Enteric viruses in drinking water supplies

A.P. Wyn-Jones*, J. Watkins**, C. Francis**, M. Laverick* and J. Sellwood***

* School of Sciences, The University, Sunderland SR1 3SD, UK

** CREH Analytical, Hoyland House, Horsforth, Leeds LS18 4RS, UK

*** Public Health Laboratory, Royal Berkshire Hospital, Reading RG1 5AN, UK

Abstract Two rural spring drinking water supplies were studied for their enteric virus levels. In one, serving about 30 dwellings, the water was chlorinated before distribution; in the other, which served a dairy and six dwellings the water was not treated. Samples of treated (40 l) and untreated (20 l) water were taken under normal and heavy rainfall conditions over a six weeks period and concentrated by adsorption/elution and organic flocculation. Infectious enterovirus in concentrates was detected in liquid culture and enumerated by plaque assay, both in BGM cells, and concentrates were also analysed by RT-PCR. Viruses were found in both raw water supplies. Rural supplies need to be analysed for viruses as well as bacterial and protozoan pathogens if the full microbial hazard is to be determined.
Keywords Drinking water; enteric pathogens; RT-PCR; viruses

Introduction

Private water supplies, defined as those not owned and maintained by the major utilities, and where the responsibility for its maintenance and repair lies with the owner or person who uses it, provide approximately 1% of the population with drinking water in the United Kingdom. There are about 60,000 such supplies and they are controlled by the Private Water Supply Regulations, 1991, under the Water Industry Act 1991. Most are small rural supplies serving fewer than 20 dwellings. Though regulations provide minimum sampling and analysis times for most types of supply, and the water must remain safe to drink, sampling may be undertaken as infrequently as yearly or less often, and often ignores high contamination risk factors such as heavy rainfall (Petrie *et al*., 1994). The possibility therefore exists that these supplies may become microbially contaminated with subsequent risk to the consumer: between 1975 and 1998, 19 outbreaks of disease were attributed to private water supplies in England and Wales (Wright, 1998), and between 1948 and 1990, 26 outbreaks were associated with private supplies in Scotland (Benton and Campbell, 1994). Humphrey and Cruikshank (1985) examined 55 rural supplies for total and faecal coliforms and total/viable count at 22°C and 37ºC; only nine sources were found to fall within EC recommended limits on all occasions, the failure rate for coliforms being 62%. Fewtrell and Kay (1996) examined 91 private supplies, taking 1100 samples in six weeks and found that half failed to meet the standard on at least one occasion, and Rutter *et al*. (2000) found that total coliforms, including *E. coli* were detected in 27% of samples from 42% of 2911 supplies on at least one occasion over a two year period. In a survey of UK local authorities by Shepherd and Wyn-Jones (1997) 64 authorities reported suspected or proven cases attributable to private supplies.

Classification of private supplies is based on the water use; Category 1 supplies are used for domestic purposes and Category 2 supplies are commercial, such as food manufacturing, or premises with changing populations such as hospitals, caravan parks and holiday camps. Categories are broken down further (Tables 1 and 2) according to degree of use, based on individuals or volume supplied (Category 1) or water used (Category 2).

Ninety-eight percent of Category 1 supplies fall into classes E and F, and 60% of

Table 1 Classification of Category 1 private supplies

Class	People supplied on any one day	Average daily volume (m³/day)
A	> 5,000	> 1,000
B	501–5,000	101–1000
C	101–500	21–100
D	25–100	2–20
E	< 25	< 5
F	single domestic property	

Table 2 Classification of Category 2 supplies

Class	Average daily volume for domestic or food use (m³/day)
1	> 1,000
2	101–1,000
3	21–100
4	2–20
5	< 2

A.P. Wyn-Jones et al.

Category 2 supplies are class 5. It is thus clear that most private supplies provide water for relatively few people in small numbers of dwellings or other buildings. The majority of supplies in the UK are surface waters, of which a third are spring supplies, 16% are boreholes and 14% are wells (Shepherd and Wyn-Jones, 1997).

