Immunomagnetic separation of Escherichia coli O157:H7 from environmental and wastewater in South Africa

EE Müller*, WOK Grabow and MM Ehlers

Department of Medical Virology, PO Box 2034, University of Pretoria, Pretoria 0001, South Africa

Abstract

Recreational and drinking water supplies polluted with sewage have become an important source of E. coli O157:H7 infection. Immunomagnetic separation (IMS) has been extensively used for the isolation of E. coli O157:H7 from food and stool samples but not for samples such as wastewater. In this study the IMS method was used in combination with the E. coli O157:H7 selective media, immunoassays, biochemical tests and PCR, to assess the prevalence of E. coli O157:H7 in selected sewage and environmental water in South Africa. Environmental and wastewater were seeded with E. coli O157:H7 to determine the sensitivity and selectivity of the enrichment-IMS-selective agar method. Naturally occurring E. coli O157:H7 organisms were recovered from selected samples by means of IMS. The IMS concentrates were plated on three selective E. coli O157:H7 media. E. coli O157:H7 was detected in seeded sewage and river water samples with numbers as low as 1.2 cfu·ml -1. The IMS procedure was used to investigate the prevalence of E. coli O157:H7 in randomly selected sewage and river water samples in South Africa. A total of 91 sewage- and 40 river water samples were tested and 17.6% and 20% yielded suspected E. coli O157:H7 colonies on CT-SMAC agar medium respectively. PCR was used to confirm the presence of genes coding for Shiga toxin-1 (Stx1), Shiga toxin-2 (Stx2), enterocyte attaching and effacing genes (eaeA) and enterohaemolysin (hly). Standard immunoassay kits specific for the O157 and H7 antigen and a biochemical indole test were used for further E. coli O157:H7 confirmation. Three colonies from one sewage sample (1.1 % of all sewage samples) agglutinated with anti-E. coli O157 and H7 antiserum and contained the genes coding for Stx2, eae A and hly. None of the colonies isolated from the river water samples were positive for E. coli O157:H7. CT-SMAC proved to have limited E. coli O157:H7 selective capabilities from samples such as sewage with high bacterial counts. Seeded sample experiments indicated that IMS is a suitable method for isolating E. coli O157:H7 from samples with high bacterial interference and low numbers of E. coli O157:H7. Evidence has been presented that the enrichment-IMS-selective agar procedure substantially increased the sensitivity of E. coli O157:H7 isolation compared to direct plating of test samples onto selective agar generally practised in the past.

Keywords: Escherichia coli O157:H7, immunomagnetic separation, river water, sewage

Introduction

Escherichia coli O157: H7 is characterised by its ability to produce shiga toxins that are cytotoxic to monkey kidney (Vero) cells and human cervical cancer (HeLa) cells (Ismaili et al., 1995). E. coli O157:H7 produces a variety of clinical syndromes including bloody and non-bloody diarrhoea, haemorrhagic colitis (HC) and haemolytic uraemic syndrome (HUS) (Karmali et al., 1985). Infections caused by E. coli O157:H7 are recognised frequently, which resulted in an increased interest in the incidence and detection of this organism (Griffin and Tauxe, 1991; Boyce et al., 1995; Goldwater and Bettelheim, 1998; Nataro and Kaper, 1998). A considerable number of epidemiological, clinical and laboratory investigations have been carried out on E. coli O157:H7 infections (Nataro and Kaper, 1998). The failure of clinical laboratories to screen for this organism, with subsequent under-reporting of enterohaemorrhagic E. coli (EHEC) infections, complicates estimates on the burden of disease caused by E. coli O157:H7. The US Center for Disease Control (CDC) estimated the annual disease burden of E. coli O157:H7 in the United States to be more than 73 000 infections and as many as 61 deaths (CDC, 2001). E. coli O157:H7 is the pathogen most frequently isolated from stool specimens that contain visible blood (Slutsker et al., 1997). The World Health Organisation (WHO) is particularly concerned about

this because bloody diarrhoea is a major cause of morbidity and

mortality among children in developing countries in the southern

and more than 2000 people fell ill (ProMed, 2000). The high number of enterohaemorrhagic E. coli organisms isolated from the faeces of patients (Takeda, 1997), has led to the concern that these organisms, especially E. coli O157:H7, could pose a significant

health risk when sewage leaks into water supplies.

