WATER RESEARCH 45 (2011) 1025–1038

Available at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Surveillance of adenoviruses and noroviruses in European recreational waters

A. Peter Wyn-Jones [a,*], Annalaura Carducci [b], Nigel Cook [c], Martin D'Agostino [c], Maurizio Divizia [d], Jens Fleischer [e], Christophe Gantzer [f], Andrew Gawler [g], Rosina Girones [h], Christiane Höller [i], Ana Maria de Roda Husman [j], David Kay [a], Iwona Kozyra [k], Juan López-Pila [l], Michele Muscillo [m], Maria São José Nascimento [n], George Papageorgiou [o], Saskia Rutjes [j], Jane Sellwood [p], Regine Szewzyk [l], Mark Wyer [a]

[a] IGES, University of Aberystwyth, Ceredigion, SY23 3DB, UK
[b] Department of Biology, Università di Pisa, Italy
[c] Food and Environment Research Agency, FERA, York, UK
[d] Faculty of Medicine, Tor Vergata University, Rome, Italy
[e] Landesgesundheitsamt Baden-Württemberg, Germany
[f] Faculté de Pharmacie, University Henri Poincaré, Nancy, France
[g] Environment Agency, United Kingdom
[h] Department of Microbiology, Faculty of Biology, University of Barcelona, Spain
[i] Bayerisches Landesamt für Gesundheit und Lebensmittelsicherheit, Germany
[j] National Institute for Public Health and the Environment (RIVM), The Netherlands
[k] National Veterinary Research Institute, Pulawy, Poland
[l] Umweltbundesamt, Berlin, Germany
[m] Istituto Superiore Sanità, Rome, Italy
[n] Faculdade de Farmácia, Universidade do Porto, Porto, Portugal
[o] Environmental Virology Laboratory, State General Laboratory, Cyprus
[p] Environmental Virology Unit, Health Protection Agency, UK

ARTICLE INFO

Article history:
Received 27 April 2010
Received in revised form
20 August 2010
Accepted 13 October 2010
Available online 29 October 2010

Keywords:
Adenoviruses
Noroviruses
Bathing water
River water
Sea water

ABSTRACT

Exposure to human pathogenic viruses in recreational waters has been shown to cause disease outbreaks. In the context of Article 14 of the revised European Bathing Waters Directive 2006/7/EC (rBWD, CEU, 2006) a Europe-wide surveillance study was carried out to determine the frequency of occurrence of two human enteric viruses in recreational waters. Adenoviruses were selected based on their near-universal shedding and environmental survival, and noroviruses (NoV) selected as being the most prevalent gastroenteritis agent worldwide. Concentration of marine and freshwater samples was done by adsorption/elution followed by molecular detection by (RT)-PCR. Out of 1410 samples, 553 (39.2%) were positive for one or more of the target viruses. Adenoviruses, detected in 36.4% of samples, were more prevalent than noroviruses (9.4%), with 3.5% GI and 6.2% GII, some samples being positive for both GI and GII. Of 513 human adenovirus-positive samples, 63 (12.3%) were also norovirus-positive, whereas 69 (7.7%) norovirus-positive samples were adenovirus-negative. More freshwater samples than marine water samples were virus-positive. Out of a small selection of samples tested for adenovirus infectivity,

* *Corresponding author*. Tel.: +44 191 384 2749.
E-mail address: pyw@aber.ac.uk (A.P. Wyn-Jones).

doi:10.1016/j.watres.2010.10.015

Recreational water
Water quality

approximately one-quarter were positive. Sixty percent of 132 nested-PCR adenovirus-positive samples analysed by quantitative PCR gave a mean value of over 3000 genome copies per L of water. The simultaneous detection of infectious adenovirus and of adenovirus and NoV by (RT)PCR suggests that the presence of infectious viruses in recreational waters may constitute a public health risk upon exposure. These studies support the case for considering adenoviruses as an indicator of bathing water quality.