Microbiological surveys of private supplies in the UK have concentrated on bacteria and protozoan parasites, and little has been done to investigate the virus content. However, there is evidence, albeit mostly circumstantial, that viruses in polluted water can lead to outbreaks of disease. Outbreaks of enteric virus disease have been linked to water at various times, and with different causes. Pipeline failures account for most incidents such as those at Bramham, UK in 1980, when 3000 individuals were affected (Short, 1988) and Naas, Ireland, where the supply to half the 11,000 residents was polluted, and waterborne outbreaks have also been reported from Europe and the US; consumption of contaminated ice, and borehole contamination (Lawson *et al*., 1991; Beller *et al*., 1997), are typical documented causes. Hepatitis E virus (HEV) has also been shown to have been transmitted by polluted water (e.g. Skidmore *et al*., 1992; Pina *et al*., 1998).

During a survey of seven private water supplies in the UK during 2000 we analysed samples from two sites for virus content. We wished firstly to determine whether the water contained infectious enteroviruses, which though not causing gastro-enteritis themselves in the absence of other disease, nevertheless act as close indicators of the presence of other enteric viruses. We also wished to screen the samples for total virus by RT-PCR, and to compare virus content with counts of indicator bacteria, *Cryptosporidium* oocysts and *Giardia* cysts.

Methods

Sites

The first site (site 1) sampled for viruses is a farm in Scotland The water supply is spring fed in fields above the farm. The water passes through field drains that feed a 6 m³ holding tank. There is no active treatment, and though the field drains were fenced off animals could still gain access.

The second site (2) is in Yorkshire. The water supply is gravity fed from a resurgent underground stream from a karst groundwater system. The catchment area incorporates an extensive rabbit warren and contains a number of swallow holes and shake holes. The immediate area is grazed by sheep and faecal matter is scattered around the area.

18

Samples

Water samples were collected, between the beginning of October and mid-November 2000. The weather deteriorated during this time, starting dry with occasional rain showers and finishing with periods of heavy continuous rain. November was the start of heavy flooding in many areas of the UK including Yorkshire.

Thirty-nine samples were taken, of which 14 (all untreated) were from site 1, and 25 (20 untreated, five treated) were from site 2. Sample volumes were 20 l (untreated) and 50 l (treated). The pH of site 1 samples ranged from 5.3 to 6.6, and that of site 2 samples ranged from 5.5 to 7.5.

Virus concentration

Viruses in all samples were concentrated by adsorption to negatively charged cellulose nitrate membranes (Sartorius; SCA, 1995). Adsorbed virus was eluted either with 0.1% (w/v) skimmed milk or 6% (w/v) beef extract, both in glycine buffer at pH 9.5. Further concentration was achieved by acid flocculation and centrifugation of the floc at $9000 \times g$ for 30 min, the protein/virus pellet being resuspended in 10 ml 0.15 mol l^{-1} disodium hydrogen phosphate buffer pH 7.4 and stored at <20ºC until required.

Virus detection and assay

Detection in cell culture. Virus concentrates were thawed and inoculated on to monolayers of Buffalo Green Monkey (BGM) kidney cells grown in 90 mm diameter dishes (SCA, 1995). Plaques were counted after three, four and five days. Since the origin of the samples suggested that animal enteroviruses might be present in the water samples which may induce a cytopathic effect (c.p.e.) in culture but not form plaques, concentrates were also inoculated into liquid culture of the same cells to detect qualitatively any non-plaquing virus in the concentrates. Recent work (UKWIR, 1999) has indicated that some sheep and a few porcine enteroviruses, in addition to bovine enteroviruses, may induce plaques in BGM cells, but that many do not. The significance to public health of finding animal enteroviruses in water is undetermined, though it is unlikely they would be a problem beyond causing false positive results in monitoring.

Cell deposits from some cultures showing a positive c.p.e. were further analysed by rapid immunofluorescence (IF) to confirm any human enterovirus detected. A portion of the infected cells were transferred to a microscope slide, dried, fixed and stained with one of a range of virus serotype specific-monoclonal antisera followed by anti-mouse IgG/FITC conjugate. Infected cells emitted a bright green granular fluorescence with the specific serotype. Antisera for polioviruses and Coxsackievirus B were used. (Rigonan *et al.*, 1998).

Detection by RT-PCR. Concentrates were also analysed by RT-PCR (Pallin *et al.*, 1997) for enteroviruses. Virus capsids in 140 µl aliquots of concentrate were lysed and the RNA extracted using QIAamp Viral RNA Mini kits (Qiagen) according to manufacturers' instructions and was stored at –20C in 20 µl amounts. Target sequences were amplified by semi-nested amplification and products analysed on ethidium bromide agarose gels. Both animal and human enterovirus sequences would be detected by this test since the primers used are well conserved across enterovirus strains.