Food-borne transmission of E. coli O157:H7 is another important source of infection in humans (Griffin, 1995). The most common vehicle of transmission is through the ingestion of faecally contaminated meat products (Griffin, 1995). Cattle are the main reservoirs of E. coli O157:H7, although it has been isolated from other animals such as chickens, pigs and sheep (Griffin and Tauxe, 1991; Griffin, 1995; Müller et al., 2002). A variety of food sources other than meat products have been implicated in the transmission of E. coli O157:H7: raw cow's milk and cheese, pasteurised milk, mayonnaise, apple cider, fruit and vegetables (Besser et al., 1993; Griffin, 1995; McCarthy, 1998; Nataro and Kaper, 1998).

☎ +2712 3192457; fax: +2712 3255550; e-mail: emuller@medic.up.ac.za Received 26 January 2001; accepted in revised form 9 July 2003.

hemisphere, including South Africa (WHO, 1997). Water-borne transmission of E. coli O157:H7 has been reported from both recreational water and contaminated drinking water (Swerdlow et al., 1992; Keene et al., 1994; ProMed, 2000). One of the most recent outbreaks of E. coli O157:H7 occurred in the water supply system of the small farming community of Walkerton, Ontario in Canada in May 2000 when six people died

^{*} To whom all correspondence should be addressed.

Once *E. coli* O157:H7 is introduced into the community through food or water it can be transmitted from person to person (Paton and Paton, 1998). Person-to-person transmission occurs in day-care centres, nursing homes or where there is close contact between individuals (Karmali, 1989; Griffin, 1995). The modes of transmission for sporadic *E. coli* O157:H7 infections appear to be similar to those for outbreaks (Griffin, 1995). Three cases of laboratory-acquired *E. coli* O157:H7 infection have been reported (Ostroff et al., 1989; Booth and Rowe, 1993; Burnens et al., 1993).

None of the previous water-borne transmission studies (Swerdlow et al., 1992; Keene et al., 1994; Ackman et al., 1997) used IMS for concentrating *E. coli* O157:H7 from water sources. Direct plating of water samples on *E. coli* O157 selective media was used to detect *E. coli* O157:H7 in these cases. This study focused on the use of the enrichment-IMS-selective agar method for the isolation of *E. coli* O157:H7 from river water and sewage samples. The presence of *E. coli* O157:H7 was confirmed with molecular and biochemical techniques.

Materials and methods

Bacterial strains

Shiga toxin 2 (Stx2)-positive strain *E. coli* O157:H7 (ATCC 43889) and Stx2-negative strain *E. coli* O157:H7 (ATCC 43888) were used as Stx2 positive and negative controls respectively (Muniesa and Jofre, 1998). Stx1-positive *E. coli* C600 and Stx1-negative *E. coli* C600 were used as Stx1 positive and negative controls. Prof. J. Jofre from the University of Barcelona, Spain, supplied all *E. coli* control cultures.

Sewage and river water

Sewage and environmental water samples (500 ml each) were collected weekly over a period of one year (September 1998 to August 1999). Sewage sampling sites included Daspoort, Zeekoegat and Baviaanspoort water purification plants near Pretoria, Gauteng, South Africa. River water samples were collected from the Levuvhu River in the Northern Province, Pienaars and Apies Rivers, situated near Pretoria, Gauteng, and Klip River (south of Johannesburg, Gauteng) in South Africa. Samples were kept at 4°C to 10°C and examined within 24 h after collection.