1. Introduction

Enteric viruses have frequently been implicated in recreational water-related gastro-intestinal (G.I.) disease (Sinclair et al., 2009). Studies in Europe and the US suggest that most infections contracted as a result of swimming, canoeing or other recreational use of sewage-polluted water may be viral in nature (e.g. Medema et al., 1995; Gray et al., 1997). Enteric viruses may cause asymptomatic or mild infections in humans, but these faecal-orally transmitted viruses may also cause more serious disease, such as hepatitis and meningitis, especially in vulnerable groups, e.g. young children (Nwachuku and Gerba, 2006). Enteric viruses are recognized as agents that can cause large outbreaks throughout the world with thousands of cases (Sarguna et al., 2007; Bucardo et al., 2007; Iijima et al., 2008; Zhang et al., 2009). Novel emerging viruses such as SARS coronavirus, human parechovirus and zoonotic influenza viruses also appear to be excreted in faeces but the evidence for enteric transmission is not always clear (Ding et al., 2004). Transmission routes for enteric viruses may be diverse such as person–-person, food- or waterborne associated with insufficient hygiene and sanitation (Koopmans et al., 2002; Wyn-Jones and Sellwood, 2001). Disease outbreaks associated with enteric viruses, such as noroviruses and astroviruses, in bathing water have been described (Hauri et al., 2005; Maunula et al., 2004). However, bathing water-related outbreaks may be easily missed due to either unidentified source or unidentified agent, or both.

Enteric viruses in water may originate from discharges of raw or treated sewage, run-off of animal manure or directly from humans or animals. Viruses commonly associated with waterborne disease include the human adenoviruses (HAdVs), noroviruses (NoVs), hepatitis A and E viruses (HAV, HEV), parvoviruses, enteroviruses, and rotaviruses (RVs). In addition, sewage, especially from slaughterhouses, may contain (for example) animal adenoviruses, sapoviruses, and HEV (Hundesa et al., 2006), which may be zoonotic. Viruses originating from (un)treated sewage can contaminate bathing water after discharge into surface waters (in)directly used for recreational water activities. All are capable of infection by ingestion. Some multiply in the intestine and may cause diarrhoea and/or vomiting, while some are associated with tissues (e.g. the liver) other than the gut. The viruses responsible for waterborne infections are not usually identified at the time of a disease outbreak following recreational water activity, and robust associations between the simultaneous presence of virus in faeces of affected individuals and in the water are only occasionally demonstrated (e.g. Hoebe et al., 2004). The epidemiological picture of disease associated with recreational use of water is therefore far from complete, and measures to limit enteric disease after exposure to recreational water are based on water quality parameters built on the detection of faecal bacterial indicator organisms (FIOs). However, it has been shown that water conforming to bacterial standards may contain high levels of human enteric viruses and that FIOs often fail to predict the risk for waterborne pathogens including enteric viruses (Gerba et al., 1979; Lipp et al., 2001). Further, several studies have shown that levels of indicator bacteria do not correlate with those of viruses, particularly when faecal indicator concentrations are low (Contreras-Coll et al., 2002). Viruses are known to be more resistant to environmental degradation than bacteria (Vasl et al., 1981; Thurston-Enriquez et al., 2003; Rzezutka and Cook, 2004; de Roda Husman et al., 2009). Together with the understanding that G.I. illness may be due to viruses rather than bacteria, this provides a case for using a viral indicator of human faecal pollution rather than to rely exclusively on bacterial parameters.

Bathing water quality in the European Union (EU) has been regulated since 1976 by the Bathing Water Directive (76/160/EEC). In 2006 this was revised (rBWD, CEU, 2006) by including enterococci (and, in fresh waters, *Escherichia coli*) as the principal microbial determinants which placed the microbiological parameters on a firmer scientific footing (Kay et al., 1994, 2004: Wiedenmann et al., 2006; WHO, 2003) and allowed classification of bathing waters to be undertaken with more confidence. When tested at sufficient frequency *E. coli* may be a useful indicator of faecal pollution and therefore of the probability of waterborne disease. However, in the EU Directive the frequency is only about once in two weeks and testing takes two days.

The earlier Directive included an enterovirus parameter which stipulated that 95% of 10-L water samples taken during the bathing season should contain no (zero p.f.u.) enteroviruses. This was based on early work (described by Farrah and Bitton, 1990) which suggested that, for poliovirus, Coxsackie A and Coxsackie B viruses, between one and twenty virus infectious units might be sufficient to cause infection. The pathogenesis of enterovirus infections is now better understood, and this belief is considered unsound in determining water quality. Further, although important pathogens in many contexts, the presence of enteroviruses in water does not necessarily correlate with the presence of pathogens such as hepatitis A virus (Dubrou et al., 1991; Pina et al., 1998). The enterovirus parameter was removed during the revision of the 1976 Directive.

Concentrations of some viruses in surface waters can be determined by cell culture monolayer plaque assays, but the technique is not applicable to most viruses of prime interest. Furthermore, cell culture is expensive and time-consuming, and detection of viruses is now done mainly by molecular methods such as reverse transcription RT-PCR or nucleic acid