A comparison of immunomagnetic separation and direct culture for the isolation of verocytotoxin-producing *Escherichia coli* O157 from bovine faeces

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Summary. Enrichment culture (EC) in modified buffered peptone water followed by immunomagnetic separation (IMS) with magnetic beads coated with an antibody against *Escherichia coli* O157 (Dynabeads anti-*E. coli* O157; Dynal, Oslo) was compared with direct culture on cefixime rhamnose sorbitol MacConkey agar (CR-SMAC) and cefixime tellurite sorbitol MacConkey agar (CT-SMAC) for the isolation of *E. coli* O157 from bovine faeces. When used to examine bovine faecal suspensions inoculated with 12 different strains of *E. coli* O157, EC-IMS was *c.* 100-fold more sensitive for detection of the organism than direct culture on either medium. During monitoring of a dairy herd, *E. coli* O157 was isolated from 84 (8.2%) of 1024 rectal swabs taken from cattle over a 4-month period; 23 (27.4% of the 84 strains were isolated by both direct culture and IMS (15 of the 23 were isolated on both media, five on CT-SMAC only and three on CR-SMAC only), whereas 61 (72.6%) strains were isolated by IMS only. IMS is a sensitive and simple technique for the isolation of *E. coli* O157 from bovine faecal samples and should prove useful in elucidating further the epidemiology of this organism.

Introduction

Verocytotoxin-producing *Escherichia coli* (VTEC) are now recognised as a major cause of haemorrhagic colitis (HC) and the haemolytic-uraemic syndrome (HUS), both conditions with high morbidity and mortality. In the UK, strains of verocytotoxin-producing (VT⁺) *E. coli* O157, the most common serogroup associated with illness in man, have been isolated from cattle^{1–3} but the epidemiology of the organism remains unclear. In view of the potential severity of infections caused by VT⁺ *E. coli* O157, it is essential that the most sensitive methods available are used in studies designed to elucidate further the reservoirs of the organism and routes of transmission of the organism to man.

VT⁺ *E. coli* O157 strains do not ferment sorbitol whereas most other serogroups of *E. coli* do and sorbitol MacConkey agar has proved useful for their isolation.⁴ Improvements to sorbitol MacConkey medium^{5,6} have resulted in increased sensitivity of isolation of *E. coli* O157 from faecal samples but efficient selective enrichment media for *E. coli* O157 have not been described.

The aim of this study was to evaluate the use of enrichment culture (EC) in modified buffered peptone water² followed by immunomagnetic separation (IMS) for the isolation of *E. coli* from bovine faeces.

Artificially inoculated faecal samples, and samples collected both during the investigation of a possible outbreak of milk-borne infection by VT⁺ *E. coli* O157 in the Sheffield area³ in May 1993 and during the subsequent monitoring of the dairy herd jointly by MAFF and PHLS, were used to compare direct culture with the IMS technique for isolation of *E. coli* O157.

Materials and methods

Preparation of inoculated faecal samples

Twelve different strains of *E. coli* O157 previously isolated from bovine faeces² were each grown overnight at 37°C in nutrient broth and the viable counts (cfu/ml) were estimated by a standard serial dilution method. The strains used were: P1394 O157:H[–] VT₁[–]VT₂⁺ phage type (PT) 2; P1396 O157:H7 VT₁[–]VT₂⁺ PT2; P1431 O157:H[–] VT₁[–]VT₂⁺ PT4; P1446 O157:H7 VT₁⁺VT₂⁺ PT4; P1426 O157:H[–] VT₁⁺VT₂⁺ PT8; P1506 O157:H7 VT₁[–]VT₂⁺ PT8; P1401 O157:H7 VT₁⁺VT₂⁺ PT14; P1523 O157:H7 VT₁[–]VT₂⁺ PT14; P1524 O157:H7 VT₁⁺VT₂[–] PT14; P1430 O157:H[–] VT₁[–]VT₂⁺ PT49; P1519 O157:H7 VT₁[–]VT₂⁺ PT49; and P1400 O157:H[–] VT₁[–]VT₂[–] PT RDNC.

Ten samples of bovine faeces containing large numbers of sorbitol-fermenting (SF) *E. coli*, but from which *E. coli* O157 could not be isolated, were used to make a suspension of faeces *c.* 50% v/v in buffered

peptone water, which was then used to prepare suspensions containing each of the above *E. coli* O157 strains at concentrations of c. 10^6 , 10^5 , 10^4 , 10^3 , 100 and 10 cfu/ml.

Collection of faecal samples from dairy cattle

Samples of faeces were collected by rectal swabs from dairy cattle; 1024 swabs were collected from 118 milking cows, 25 in-calf heifers, 16 calves and 8 dry cows. Samples were collected at approximately 2-week intervals over a 4-month period from late May until late August by colleagues from the MAFF Veterinary Investigations Centre, Thirsk, and were transported immediately to Sheffield Public Health Laboratory for microbiological examination.