Immunomagnetic separation (IMS) of E. coli O157:H7

The effectiveness of the immunomagnetic separation (IMS) method for the selective recovery of *E. coli* O157:H7 from food and stool specimens has been well established (Wright et al., 1994; Tomoyasu, 1998). Dynabeads® anti-*E. coli* (Dynabeads anti-*E. coli* O157; Dynal, Oslo) are made of uniform, superparamagnetic, polystyrene beads with adsorbed and affinity purified anti-*E. coli* O157 antibodies covalently bound to the bead particle surfaces. These magnetic antibody-coated beads are incubated with the preenrichment culture to allow the target bacteria to bind onto it. The IMS concentrates are plated on selective media and suspected colonies were confirmed with molecular and biochemical techniques.

Municipal sewage

Settled sewage samples as well as settled sewage samples ($100\,\mathrm{ml}$) seeded with *E. coli* O157:H7 ($1.2\,\mathrm{x}\ 10^0$ to $1.2\,\mathrm{x}\ 10^3\,\mathrm{cfu\cdot ml}^{-1}$) were inoculated ($100\,\mathrm{\mu l}$) of each sample) and enriched in 50 ml of

buffered peptone-saline water (Oxoid, CM509) supplemented with Vancomycin (8 mg·l·¹), Cefixime (0.05 mg·l·¹) and Cefsulodin (10 mg·l·¹) (VCC) antibiotic solution (MAST® Diagnostics) to inhibit the growth of gram-positive organisms as well as *Aeromonas* and *Proteus* spp. The sewage enrichment broth suspensions were incubated in a shaker incubator (Hub-O-Mat) for 6 h at 37°C while rotating at 100 r·min⁻¹.

River water

River water samples as well as river water samples (100 ml) seeded with *E. coli* O157:H7 (1.2 x 10^{0} to 1.2 x 10^{3} cfu·ml $^{-1}$) were filtered (100 ml of each sample) through 0.45 μ m Gelman GN-6 Metricel filter membranes (Prod no. 66191), placed into, incubated and enriched in VCC-supplemented buffered peptone-saline water (Difco) for 6 h at 37°C.

Aliquots (1 ml) of the pre-enriched samples with the addition of $20\,\mu l$ of Dynabead® suspension were incubated at room temperature for 10 min with continuous mixing. This step was performed to allow the O157-specific antibodies coated onto the beads to bind to the target bacteria. The bead-bacteria complexes were separated using a magnetic particle concentrator, Dynal® MPC-M for 3 min (Dynal, Oslo). After discarding the supernatant and resuspending the bead-particles in a phosphate-buffered saline-Tween, pH 7.0 (PBS-Tween) (Sigma) solution, the process was repeated four times (Dynal® product brochure, 1995). The final bead-bacteria complexes were resuspended in 100 μl washing buffer (PBS-Tween).

Isolation of E. coli O157

After immunomagnetic separation, 10 µl and 50 µl volumes of each sewage and river water IMS concentrates were transferred to *E. coli* O157 selective media. The *E. coli* O157:H7 selective media used in this study were Cefixime-tellurite Sorbitol-MacConkey agar (CT-SMAC) (Oxoid), CHROMagar O157 (Dynal®) and Rainbow agar O157 (Biolog). *E. coli* O157:H7 strains produced typical colourless colonies on CT-SMAC, red/pink colonies on CHROMagar O157 and grey/black colonies on Rainbow O157 agar after 24 h of incubation at 37°C.

All suspect colonies were subcultured on CT-SMAC to confirm its non-sorbitol fermenting properties. Non-sorbitol fermenting colonies were examined for the presence of the genes coding for Stx1, Stx2, enterohaemolysin and *eae*A. A loopful of these colonies was dispersed in 500 µl ultra-high quality (UHQ) water and boiled at 99°C for 10 min without further treatment to obtain bacterial DNA for amplification with PCR (Sambrook et al., 1989).

