Available online at www.sciencedirect.com
ScienceDirect
Int. J. Hyg. Environ.-Health 211 (2008) 454–457
International Journal of Hygiene and Environmental Health
www.elsevier.de/ijheh

SHORT COMMUNICATION

Prevalence of Shiga toxin-, intimin- and haemolysin genes in *Escherichia coli* isolates from drinking water supplies in a rural area of Austria

Milad Halabi[a,*], Dorothea Orth[b], Katharina Grif[b], Margit Wiesholzer-Pittl[a], Martina Kainz[a], Johann Schöberl[a], Manfred P. Dierich[b], Franz Allerberger[b], Reinhard Würzner[b]

[a]*Department of Pathology and Microbiology, Krankenhaus der Barmherzigen Schwestern, Schlossberg 1, 4910 Ried im Innkreis, Austria*
[b]*Department of Hygiene, Microbiology and Social Medicine, Innsbruck Medical University and Austrian Reference Centre for EHEC/STEC/VTEC, Innsbruck, Austria*

Received 2 February 2007; received in revised form 31 May 2007; accepted 7 August 2007

Abstract

Literature harbours several reports of potable water-associated outbreaks. We studied the prevalence of Shiga toxin- (*stx*1/2), intimin- (*eae*) and haemolysin (*hly*A) genes in *Escherichia coli* isolates from drinking water of private and public water supplies in a rural area of Upper Austria; 2633 water samples were gained between November 2000 and December 2003. Two hundred and eighty of these water samples were positive for *E. coli* (10.6%). Of these, 101 samples were drawn from drilled wells (36%), 96 from dug wells (34%), 61 from springs (22%) and 22 from water supplies without available information on technical details (8%); 141 of the samples were from public water supplies, 139 from private water supplies. Eleven of the *E. coli* isolates were found to be positive for one of the investigated virulence genes (3.9%): one isolate yielded *stx*2, seven *eae*, and three isolates had *hly*A. The presence of these genes underlines the importance of control of water quality in public and also private water supplies.

Keywords: EHEC; Shiga toxin; Water supplies; Drilled wells; Potable water

Introduction

In 1982, two outbreaks of bloody diarrhoea led to the identification of a new Shiga toxin producing *Escherichia coli* O157:H7 (Riley et al., 1983). This group of enteropathogenic *E. coli* is nowadays termed enterohaemorrhagic *E. coli* or EHEC (Orth and Würzner, 2006). Since then this pathogen has emerged as a major public health concern throughout the world and nowadays serves as a major cause of haemorrhagic colitis and haemolytic uraemic syndrome (HUS) (Griffin and Boyce, 1998). Transmission of EHEC occurs via person-to-person contact, direct zoonotic or environmental transmission or through consumption of contaminated food, mostly from undercooked contaminated beef or raw milk (Chalmers et al., 2000).

The first documented outbreak of an EHEC infection due to *E. coli* O157:H7 associated with potable water

*Corresponding author. Tel.: +43 7752 602 92406; fax: +43 7752 602 95406.
E-mail address: milo.halabi@bhs.at (M. Halabi).

1438-4639/$ - see front matter © 2007 Elsevier GmbH. All rights reserved.
doi:10.1016/j.ijheh.2007.08.002

took place in Missouri in 1989 (Swerdlow et al., 1992). Out of 3126 people 243 developed symptoms, 86 with bloody diarrhoea, two with HUS, and a total of four cases were fatal. The follow-up investigation revealed that the common source of possible infection was the municipal water supply, where main water pipe leakages had occurred somewhat earlier. However, the investigators could not isolate EHEC from the municipal water.

Another EHEC-associated outbreak occurred in Wyoming in 1998 during a family reunion (Olsen et al., 2002), where a total of 71 persons out of 157 suffered from diarrhoea. The common source was again the municipal water supply, which in this case was contaminated by faeces of deer and elks.

