from the aquatic environment were identified. Of these, 128 were found to be polioviruses; 27 type 1, 73 type 2, and 28 type 3. The remaining 406 were identified as coxsackieviruses group B (CVB); 26 CVB2, 89 CVB3, 209 CVB4 and 82 CVB5. Fifty-two CVB were isolated from clinical specimens sent to the Regional Virus Laboratory, Belfast during the study period. The same serotypes were found in a similar temporal distribution.

DISCUSSION

Analysis of coastal and inland water samples suggested that enteroviral contamination of Northern Ireland recreational waters was common and widespread. Twelve of the 16 designated coastal bathing waters, tested in 1987, failed the viral standard stated in the EEC directive (76/160/EEC). The highest numbers of enterovirus plaque-forming units were detected in coastal water samples from Ballycastle, Ballyholme and, most frequently, from Newcastle, a major tourist resort in Northern Ireland. Of the bathing waters passing the standard, two (Crawfordsburn and Helen's Bay) were thought to have been under-sampled (Table 1). The 1987 coastal water results provided a more representative

overview of the viral status of Northern Ireland coastal waters than the 1986 results due to improved methodology, as previously described.

Viral and bacterial analyses indicated that sewage outfalls were responsible for microbial contamination of adjacent coastal bathing waters. The highest levels of enteroviruses detected at Ballycastle were at a sampling station adjacent to a sewage outfall. Rivers were also a source of viral contamination of certain coastal bathing waters. Both sewage outfalls and rivers were observed to be commonly used by young children at coastal resorts.

Coliform counts and viral yields of coastal bathing water samples, in 1987, often highlighted the same microbially contaminated sites but more samples passed the EEC coliform standards than the EEC viral standard. This finding questioned the acceptability of coliforms as a microbial standard for bathing waters and was in accordance with previous reports of poor correlation between the presence of enteroviruses and bacteria in water [9–12].

Enteroviruses were recovered from three inland waters used for water sports. Levels of enteroviruses detected in the River Lagan, however, were often higher than those detected in the Balancing Lakes and Kinnego Bay. Effluents from several sewage treatment works are discharged into the River Lagan. A canoe slalom course has been developed at one of the sampling sites along the River Lagan. Children have also been seen both swimming and totally immersed in the river water at this site.

The public health risk constituted by the presence of viruses in water depends on several factors including the immune status and age of the host and the amount of water ingested. Shuval [13] states that swimmers may ingest between 10 and 50 ml of water per bathing period. The significance of low numbers of enteroviruses in water to public health is controversial [1] although it is believed that one enterovirus particle may infect a susceptible person [14].

The efficiency of virus recovery seemed to be affected by the number of virus plaque-forming units seeded and possibly the type of beef broth utilized. Overall, our experiments showed that the efficiency of recovery of viruses from water samples may be very low. Rao and Melnick [12] report an average estimate of below 20% for the recovery of enteroviruses from water. The levels of viruses detected in the coastal and inland recreational water samples tested may, therefore, greatly underestimate the actual number of enteroviruses that were present in the water samples.

Over 120 different types of enteric viruses are excreted by humans [12]. Only a limited number, however, could be detected by the cell culture used in this study. Although the survival rate of different enteroviruses and enteric viruses in the environment may vary, the possibility exists that other viruses were also present in the same samples.

Sampling of surface waters alone may not give a true indication of the potential viral hazard of the sampling site. Enteric viruses are known to attach to solids in water. Laboratory and field experiments indicate that an association with a solid confers protection on the virus, resulting in enhanced survival of the virus [15–18]. Viruses adsorbed to fluffy sediments may thus pose a greater public health risk than those in water [18] as the water/sediment interface is unstable and sediment particles can be easily resuspended.

The viruses identified in Northern Ireland recreational waters are potentially pathogenic viruses. The polioviruses were probably derived from immunized babies and children and their infected contacts. Genetic instability and reversion to neurovirulence of vaccine strains during human intestinal passage have been reported for the Sabin strains of the three poliovirus serotypes [19–22]. Therefore, the polioviruses recovered from sewage-contaminated water may have been more neurovirulent than vaccine strains. The neurovirulence of the polioviruses isolated could have been assessed since it is possible to differentiate wild-type polioviruses from vaccine strains [21, 23–25]. Coxsackieviruses group B are associated with a wide range of illnesses including aseptic meningitis, encephalitis, pleurodynia, myocarditis and pericarditis, although most CVB infections are asymptomatic, or cause an undifferentiated febrile illness or mild upper respiratory symptoms. Consequently, it is postulated that the 'ubiquitous' appearance of enteroviruses in the water should not be accepted with complacency.

ACKNOWLEDGEMENTS

We thank the Department of the Environment for Northern Ireland for funding this project. The views expressed are those of the authors and do not necessarily represent those of the Department of the Environment for Northern Ireland.

