Detection of Escherichia coli serogroups O26, O103, O111 and O145 from bovine faeces using immunomagnetic separation and PCR/DNA probe techniques

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Detection of *Escherichia coli* serogroups O26, O103, O111 and O145 from bovine faeces using immunomagnetic separation and PCR/DNA probe techniques

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ABSTRACT

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Aims: The aim of this study was to isolate *Escherichia coli* O26, O103, O111 and O145 from 745 samples of bovine faeces using (i) immunomagnetic separation (IMS) beads coated with antibodies to lipopolysaccharide, and slide agglutination (SA) tests and (ii) PCR and DNA probes for the detection of the Verocytotoxin (VT) genes.

Methods and Results: IMS-SA tests detected 132 isolates of presumptive *E. coli* O26, 112 (85%) were confirmed as serogroup O26 and 102 had the VT genes. One hundred and twenty-two strains of presumptive *E. coli* O103 were isolated by IMS-SA, 45 (37%) were confirmed as serogroup O103 but only one of these strains was identified as Verocytotoxin-producing *E. coli* (VTEC). Using the PCR/DNA probe method, 40 strains of VTEC O26 and three strains of VTEC O103 were isolated. IMS-SA identified 21 strains of presumptive *E. coli* O145, of which only four (19%) were confirmed as serogroup O145. VTEC of this serogroup was not detected by either IMS-SA or PCR/DNA probes. *E. coli* O111 was not isolated by either method.

Conclusion: IMS beads were 2·5 times more sensitive than PCR/DNA probe methods for the detection of VTEC O26 in bovine faeces.

Significance and Impact of the Study: IMS-SA is a sensitive method for detecting specific *E. coli* serogroups. However, the specificity of this method would be enhanced by the introduction of selective media and the use of tube agglutination tests for confirmation of the preliminary SA results.

Keywords: *E. coli*, VTEC, IMS, PCR/DNA probes.

INTRODUCTION

Verocytotoxin-producing *Escherichia coli* (VTEC) O157 is the most common VTEC serogroup associated with human diarrhoeal disease in the UK (Willshaw *et al.* 2001) and cattle are regarded as major reservoir of these organisms (Chapman *et al.* 1993). Non-O157 VTEC serogroups, most commonly O26, O103, O111 and O145, have been shown to cause diarrhoea in humans (Paton *et al.* 1996; Schmidt *et al.* 1999; McMaster *et al.* 2001; Scheutz *et al.* 2001; Tozzi *et al.* 2003) and have been isolated from the faeces of cattle in many countries (Beutin *et al.* 1993; Willshaw *et al.* 1993; Kobayashi *et al.* 2001). While there have been some outbreaks caused by non-O157 VTEC (such as O22 and O104) where circumstantial evidence for a link with cattle has been shown (Bockemuhl *et al.* 1992; Anon 1995), the

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source of non-O157 VTEC in human infection is largely unknown or unreported.

In the Laboratory of Enteric Pathogens (LEP), the detection of non-O157 VTEC is carried out using a combination of PCR and DNA probe techniques to detect Verocytotoxin (VT) genes (Willshaw *et al.* 2001). However, these tests are not routinely available in laboratories in the UK. Rapid and affordable tests for non-O157 VTEC from food samples, and bovine and human faecal samples would improve the detection and epidemiological surveillance of VTEC.

The detection of *E. coli* O157 from faeces and foods using immunomagnetic separation (IMS) is well established (Synge and Paiba 2000; Chapman *et al.* 2001). However, recently IMS beads coated with polyclonal antibodies to the lipopolysaccharide (LPS) of *E. coli* O26, O103, O111 and O145 have become available. These are the non-O157 VTEC serogroups most commonly associated with haemolytic uraemic syndrome (Paton *et al.* 1996; Schmidt *et al.* 1999; McMaster *et al.* 2001; Scheutz *et al.* 2001; Tozzi *et al.* 2003). The O26 and O111 IMS beads have been evaluated for the detection of these *E. coli* serogroups in vegetables (Safarikova and Safarik 2001) and O103 IMS beads have been used to detect *E. coli* O103 in sheep faecal samples (Urdahl *et al.* 2002). However, field evaluation of these IMS beads for the detection of these serogroups in bovine faecal samples has not been reported to date.

The IMS protocol used in this study detected *E. coli* (VTEC and non-VTEC) expressing the LPS of O26, O103, O111 and O145, whereas the PCR and DNA probe methods detect VT genes, therefore identifying VTEC regardless of serogroup. The aim of this study was to evaluate the detection of *E. coli* O26, O103, O111 and O145 from bovine faecal samples using IMS and slide agglutination (IMS-SA) and make comparisons with the PCR/DNA probe method.

MATERIALS AND METHODS

Faecal sampling

Rectal faecal samples were taken from 49 calves and their 44 dams on a cattle farm in northern Scotland. The calves were born between August and November 2001 and were sampled weekly from birth until the end of January 2002. Cows were sampled at the time of birth and at the end of the sampling period. All calves and dams were healthy at the time of sampling. Seven hundred and forty-five samples were examined by IMS-SA and PCR/DNA probes.