Oligonucleotide primers (Sigma-Genosys Ltd.) specific for Stx1 (VT1), Stx2 (VT2), EHEC 1 and 2 (eaeA primers) and EHEC P1 and P2 (enterohaemolysin plasmid primers) were used in the polymerase chain reaction (PCR) (Pollard et al., 1990; Gannon et al., 1992; Fratamico et al., 1995) (Table 1). Each 90 µl PCR reaction mixture contained: 10 µl of Mg-free 10x amplification buffer (Promega); 6 µl of 25 mM MgCl₂ (Promega); 2 µl of 10 mM dNTP (Promega); 100 pmol of VT1 and VT2 primers; 2.5 U of Taq DNA polymerase (Promega) and 10 µl of template DNA. An additional PCR was performed with the same PCR reaction mixture for the detection of the eaeA (100 pmol EHEC/P1 and 100 pmol EHEC/P2 primers) genes. The mixtures were overlaid with one drop of sterile mineral oil and placed in an automated thermocycler (Hybaid). The PCR cycle consisted of an initial 5 min DNA

TABLE 1 Primer sequences and predicted sizes of PCR amplified products for the detection of EHEC O157, the haemolysin plasmid and Stx (VT)-specific genes of E. coli O157:H7

Primer	Oligonucleotide sequence (5'-3')	Target(s)	Size of amplified product (base pairs)	Reference
VT1a VT1b	GAAGAGTCCGTGGGATTACG AGCGATGCAGCTATTAATAA	Stx 1	130	Pollard et al. (1990)
VT2a VT2b	TTAACCACACCCACGGCAGT GCTCTGGATGCATCTCTGGT	Stx2	346	Pollard et al. (1990)
EHEC 1* EHEC 2*	CAGGTCGTCGTGTCTGCTAAA TCAGCGTGGTTGGATCAACCT	eaeA	1087	Gannon et al. (1993)
EHEC/P1# EHEC/P2#	ACGATGTGGTTTATTCTGGA CTTCACGTCACCATACATAT	60-MDa plasmid	166	Fratamico et al. (1995)

^{* =} EHEC genes specific for E. coli O157

Primers from Sigma-Genosys Ltd. London Road, Papisford, Cambridgeshire,

CB2 4EF, UK

Figure 1

The detection of Stx1(130 bp product) and Stx2 (346 bp product) amplicons of E. coli O157:H7 isolates from sewage (Daspoort Water Purification Plant, Pretoria, South Africa) using gel electrophoresis.

Lane 1: 100 bp ladder

Lane 2: E. coli colony 1 from Daspoort sewage west intake

Lane 3: E. coli colony 2 from Daspoort sewage west intake

Lane 4: E. coli colony 3 from Daspoort sewage west intake

Lane 5: E. coli colony 4 from Daspoort sewage west intake

Lane 6: Stx1 positive control (130 bp)

Lane 7: Stx2 positive control (346 bp)

Lane 8: Negative control



denaturation cycle at 94°C followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 1 min and extension at 72°C for 1 min (Pharmacia LKB Thermocycler). The amplicons (20 μl aliquots from each amplification) were detected by gel electrophoresis using a 2% agarose (SeaKem® LE) gel suspension stained with ethidium bromide (Sigma). A 100 base pair DNA molecular size marker (Promega) was used. The amplified products were visualised by UV-transillumination (UVP -Transilluminator) and the image was captured using the UVP Image store 5000 gel documentation system (Fig. 1).

Suspect E. coli O157:H7 colonies isolated from all selective media (CT-SMAC, Rainbow agar O157 and CHROMagar O157) were individually tested for agglutination using a commercial E. coli O157 slide agglutination kit with antisera against E. coli O157 (Mast Assure, Mono Factor O157, code:M12030). In addition, all colonies were biochemically confirmed as E. coli by their ability to produce indole from tryptophan using Kovac's reagent (ISO, 2001).