Results

Cytopathic effects (c.p.e.) characteristic of virus infection were seen in 26 of the 39 samples tested by liquid culture. However the number of cultures showing plaques in the monolayer plaque assay was much less, only two samples showing plaques characteristic

of enterovirus infection, and even here the count was low, one plaque forming unit (pfu) being found in one 20 l sample and two pfu in another. Six of the samples from liquid culture were tested for human enteroviruses by IF, but none confirmed positive.

Two samples when tested by semi-nested RT-PCR were positive for enterovirus sequence (Figure 1), both samples had given a positive cpe in liquid culture and one had given plaques in the plaque assay.

In respect of sampling sites, 10 out of 14 samples from site 1 were positive in liquid culture, of which one was confirmed by RT-PCR. In site 2, 16 out of 25 samples were positive in liquid culture, again one being confirmed by RT-PCR. Two produced plaques in the monolayer assay. The results are summarised in Table 3.

Discussion

Pollution of rural water supplies can originate either from contamination with human sewage if the supply is near a faulty septic tank or other sewage conduit, or it may arise through animal faecal contamination of the ground and percolation of micro-organisms into the water. In this study sample concentrates were analysed in several ways to facilitate the detection of waterborne viruses. Liquid culture will permit the growth of a range of enteric viruses in addition to enteroviruses, principally reoviruses, though not all will
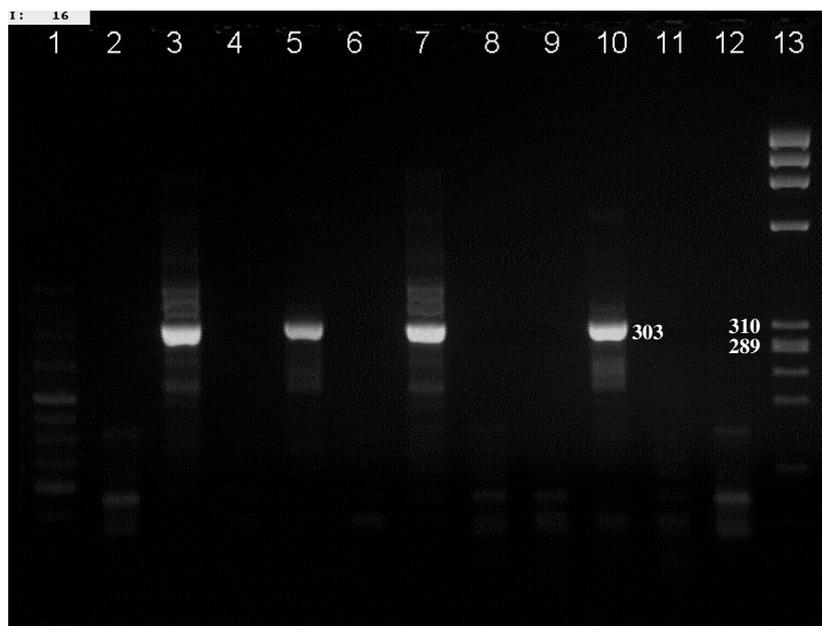

Figure 1 RT-PCR analysis of water samples. Seven samples gave positive bands on first round amplification and these were tested by semi-nested amplification. Two samples confirmed as positive. Lane 1 500bp ladder; lanes 2 and 12 negative control; lanes 3 and 7 positive control (100 pfu Coxsackievirus B4); lanes 4–6, 8–11 test samples; lane 13 $\Phi \times 174$ *Hae* digest

Table 3 Enteroviruses in drinking water supplies: summary of infectivity and RT-PCR analysis

Site	Samples	Positive samples		
		Liquid culture	Plaque assay	RT-PCR
1	14	10	0	1
2	25	16	2	1

produce plaques. Hence both approaches were chosen, the latter to afford some indication of the quantity of virus present. Confirmation of enterovirus identity was done in two ways, by immunofluorescence, which is a rapid technique which would confirm the identity of enteroviruses, but only of human serotype, and RT-PCR, which detects all enterovirus sequences apart from echovirus 22.