The largest outbreak occurred in 2000 among residents of the town of Walkerton in Ontario, Canada (Anonymous, 2000), where more than 2300 cases were identified with 27 cases of HUS and a total of seven fatal cases. Again, consumption of faecally contaminated drinking water was assumed to represent the source of infection.

Other outbreaks associated with potable water are also well documented (Bruce et al., 2003; Chalmers et al., 2000; Dev et al., 1991; Hunter, 2003; Rangel et al., 2005).

At the time the water was analysed, the causing agent usually could not be isolated from the water itself.

The aim of our prospective study was to investigate the prevalence of EHEC genes in private and public water supplies of a rural area of the province Upper Austria, where water supplies are mostly wells and springs. Such water supplies are amenable to contamination with surface water and animal faeces.

Material and methods

Two thousand six hundred and thirty-three water samples from private and public water supplies were collected between November 2000 and December 2003. For sampling, the methodology of the "Drinking water guideline" of the Austrian Health Authorities (based on the EU guideline 93/98) and the national standard ÖNORM M6252 was followed. Processing of the samples was done according to ISO 9308-1:2000.

Isolate identification was confirmed using conventional biochemical tests (API 20E; bioMerieux, Marcy-l'Etoile, France). All *E. coli* strains were investigated for the presence of Shiga toxins using a commercial EIA (Premier EHEC; Meridian, Milano, Italy). Testing for *stx*1 and *stx*2 genes and of the genes encoding EHEC haemolysin (*hly*A) and intimin (*eae*) was done by PCR (Gerber et al., 2002). O- and H-serotyping of the isolates was performed according to the methods of Ørskov and Ørskov (1984) using Statens Serum Institute (SSI, Copenhagen, Denmark) diagnostic antisera. Pulsed-field gel electrophoresis (PFGE) using *Xba*I as restriction enzyme was performed according to the CDC standardized protocol (PulseNet, CDC, Atlanta, GA, USA). Sorbitol fermentation and enterohaemolysis were diagnosed after overnight incubation on Sorbitol MacConkey agar (Oxoid, Basingstoke, UK) and on Enterohaemolysin agar (Heipha, Eppelheim, Germany), respectively.

Results

Between November 2000 and December 2003, 2633 water samples were collected. Out of these, 280 were positive for *E. coli* (i.e. 10.6%). Of the 280 samples, 101 were drawn from drilled wells (36%), 96 from dug wells (34%), 61 from springs (22%) and 22 from water supplies without available information on technical details (8%); 141 samples were from public water supplies, 139 from private water supplies. Of the 280 *E. coli* isolates, eleven isolates were found to be positive for one of the investigated virulence genes (3.9%): one *stx*2-containing isolate, seven *eae*-positive isolates and three isolates containing *hly*A (Table 1). None of the isolates contained *stx*1. Eight of these were from private and three from public water supplies, respectively.

These eleven isolates belonged to ten different *E. coli* serotypes. Except for the two isolates of serotype O26:H11, no typical EHEC serotypes could be found. All eleven *E. coli* isolates fermented sorbitol and only three isolates showed enterohaemolysis.

All isolates showed different PFGE patterns (isolate 105/02 was not typeable) (Fig. 1). In addition, all isolates showed unique PFGE patterns compared with our collection of 240 human, animal and food isolates.

Discussion

The aim of our study was to gain knowledge on the prevalence of Shiga toxin-, intimin- and haemolysin genes in *E. coli* isolates from drinking water supplies in a rural area of Austria. This study is one of the rare reports on detection of EHEC genes in potable water: in most waterborne outbreaks EHEC was isolated from infected patients but not from the suspected water source itself (Wang and Doyle, 1998). Some papers in literature describe associations to sewage (Chalmers et al., 2000), natural river or recreational water (Hunter, 2003). Our finding of 10.6% water supply systems contaminated with *E. coli* as the general indicator of contamination in a mostly agricultural area of the federal state of Upper Austria was not unexpected, especially in the light of the fact that the soil is mostly manured with animal faeces. Faeces of wild or farmed animals represent the most likely source of