Results

Assessment of the sensitivity of the enrichment-IMS-selective agar procedure revealed that E. coli O157:H7 colonies were recovered from sewage and river water samples with average counts of seeded E. coli O157:H7 as low as 1.2 cfu·ml -1. The enrichment procedure increased average counts of E. coli O157:H7 in seeded sewage samples from 1.2 to 45 cfu·ml ⁻¹ (3 650%) and in seeded river water samples from 1.2 to 72 cfu·ml⁻¹ (5 900%). In the case of samples seeded with higher numbers of E. coli O157:H7 the percentage increase in counts of E. coli O157:H7 accomplished by enrichment was lower (Table 2). In the case of sewage seeded with E. coli O157:H7 to average counts of 1 200 cfu·ml -1, the percentage

^{# =} Haemolysin plasmid

TABLE 2
Assessment of the sensitivity of the enrichment-IMS-selective agar method using testson seeded samples

Seeded	Count of seeded <i>E. coli</i> O157:H7 cfu·ml ⁻¹					
samples	Before Enrichment	After Enrichment	Percentage Increase (%)	Presence- absence after IMS		
Sewage	1 200 120 12 1.2	1 740 170 52 45	45 42 333 3650	+ + + +		
River water	1 200 120 12 12 1.2	2 950 890 140 72	146 641 1 067 5900	+ + + +		

Enrichment was on CT-SMAC Agar. Counts are averages of tests carried out in triplicate.

E. coli O157:H7 was detected in all seeded samples.

- + = Present
- = Absent

increase was only 45%.

After the above determination of the sensitivity of the enrichment-IMS-selective agar method, the procedure was applied in studies on the incidence of naturally occurring E. coli O157:H7 in selected samples. A total of 91 sewage and 40 river water samples was examined. Suspect E. coli O157:H7 colonies were isolated from 16 sewage (17.6%) and 8 river water (20%) samples using the three selective media. No E. coli O157:H7 colonies were isolated when sewage and river water were directly plated on the selective media due to interfering growth and overwhelming numbers of non-pathogenic strains of E. coli. Three colonies isolated on CT-SMAC from one sewage sample of the Daspoort west intake (Table 2) agglutinated with the anti-E. coli O157 antisera, tested positive for indole using Kovac's reagent and contained the genes coding for Stx2, hly and eaeA after PCR. These three isolates imply that 6.25% of suspect E. coli O157:H7 colonies isolated from sewage were confirmed to be E. coli O157:H7, and that E. coli O157:H7 colonies were isolated from 1.1% of sewage samples analysed. No suspect E. coli O157:H7 colonies were isolated from any of the 40 river water samples analysed.

Although not confirmed by statistically meaningful results, CT-SMAC proved the agar medium of choice for the selective cultivation of *E. coli* O157:H7. The choice is predominantly based on the morphology and colour of suspect colonies, and the extent to which their detection was obscured by background growth.

Discussion

Evidence has been presented that the enrichment-IMS-selective agar procedure substantially increased the sensitivity of *E. coli* O157:H7 isolation compared to direct plating of test samples on selective agar. Comparative tests revealed that the enrichment step increased counts of *E. coli* O157:H7 seeded into samples of sewage and river water by up to 5 900% (Table 2). The higher percentage increase in counts of *E. coli* O157:H7 in samples seeded with low

numbers of the organism, is probably due to normal population dynamics and maximum numbers of organisms attainable in a steady-state culture. This phenomenon serves the objectives of the enrichment procedure because initially low numbers of E. coli O157:H7 require higher levels of enrichment for detection. Since E. coli O157:H7 colonies were recovered from all seeded samples, the lowest numbers of the organisms detectable in the samples concerned have unfortunately not been established. Determination of the lowest number of E. coli O157:H7 detectable would require tests in which samples are seeded with numbers of E. coli O157:H7 even lower than those in Table 2. However, the results indicated that E. coli O157:H7 would be detectable when present in numbers as low as 1.2 cfu·ml -1. Although not confirmed by statistically meaningful results, the CT-SMAC agar medium seemed to yield the best results of the three media used for the selective cultivation of E. coli O157:H7 bacteria. In addition, CT-SMAC agar was less expensive than Rainbow Agar O157 and CHROMagar O157.