Samples from both sites contained virus which gave positive tests in at least one type of analysis. In site 1 ten out of 14 samples gave a positive test in liquid culture, though only one was confirmed as enterovirus positive by RT-PCR and none produced plaques in cell culture nor confirmed in IF. This would indicate the presence of a cpe-inducing agent other than enterovirus. Reoviruses are consistent inhabitants of the gut, grow slowly in culture and would not confirm in any of the tests used here. It is thus possible that this group was present in the samples.

In site 2 again over half the samples induced cpe in liquid culture. Two samples produced plaques in the monolayer assay, though the titres were low. One sample showed 1 pfu and the other 2 pfu, both in a volume of 20 l untreated water. The second of these confirmed as enterovirus positive by RT-PCR. The first did not, which suggests that a non-enterovirus agent was present. Reoviruses take longer to produce plaques than enteroviruses, and though the tests were left on six days it is still not possible to conclude that this group was present.

The growth of human enteroviruses in BGM cells is well documented, but the number of animal types that will grow is not well determined. It has recently been shown (UKWIR, 1999) that porcine and ovine, as well as bovine serotypes will multiply in this cell line, though not all induce plaque formation. The high proportion of positives in this study would indicate that most of the positive reactions in culture are indeed due to animal viruses not human enteroviruses, though the possibility of human agents other than enteroviruses cannot be excluded.

Acknowledgements

References

Beller, M., Ellis, A., Lee, S.H., Drebot, M.A., Jenkerson, S.A., Funk, E., Sobsey, M.D., Simmons, O.D., Monroe, S.S., Ando, T., Noel, J., Petric, M., Middaugh, J.P. and Spika, J.S. (1997). Outbreak of viral gastroenteritis due to a contaminated well: international consequences. *Journal of the American Medical Association* **278**, 563–8.

Fewtrell, L. and Kay, D. (1996). *Health risks from private water supplies*. EPG 1/9/79. A CREH Report for the Drinking Water Inspectorate.

Humphrey, T.J. and Cruickshank, J.G. (1985). The potability of rural water supplies – a pilot study. *Community Medicine* **7**, 43–47.

Lawson, H.W., Braun, M.M. and Glass, R.I.M. *et al*. (1991). Waterborne outbreak of Norwalk virus gastroenteritis at a southwest US resort: role of geological formations in contamination of well water. *Lancet* **337**, 1200–1204.

Pallin, R., Place, B.M., Lightfoot, N.F. and Wyn-Jones, A.P. (1997). The detection of enteroviruses in large volume concentrates of recreational waters by the polymerase chain reaction. *Journal of Virological Methods* **57**, 67–77.

Petrie, A.S., Horan, N.J., Clapham, D.B. and Cram A.G. (1994). Seasonal variations in the quality of spring waters used as private water supplies. *Journal of the Institute of Water an Environmental Management* **8**, 320–326.

Pina, S., Puig, M., Lucena, F., Jofre, J. and Girones, R. (1998). Viral pollution in the environment and in shellfish: human adenovirus detection by PCR as an index of human viruses. *Appl & Environ Microbiol.* **64**, 3376–3382.

Rigonan, A.S., Mann, L. and Chonmaitree, T. (1998). Use of monoclonal antibodies to identify serotypes of enterovirus isolates. *J. Clin. Microbiol.* **36**, 1877–1881.

SCA (1995). *Methods for the isolation and identification of human enteric viruses from waters and associated material 1995*. HMSO, 1995.

Shepherd, K.M. and Wyn-Jones, A.P. (1997). Private water supplies and the local authority role: results of a national survey. *Water Science & Technology* **35**(11–12), 41–45.

Short, C.S. (1988). The Bramham incident, 1980: an outbreak of waterborne infection. *Journal of the Institute of Water an Environmental Management* **2**, 383–390.

Skidmore, S.J., Yarborough, P.O. and Gabor, K.A. (1992). Hepatitis E virus: the cause of a waterborne hepatitis outbreak. *J. Med. Virol.* **37**, 58–60.

UKWIR (1999). *Virus sensitivity of the plaque assay technique*. Report for The UK Water Industry Research Ltd, London. Report No. WW-11F.

Wright, F.W. (1998). *Outbreaks associated with private drinking water supplies in England and Wales: a critical review of the evidence*. Communicable Diseases Surveillance Centre, UK Public Health Laboratory Service.

A.P. Wyn-Jones *et al.*

22