Immunomagnetic separation-slide agglutination

Samples were refrigerated at 5°C within 2 h of sampling. Within 48 h of sampling, 1 g of faeces from each sample was suspended in 20 ml buffered peptone water (BPW), and incubated at 37°C for 6 h. Following incubation, 1 ml of BPW was added to 20 μl of four different sets of serogroup specific IMS beads (serogroups O26, O103, O111, O145) (IDG Plc, Bury, Lancashire, UK) in four separate screw capped microcentrifuge tubes (Fig. 1). Tube contents were mixed on a blood tube rotator for 30 min then tubes were placed in IMS magnet racks for 5 min. Beads were then washed three times as follows: from each tube, supernatant was removed and beads resuspended in 1 ml phosphate buffered saline (PBS) with 0·05% Tween (PBST); each tube was inverted gently 4–5 times and then placed in a magnet rack for 3 min.

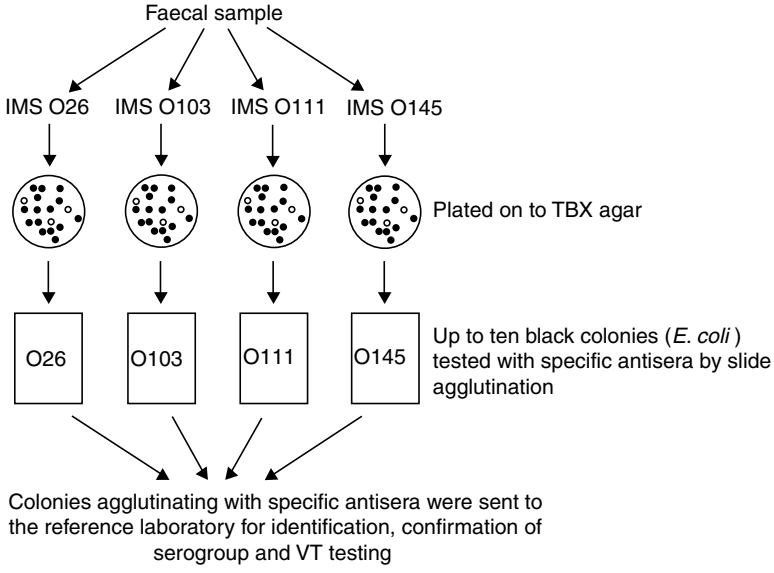

Fig. 1 Flow diagram of the steps involved in the IMS-SA method. Black colonies represent isolates of *E. coli* (blue/violet colonies on Chromocult TBX agar). White colonies represent non-*E. coli* isolates

Following the final wash, supernatant was removed and beads were resuspended in 50 μl PBST. To ensure beads were thoroughly suspended, tubes were held upright and flicked gently several times. Fifty microlitres suspensions of serogroup O26, O103, O111 and O145 beads were plated on Chromocult TBX plates (Merck, Poole, Dorset, UK) (Frampton *et al.* 1988) (Fig. 1). Chromocult agar contains two chromogenic substrates, which allows for the identification of total coliforms and *E. coli*. The characteristic enzyme for coliforms, β-D-galactosidase cleaves the salmon-GAL substrate and causes a salmon to red colour. The substrate X-glucuronide is used for the identification of β-D-glucuronidase, which is characteristic of *E. coli* and these colonies are dark blue to violet colour. Plates were incubated at 37°C overnight. From each plate, all morphologically different blue to violet colonies, but not more than 10, were tested with serogroup specific antisera (Statens Serum Institut, Copenhagen, Denmark) by SA (Fig. 1). Those colonies showing a dark blue to violet colour, characteristic of *E. coli*, and agglutinating with the specific antisera, were considered putative *E. coli* O26, O103, O111 or O145 and sent to the reference laboratory for serogroup confirmation.

Identification of samples containing VT genes by PCR and isolation of VTEC by DNA probes

One gram of faeces from a second sample taken at the same time as the IMS-SA sample was suspended in 4 ml of PBS and 200 μl were added to 10 ml BPW, prior to 6 h incubation at 37°C. The cultures were plated onto MacConkey agar (Oxoid, Basingstoke, UK) and incubated overnight at 37°C. Nutrient broths (Oxoid, Basingstoke, UK) were inoculated with a sweep of mixed colonies from the MacConkey plates, incubated at 37°C for 2–4 h and examined for VT1 and VT2 sequences by PCR, as described previously (Willshaw *et al.* 2001). Mixed colonies from the original MacConkey agar plate, shown to contain VT genes by PCR, were transferred by replica plating onto a nylon membrane (Amersham Biosciences, Little Chalfont, UK) placed on a nutrient agar plate and incubated at 37°C for 4–6 h. The membranes were prepared for hybridisation by the method of Maniatis *et al.* (1982). Individual VTEC colonies were identified by colony DNA hybridisation with a mixture of VT1 and VT2 polynucleotide probes (Willshaw *et al.* 1987; Thomas *et al.* 1991). VTEC colonies detected were marked on the master plate and inoculated onto MacConkey agar. PCR was carried out, as described above, on pure cultures to confirm that VTEC had been isolated.