The higher efficiency of the enrichment-IMS-selective agar procedure for the recovery of naturally occurring *E. coli* O157:H7 in sewage and river water would appear to be supported by the isolation of at least one *E. coli* O157:H7 organism from a sewage sample in this study. In comparison, the survey de-

scribed by Müller and colleagues (2001) using conventional plating of test samples on selective media, failed to recover the pathogen from any of the samples analysed.

Enterohaemorrhagic *E. coli* bacteria have been isolated from sewage in Germany by direct plating of test samples onto selective media (Höller et al., 1999). *E. coli* O157:H7 has been isolated from patient stool specimens as well as samples of meat products and milk associated with infections (Nataro and Kaper, 1998). However, as far as can be established this is the first report on the isolation of *E. coli* O157:H7 from sewage in South Africa, and the first application of the enrichment-IMS-selective agar procedure for the isolation of *E. coli* O157:H7 from wastewater anywhere in the world.

One out of 16 samples (6.3%) of suspect E. coli O157:H7 were confirmed as E. coli O157:H7. This confirms the shortcomings of the agar media for the selective cultivation of E. coli O157:H7. The three E. coli O157:H7 colonies isolated from the same sewage sample were probably the offspring from the same original organism which multiplied during the initial enrichment stage. This is confirmed by identical features such as the toxicity factors that they carried (Table 3). Shortcomings of media for the selective cultivation of E. coli O157:H7 in test samples with heavy background growth such as sewage and river water have been reported by other researchers (Bettelheim, 1998). Bettelheim (1998) pointed out that the black colonies of E. coli O157:H7 were difficult to distinguish on Rainbow Agar O157 in the presence of large numbers of other E. coli colonies of different colours. These observations call for further improvement of methods for the selective cultivation of E. coli O157:H7 in the presence of large numbers of wild type E. coli and other bacteria capable of growing on the selective media. Resistance to the antibiotics used for the suppression of bacteria other than E. coli O157:H7 may largely be accountable for the problem. One solution may therefore be to find antimicrobial agents which more efficiently suppress background growth and strains of E. coli other than E. coli O157:H7.

TABLE 3
Isolation of *E. coli* O157:H7 from selected samples of sewage and river water

Samples	Total number of samples	Number of isolates (percentage of total)		Toxicity factors		
	samples	Suspect isolates	Confirmed isolates	Stx2	Hly	eaeA
Sewage River water Total	91 40 181	16 (17.6 %) 0 16 (8.8 %)	1 (1.1 %) 0 1 (0.6 %)	1 0 1	1 0 1	1 0 1

Despite shortcomings of the growth media for the selective cultivation of $E.\ coli\ O157:H7$, evaluation of the sensitivity of the enrichment-IMS-selective agar procedure in seeding experiments confirmed that it was capable of detecting numbers of $E.\ coli\ O157:H7$ as low as $1\ cfu\cdot ml^{-1}$ in any of the sewage and river water samples analysed (Table 2). These results confirmed that $E.\ coli\ O157:H7$ bacteria did occur in low numbers in the samples under investigation.

Data reported on the isolation of at least one E. coli O157:H7 organism from sewage and results of the seeding experiments, confirmed that a procedure is available for the relatively sensitive isolation of E. coli O157:H7 from a variety of environmental samples with heavy background growth and large numbers of wild type E. coli and related bacteria. This enrichment-IMS-selective agar technique could be applied for purposes such as analysis of samples related to E. coli O157:H7 infections. The enrichment-IMS-selective agar procedure developed in this study is superior to methods used in the past, but there is potential for further improvement. This refers in particular to the final step for the selective cultivation of E. coli O157:H7 isolates. Selection may be improved by using alternative antimicrobial agents which more efficiently suppress interfering background growth and wild type E. coli. The development of a multiplex PCR for the simultaneous detection of Stx1, Stx2, eaeA and the enterohaemolysin plasmid would improve the time efficiency of the molecular detection method and should be investigated.

Acknowledgements

The authors would like to thank Rand Water and staff of the Department of Medical Virology, University of Pretoria, for technical assistance. The Water Research Commission funded this study.

