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Absence of *Escherichia coli* O157:H7 in waters

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Abstract *Escherichia coli* O157:H7 produces a toxin called Shiga-like toxin, which has been associated with haemolytic uremic syndrome (HUS), haemorrhagic colitis and thrombocytopaenic purpura. Infections in humans are associated with consumption of contaminated food such as meat and raw milk. Early in the year 1996, several cases of HUS in children were reported in Argentina. In most cases, the infection source could not be determined. Consequently, several health centres began to distrust water as a transmission media. A total of 493 water samples from different regions, industries and institutions in Santa Fe Province were analysed. None of the analysed samples showed the presence of *E. coli* O157:H7 and the mechanism of its transmission through water remains unknown.

INTRODUCTION

Escherichia coli O157:H7 (*E. coli* O157:H7) is the prototype of the entero-haemorrhagic group (EHEC). The serotypes defined as EHEC produce a toxin called Shiga-like toxin. The most serious manifestations are haemolytic uremic syndrome (HUS), haemorrhagic colitis and thrombocytopaenic purpura (Cahoon & Thompson, 1987). Children younger than five years of age, and adults sixty years and older, constitute the highest risk groups for HUS (Rivas, 1995). Argentina reports an annual incidence rate of 7.8 per 100 000 children of five years age and younger, the highest in the world (Rivas, 1995). The infections in humans are associated with consumption of contaminated foods such as meats and raw milk. Epidemiological reports showed that cattle can excrete this toxin in their faeces, which represents an important reservoir and infection source.

E. coli O157:H7 has been considered a human pathogen since 1982 following reports of two outbreaks in United States which were associated with hamburger consumption in fast food restaurants (Bell *et al.*, 1994).

On 5 July 1995, the Winnebago County Health Department (WCHD) in northern Illinois received a report from the local hospital of five cases of *E. coli* O157:H7 infection among children who resided in Rockford. Interviews with the children's parents revealed no common food source. However, on 24–25 June, they all had visited a lake at an Illinois State park. On 6 July, the Illinois Department of Public Health (IDPH) closed the swimming beach because of suspected transmission of infection through lake water. WCHD and IDPH investigated the outbreak to assess risk factors for illness and determine the source of infection. Their report summarizes the findings of the investigation, which indicates that ingesting contaminated and untreated lake water can result in infection (Centers for Disease Control and Prevention, 1996).

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The goal of this study was to investigate the presence of this serotype in waters and aerosols produced in industrial waste treatment plants.

METHODS

Sample collection

A total of 493 water samples were analysed. These included 118 groundwater samples from below/close to meat processing factories, 103 surface water samples from milk processing factories, 26 samples from lakes into which meat processing factories discharge some waste, 38 water samples from the Paraná River at Rosario, 18 samples from natural and artificial lakes adjacent to dairy operations, 17 liquid effluent samples from meat processing factories, and 173 water samples from swimming pools.

Determinations

The following determinations were made: Heterotrophic plate count, Total coliforms, *E. coli* and *Pseudomonas aeruginosa* as directed in methods 9215B, 9221B, 9221E and 9213F respectively of APHA (1992).

Methodology for the detection of *E. coli* O157

To investigate the presence of *E. coli* O157:H7, 1 ml of Fluorocult Brila broth was extracted from each one of the positive and negative tubes of the coliforms test. The broth portions extracted were gathered in a single sterile tube. Streaks were made out of this broth on sorbitol Mac Conkey (SMAC) (Difco) and Fluorocult *E. coli* O157:H7 (FEC) agars (Szabo *et al.*, 1986). Plates were incubated at 35°C for 24 h. Sorbitol non-fermenting colonies in SMAC and greenish colonies in FEC, which were not fluorescent to ultraviolet light, were selected. All selected colonies were transferred to Tryptic soy Agar and incubated at 35°C for 24 h. Presumptive primary tests involving Triple Sugar Iron Agar, Simmons Citrate Agar, MR-VP Medium and SIM Medium were performed. In some cases, Kit API system (BioMérieux) was inoculated with the isolated pure cultures.

Finally, serology was conducted on isolated colonies from the different agars that demonstrated a similar fermentation scheme to *E. coli* (Holt *et al.*, 1994). Difco's Antiserum, Bacto *E. coli* O Antiserum O157, and Bacto *E. coli* H Antiserum H7, were employed for serology. As a positive control test, water samples were inoculated with an *E. coli* O157:H7 ATCC 35.150 strain, and then the recovery procedure described above was used. The recovery of *E. coli* O157:H7 was 90%.

RESULTS

E. coli O157:H7 was not found in any of the samples which were analysed (Table 1).

Table 1 Results.

Type of analysed water	No. of samples analysed	No. of samples that contained total coliforms	No. of samples that contained *E. coli*	No. of samples that contained *E. coli* O157:H7
Groundwater	118	23	4	0
Surface water (milk processing)	103	10	1	0
Lakes (meat processing)	26	26	26	0
Paraná River	38	38	38	0
Lakes (dairies)	18	18	18	0
Meat processing factory liquid effluents	17	10	8	0
Swimming pools	173	13	1	0

The absence of *E. coli* O157:H7 may be explained by a complex set of environmental factors and, perhaps, by competitive inhibition in the presence of native microbial populations. The same environment would possibly permit bacteria to be found in viable, but unculturable, form. Transmission through water still continues to be an unknown mechanism.

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