Direct culture of E. coli O157

Samples (either rectal swabs or 20 μ l of inoculated faecal suspension) were inoculated on to cefixime rhamnose sorbitol MacConkey (CR-SMAC) medium⁵ and cefixime tellurite sorbitol MacConkey (CT-SMAC) medium.⁶ After incubation overnight at 37°C, apparently sorbitol and rhamnose non-fermenting colonies from CR-SMAC and apparently sorbitol non-fermenting colonies from CT-SMAC were tested for agglutination with a latex test kit (Oxoid; DR622) for detecting *E. coli* O157.⁷ Isolates that gave positive results with this test were further characterised as described below.

Immunomagnetic separation of E. coli O157

After direct culture, swabs (or 100 μ l of inoculated faecal suspension) were placed in 5 ml of buffered peptone water (Oxoid; CM509) supplemented with vancomycin 8 mg/L, cefixime 0.05 mg/L and cefsulodin 10 mg/L to inhibit the growth of gram-positive organisms, aeromonads and *Proteus* spp., respectively.² After vortex mixing, broths were incubated at 37°C for 6 h and 1 ml of broth was added to 20 μ l of magnetic beads coated with an antibody against *E. coli* O157 (Dynabeads anti-*E. coli* O157; Dynal, Oslo) in a 1.5-ml microcentrifuge tube. The beads were suspended evenly in the broth culture by vortex mixing and were placed in a rotating mixer so that they were mixed by inversion every 2–3 s for 30 min at ambient temperature. Tubes were placed in a magnetic separator rack (MPC-10; Dynal, Oslo) and the magnets were placed in position and left for 5 min. The culture supernate was removed by aspiration with a pasteur pipette, the magnetic slide was removed from the rack, the beads were washed by resuspension in 1 ml of PBS, pH 7.2, with Tween 20 0.002% v/v (PBST) and the magnetic slide was replaced for 2 min. The beads were washed in PBST in this way a further two times, the magnetic slide was replaced for 2 min, the supernate was removed and the beads were resuspended in c. 25 μ l of PBS. Beads were inoculated on to CT-SMAC

medium and incubated overnight at 37°C. Sorbitol non-fermenting colonies were examined as above.

Characterisation of isolates

Identification. As described previously,⁷ isolates that gave a positive latex test result were confirmed as *E. coli* by biochemical tests and confirmed as serogroup O157 by agglutination to titre with antiserum to *E. coli* O157 (Laboratory for Microbiological Reagents, Central Public Health Laboratory, 61 Colindale Avenue, London).

Verocytotoxin production. Toxigenicity was determined by Vero cell culture assay.¹ Toxin type was determined by specific hybridisation with DNA probes for the VT₁ and VT₂ genes. DNA specific for the A cistrons of the VT₁ and VT₂ genes was prepared by the polymerase chain reaction, random-prime labelled with digoxigenin-11-dUTP, and used in colony hybridisation reactions as described previously.^{2,8} Known VT₁⁺, VT₂⁺ and VT⁻ strains were included as controls in each batch of tests.

Plasmid analysis. Plasmids were extracted by an alkaline detergent method⁹ and were separated by submerged gel electrophoresis in Tris-acetate-EDTA buffer with agarose 1%, stained by ethidium bromide and visualised on an ultraviolet transilluminator. A control *E. coli* K-12 strain (NCTC 50192-39R861) harbouring plasmids of 148, 63.4, 36 and 6.9 kb was included with each batch of tests. For this control strain, log₁₀ of plasmid size was plotted against distance migrated through the agarose gel and approximate sizes of plasmids from strains of *E. coli* O157 were estimated from this graph.

Phage typing. All *E. coli* O157 strains were phage typed by the Laboratory for Enteric Pathogens, Central Public Health Laboratory.

Results

Preliminary evaluation of IMS

The detection limits and approximate ratios of SF *E. coli*:*E. coli* O157 for both direct culture and EC-IMS are shown in detail in table I. EC-IMS was c. 100-fold more sensitive than direct culture on either CR-SMAC or CT-SMAC for the recovery of most of the strains of *E. coli* O157.

Isolation of E. coli O157 from cattle in a dairy herd

E. coli O157 was isolated from 84 (8.2%) of 1024 bovine rectal swabs: 23 (27.4%) of the 84 strains were isolated by both direct culture and EC-IMS (15 of the 23 were isolated on both media, five on CT-SMAC only and three on CR-SMAC only), whereas 61 (72.6%) strains were isolated by EC-IMS only (table II).