Reference

- ACKMAN D, MARKS S, MACK P, CALDWELL M, ROOT T and BIRKHEAD G (1997) Swimming associated haemorrhagic colitis due to *Escherichia coli* O157:H7 infection: evidence of prolonged contamination of a fresh water lake. *Epidemiol. Infect.* 119 1-8.
- BESSER RE, LETT SM, WEBER JT, DOYLE MP, BARRET TJ, WELLS JG and GRIFFIN PM (1993) An outbreak of diarrhea and hemolytic uremic syndrome from *Escherichia coli* O157:H7 in fresh-pressed apple cider. *JAMA* **269** 2217-2220.
- BETTELHEIM KA (1998) Studies of *Escherichia coli* cultured on Rainbow™ Agar O157 with particular reference to enterohaemorrhagic *Escherichia coli* (EHEC). *Microbiol. Immunol.* **42** 265-269.
- BOOTH L and ROWE B (1993) Possible occupational acquisition of Escherichia coli O157 infection. Lancet 342 1298-1299.

- BOYCE TG, PEMBERTON AG, WELLS JG and GRIFFIN PM (1995) Screening of *Escherichia coli* O157:H7 - a nationwide survey of clinical laboratories. *J. Clin. Microbiol.* 33 1868-1869.
- BURNENS AP, ZBINDEN R, KAEMP FL, HEINZER I and NICOLET J (1993) A case of laboratory acquired infection with *Escherichia coli* O157:H7. *Zentralbl. Bakteriol.* **279** 512-517.
- CDC (2001) Escherichia coli O157:H7. Division of Bacterial and Mycotic Diseases. Disease Information. http://www.cdc.gov/ncidod/dbmd/diseaseinfo/escherichiacoli g.htm.
- FRATAMICO PM, SACKITEY SK, WIEDMANN Mand DENG MY (1995) Detection of *Escherichia coli* O157:H7 by multiplex PCR. *J. Clin. Microbiol.* **33** 2188-2191.
- GANNON VPJ, RASHED M, KING RK and THOMAS EJG (1992) Detection and characterization of the *eae* gene of shiga-like toxin-producing *Escherichia coli* using polymerase chain reaction. *J. Clin. Microbiol.* 31 1268-1274.
- GOLDWATER PN and BETTELHEIM KA (1998) New perspectives on the role of *Escherichia coli* 0157:H7 and other enterohaemorrhagic *E. coli* serotypes in human disease. *J. Med. Microbiol.* **47** 1039-1045.
- GRIFFIN PM and TAUXE RV (1991) The epidemiology of infections caused by *Escherichia coli* O157:H7, and other enterohemorrhagic *E. coli*, and the associated hemolytic uremic syndrome. *Epidemiol. Rev.* **13** 60-98.
- GRIFFIN PM (1995) Escherichia coli O157:H7 and other enterohaemorrhagic Escherichia coli. In: Blaser MJ, Smith PD, Ravdin JI, Greenberg HB, and Guerrant RL (eds.) Infections of the Gastrointestinal Tract. Raven Press, New York, N.Y. 739-761.
- HÖLLER C, KOSHINSKY S and WITTHUHN D (1999) Isolation of enterohaemorrhagic *E. coli* from municipal sewage. *Lancet* **353** 2039.
- ISMAILI A, PHILPOTT DJ, DYTOC MT and SHERMAN PM (1995) Signal transduction responses following adhesion of verocytotoxinproducing *Escherichia coli. Infect. Immun.* **63** 3316-3326.
- ISO (2001) Microbiology of food and animal feeding stuffs Horizontal method for the detection of Escherichia coli O157. ISO/FDIS 16654.
- KARMALI MA, PETRIC M, LIM C, FLEMING PC, ARBUS GS and LIOR H (1985) The association between idiopathic haemolytic uraemic syndrome and infection by verotoxin-producing *Escherichia coli*. *J. Infect. Dis.* **151** 775-782.
- KARMALI M (1989) Infection by verocytotoxin-producing *Escherichia coli. Clin. Microbiol. Rev.* **2** 15-38.
- KEENE WE, MCANULTY JM, HOESLY FC, WILLIAMS LP, HEDBERG K, OXMAN GL, BARRET TJ, PFALLER MA and FLEMING DW (1994) A swimming-associated outbreak of haemorrhagic colitis caused by *Escherichia coli* O157:H7 and *Shigella sonnei*. New Eng. J. Med. 331 579-584.
- MCCARTHY M (1998) E. coli O157:H7 outbreak in USA traced to apple juice. Lancet. 348 1299.
- MÜLLER EE, EHLERS MM. and GRABOW WOK (2001) The Occurrence of *E. coli* O157:H7 in South African Water Sources Intended for Direct and Indirect Human Consumption. *Water Res.* **35** 3085-3088.
- MÜLLER EE, TAYLOR MB, GRABOW WOK and EHLERS MM (2002) Isolation and characterization of *Escherichia coli* O157:H7 and Shiga toxin-converting bacteriophages from strains of human, bovine and porcine origin. *Water Sci. Technol.: Water Supply* 2 29-38.
- MUNIESA M and JOFRE J (1998) Abundance in sewage of bacteriophages that infect *Escherichia coli* O157:H7 and that carry the shiga toxin 2 gene. *Appl. Env. Microbiol.* **64** 2443-2448.
- NATARO JP and KAPER JB (1998) Diarrheagenic *Escherichia coli. Clin. Microbiol. Rev.* 11 142-201.
- OSTROFF SM, KOBAYASHI JM and LEWIS JH (1989) Infections with Escherichia coli O157:H7 in Washington State: the first year of statewide disease surveillance. JAMA. 262 355-359.
- PATON JC and PATON AW (1998) Pathogenesis and diagnosis of shiga toxin-producing *Escherichia coli* infections. *Clin. Microbiol. Rev.* 11 450-479.
- POLLARD DR, JOHNSON WM, LIOR H, TYLER SD and ROZEE KR (1990) Rapid and specific detection of verotoxin genes in Escherichia coli by the polymerase chain reaction. *J. Clin. Microbiol.* **28** 540-545.