REFERENCES

1. Tyler JM. Occurrence in water of viruses of public health significance. J Appl Bacteriol Symp Suppl 1985: 37S–46S.
2. Freeman L. Keeping the viruses at bay. Water 1978: **20**: 11–13.
3. Slade JS. Enteroviruses in slow sand filtered water. J Inst Wat Engr Sci 1978: **32**: 530–5.
4. Payment P, Fortin S, Trudel M. Ferric chloride flocculation for nonflocculating beef extract preparations. Appl Environ Microbiol 1984: **47**: 591–2.
5. Barron AL, Olshevsky C, Cohen MM. Characteristics of the BGM line of cells from African Green Monkey Kidney. Arch Virusforsch 1970: **32**: 389–92.
6. Dahling DR, Wright BA. Optimization of suspended cell method and comparison with cell monolayer techniques for virus assays. J Virol Meth 1988: **20**: 169–79.
7. Cooper PD. A method for producing plaques in agar suspensions of animal cells. Virology 1955: **1**: 397–401.
8. Slade JS, Chisholm RG, Harris NR. Detection of enteroviruses in water by suspended-cell cultures. In: Grainger JM, Lynch JL, eds. Microbiological methods for environmental biotechnology. London: Academic Press, 1984: 365–74.
9. Berg G, Dahling DR, Brown GA, Berman D. Validity of fecal coliforms, total coliforms and fecal streptococci as indicators of viruses in chlorinated and primary sewage effluents. Appl Environ Microbiol 1978: **36**: 880–4.
10. Payment P, Lemieux M, Trudel M. Bacteriological and virological analysis of water from four fresh water beaches. Water Res 1982: **16**: 939–43.
11. Vaughn JM, Landry EF, Thomas MZ. Entrainment of viruses from septic tank leach fields through a shallow soil aquifer. Appl Environ Microbiol 1983: **45**: 1474–80.
12. Rao VC, Melnick JL. Environmental virology. Wokingham: Van Nostrand Reinhold (UK) Co Ltd, 1986: 1–9, 41–54.
13. Shuval HI. Water needs and usage. In: Berg G, Bodily HL, Lennette EH, Melnick JL, Metcalf TG, eds. *Viruses in water*. Washington, DC: American Public Health Association Ind., 1976: 21–2.
14. Schiff GM, Stefanovic GM, Young B, Pennekamp JK. Minimum human infectious dose of enteric virus (echovirus – 12) in drinking water. In: Melnick JL ed. Enteric viruses in water. Monographs in Virology, vol. 15. New York, Basel: Karger, 1984: 222–9.

15. Smith EM, Gerba CP, Melnick JL. Role of sediment in the persistence of enteroviruses in the estuarine environment. Appl Environ Microbiol 1982; **43**: 1440–50.
16. Dahling DR, Safferman RS. Survival of enteric viruses under natural conditions in a subarctic river. Appl Environ Microbiol 1979; **38**: 1103–10.
17. Goyal SM, Adams WN, O'Malley ML, Lear DW. Human pathogenic viruses at sewage sludge disposal sites in the middle Atlantic region. Appl Environ Microbiol 1984; **48**: 758–63.
18. Rao VC, Seidel KM, Goyal SM, Metcalf TG, Melnick JL. Isolation of enteroviruses from water, suspended solids and sediments from Galveston Bay: survival of poliovirus and rotavirus adsorbed to sediments. Appl Environ Microbiol 1984; **48**: 404–9.
19. Dane DS, Dick GWA, Briggs EM, et al. Changes in Sabin type 1 oral vaccine virus after multiplication in the intestinal tract. Br Med J 1961; **2**: 259–65.
20. Minor PD, John A, Ferguson M, Icenogle JP. Antigenic and molecular evolution of the vaccine strain of type 3 poliovirus during the period of excretion by a primary vaccinee. J Gen Virol 1986; **67**: 693–706.
21. Minor PD, Dunn G. The effect of sequences in the 5′ non-coding region of the replication of polioviruses in the human gut. J Gen Virol 1988; **69**: 1091–6.
22. Dunn G, Begg NT, Cammack N, Minor PD. Virus excretion and mutation by infants following primary vaccination with live oral poliovaccine from two sources. J Med Virol 1990; **32**: 92–5.
23. Nakano JH, Hatch MH, Thieme ML. Parameters for differentiating vaccine-derived and wild poliovirus strains. Prog Med Virol 1978; **24**: 178–206.
24. Minor PD. Comparative biochemical studies of wild type 3 poliovirus. J Virol 1980; **34**: 73–84.
25. Ferguson M, Magrath DI, Minor PD, Schild GC. WHO collaborative study on the use of monoclonal antibodies for the intratypic differentiation of poliovirus strains. Bull WHO 1986; **64**: 239–46.