

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, *eaeA* 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 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 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).

* To whom all correspondence should be addressed.

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