ISSN 0378-

- PROMED (2000) www.promedmail.org. E. coli, EHEC Canada (Ontario) (09) 16 Aug.
- SAMBROOK J, FRITSCH EF and MANIATIS T (1989) *Molecular cloning: A Laboratory Manual* (2nd edn.). Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
- SLUTSKER L, RIES AA, GREENE KD, WELLS JG, HUTWAGENER L and GRIFFIN PM (1997) *Escherichia coli* O157:H7 diarrhea in the United States: Clinical and epidemiological features. *Ann. Intern. Med.* **126** 505-513.
- SWERDLOW, DL, WOODRUFFBA, BRADY RC, GRIFFIN PM, TIPPEN S, DONNEL HD, GELDREICH E, PAYNE BJ, MEYER A and WELLS JG (1992) A waterborne outbreak in Missouri of *Escherichia coli* O157:H7 associated with bloody diarrhea and death. *Ann. Intern. Med.* **117** 812-819.
- TAKEDA Y (1997) Enterohaemorrhagic Escherichia coli. Rapp. Trimest. Statist. Sanit. Mond. 50 74-80.
- TOMOYASU T (1998) Improvement of the immunomagnetic separation method selective for *Escherichia coli* O157 strains. *Appl. Env. Microbiol.* **64** 376-382.
- WHO (1997) Consultations and Workshops. Prevention and Control of Enterohaemorrhagic Escherichia coli (EHEC) Infections. Report of a WHO Consultation, Geneva, Switzerland. 8 April-1May.
- WRIGHT DJ, CHAPMAN PA and SIDDONS CA (1994) Immunomagnetic separation as a sensitive method for isolating *Escherichia coli* O157 from food samples. *Epidemiol. Infect.* **113** 31-39.