All strains were confirmed as VT producers by a cell culture assay, hybridised with the DNA probe for VT₂

Table I. Evaluation of IMS with 12 different strains of *E. coli* O157

Strain no.	CR-SMAC		CT-SMAC		IMS	
	Limit	Ratio	Limit	Ratio	Limit	Ratio
P1394	10 ⁵	+++ / +	10 ⁵	+++ / +++	100	+++ / +++
P1396	10 ⁵	+++ / +	10 ⁵	+++ / +++	10 ³	- / +++
P1431	10 ⁴	+++ / +	10 ⁵	+++ / +++	100	+++ / +++
P1446	10 ⁵	+++ / +	10 ⁵	+++ / +	100	+++ / +++
P1426	10 ⁴	+++ / +	10 ⁴	+++ / +++	100	+++ / +++
P1506	10 ⁵	+++ / +	10 ⁵	+++ / +	10 ³	- / +++
P1401	10 ⁵	+++ / +	10 ⁵	+++ / +++	10 ³	- / +++
P1523	10 ⁵	+++ / +	10 ⁵	+++ / +++	10 ³	- / +++
P1524	10 ⁵	+++ / +	10 ⁵	+++ / +	10 ³	- / +++
P1430	10 ⁵	+++ / +	10 ⁴	+++ / +++	100	+++ / +++
P1519	10 ⁵	+++ / +	10 ⁵	+++ / +	10 ³	- / +++
P1400	10 ⁵	+++ / +	10 ⁴	+++ / +	10 ³	- / +++

Limit, lowest detectable number of *E. coli* O157/ml of faecal suspension; ratio, approximate ratio of colonies of sorbitol-fermenting *E. coli*/ *E. coli* O157; -, not detected; +, < 10 colonies; ++, 10-100 colonies; +++, > 100 colonies.

Table II. Isolation of *E. coli* O157 from 1024 bovine rectal swabs

Isolation achieved by			Number of isolates
CR-SMAC	CT-SMAC	EC-IMS	
+	+	+	15
-	+	+	5
+	-	+	3
-	-	+	61
Total			84

CR-SMAC, direct culture on cefixime rhamnose sorbitol MacConkey agar; CT-SMAC, direct culture on cefixime tellurite sorbitol MacConkey agar; EC-IMS, pre-enrichment culture in modified buffered peptone water, immunomagnetic separation of *E. coli* O157 and subculture on CT-SMAC; +, *E. coli* O157 isolated; -, *E. coli* O157 not isolated.

but not with that for VT₁, harboured a single 92-kb plasmid and were of phage type 2.

Discussion

Cattle appear to be a major reservoir of VT⁺ *E. coli* O157 and the organism has been isolated from apparently healthy animals while investigating sources of human infection in Canada,¹⁰ the USA¹¹⁻¹³ and England.¹⁻³ Therefore, sensitive methods for detecting VT⁺ *E. coli* O157 in bovine faeces are necessary for epidemiological studies. VT⁺ *E. coli* O157 strains do not ferment sorbitol, whereas most other *E. coli* strains do, and sorbitol MacConkey (SMAC) medium has become widely used for their isolation. However, SMAC medium relies entirely on differential sugar fermentation and does not select VT⁺ *E. coli* O157 from other *E. coli* or sorbitol non-fermenting genera and, therefore, lacks sensitivity. SMAC media incorporating cefixime and rhamnose (CR-SMAC)⁵ or cefixime and tellurite (CT-SMAC)⁶ have been shown previously to be more efficient than unmodified SMAC medium for the isolation of the organism from human

faeces and bovine faeces, respectively. In this study, the sensitivities of CT-SMAC and CR-SMAC were not apparently different for the isolation of 12 different strains of *E. coli* O157 from artificially inoculated bovine faecal suspensions, but the greater ratio of *E. coli* O157: SF *E. coli* usually observed on CT-SMAC meant that the organism was easier to obtain in pure culture from the CT-SMAC medium.

A 6-h period of enrichment culture in buffered peptone water supplemented with vancomycin, cefixime and cefsulodin followed by subculture to CR-SMAC was more sensitive than direct culture on CR-SMAC for detecting *E. coli* O157 on beef carcasses,² but was less sensitive than direct culture on CR-SMAC for detecting the organism in faecal samples due to heavy overgrowth of other organisms (Sheffield PHL, unpublished data).

IMS has been shown to be more sensitive than direct culture for the isolation of *E. coli* O157 from artificially mixed cultures and inoculated meat samples.^{14,15} The present study has confirmed that EC followed by IMS is c. 100-fold more sensitive than direct culture on either CR-SMAC or CT-SMAC for detecting a range of types of *E. coli* O157 in inoculated bovine faeces. On examination of 1024 bovine rectal swabs, *E. coli* O157 was isolated from 23 by direct culture; 15 of the 23 were isolated on both CR-SMAC and CT-SMAC, five on CT-SMAC only and three on CR-SMAC only. *E. coli* O157 was isolated from 84 swabs by EC-IMS and these included the 23 that were isolated by direct culture; this provides further evidence of the enhanced sensitivity of the EC-IMS technique. All strains were confirmed as typical VT⁺ *E. coli* O157 by phage typing, plasmid content and DNA hybridisation for toxin genes.

Other immunological methods have been described for the detection of *E. coli* O157, including enzyme immunoassay^{16,17} and immunoblot techniques.^{18,19} Although sensitive, these methods may be time-consuming, technically demanding, expensive and prone to give positive results that cannot be confirmed

by culture. In contrast, EC-IMS is rapid, technically simple and, most importantly, yields an isolate of *E. coli* that may be useful in epidemiological studies.

Monitoring of the dairy herd is continuing to help understand further the epidemiology of this organism.

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