Serotyping and VT typing

Strains detected by IMS-SA and PCR/DNA probes were biochemically confirmed as *E. coli* using the tests described in Edwards and Ewing's Identification of Enterobacteriaceae (Ewing 1986), and serotyped using the LEP serotyping scheme that depends on the identification of the heat stable LPS somatic ('O') and the flagellar ('H') antigens (Gross and Rowe 1985). Provisional new serogroups, formal 'O' group pending, were given an 'E' prefix and strains that could not be serogrouped as O1–O173 were designated 'O'? Each isolate was tested for VT1 and VT2 sequences by PCR, as described previously (Willshaw *et al.* 2001).

Statistics

Statistical analysis was done using SAS v8.2. Rates of VTEC isolation using the IMS-SA and the PCR-DNA probes were compared using the paired exact test and the kappa statistic.

RESULTS

Seven hundred and forty-five faecal samples were examined by IMS-SA and PCR/DNA probes and the numbers of strains of *E. coli* (VTEC and non-VTEC) belonging to serogroups O26, O103, O111 and O145 isolated are summarised in Table 1. *E. coli* O26, O103 and O145 were isolated more frequently from faecal samples using IMS-SA than using PCR-DNA probes. *E. coli* O111 was not detected. Of the 161 strains of *E. coli* isolated by IMS-SA and confirmed as serogroup O26, O103 or O145, 64% (103 strains) had the VT genes (Table 1). A total of 169 strains of VTEC, including at least 15 different serogroups, were isolated using the PCR/DNA probe method (Table 2).

The isolation rate of strains of VTEC O26 was significantly different between the two protocols (paired exact, $P < 0{\cdot}001$: $\kappa = 0{\cdot}38$), with the IMS-SA method detecting 2·5 times more isolates of VTEC O26 than the PCR/DNA probe technique (Table 1). Four strains of VTEC O103 were isolated, one using IMS-SA and three by PCR/DNA probes. VTEC O111 or O145 were not detected by IMS-SA

Table 1 Summary of strains of *E. coli* isolated using Immunomagnetic separation-slide agglutination (IMS-SA) and PCR/DNA probe methods

	Number of isolates			
	IMS-SA			
Serogroup	Putative serogroup	Confirmed serogroup	VTEC	PCR/DNA probe
O26	132	112	102	40
O103	122	45	1	3
O111	0	0	0	0
O145	21	4	0	0
Total	275	161	103	43

Table 2 VTEC serogroups isolated using PCR/DNA probe method

Serogroup	No.
O2	12
O8	2
O15	1
O20	1
O26	40
O84	4
O91	6
O103	3
O113	7
O128ab	1
O162	1
O168	2
†E874/85	17
†E54071/88	21
O?	50
Total	168

†Provisional new serogroups, formal 'O' group pending.
Strains that could not be serogrouped as O1–O173 were designated 'O?'.

or PCR/DNA probes (Table 1). Although IMS-SA detected more VTEC O26 than PCR/DNA probes overall, nine faecal samples found to contain VTEC O26 by PCR/DNA probes were negative using IMS-SA. Similarly, using the PCR/DNA probe method, VTEC O103 were detected in three faecal samples but were not detected in the same three samples using IMS-SA.

The proportion of isolates detected by IMS-SA whose putative serogroup was confirmed by the LEP serotyping scheme was significantly different between serogroups (Fisher's exact, $P < 0{\cdot}001$). Of the strains isolated using IMS-SA, 112 of 132 (85%) of putative serogroup O26 were confirmed as O26, 45 of 122 (37%) of putative serogroup O103 were confirmed as *E. coli* O103 and four of 21 (19%) of putative *E. coli* O145 were confirmed as such (Table 1). *E. coli* serogroups that appeared to agglutinate with the specific antisera but were not confirmed as *E. coli* O26, O103, O111 and O145 are shown in Table 3.

DISCUSSION

This paper is the first description of the use of IMS beads for the detection of *E. coli* O26, O103, O111 and O145 in faeces of naturally infected healthy cattle. In this study, IMS-SA was more sensitive than PCR-DNA probes for the detection of *E. coli* O26, O103 and O145, although direct comparisons of sensitivity and specificity between the two methods are difficult as IMS-SA detects LPS ('O' serogroup) and PCR/DNA probes detect VT genes. However, direct comparisons can be made for the detection of VTEC

Table 3 Non-target *E. coli* serogroups detected using IMS beads for O26, O103 and O145 and agglutinating with the corresponding specific antisera in SA tests

Non-target *E. coli* serogroups	IMS O26 beads	IMS O103 beads	IMS O145 beads
O1	0	1	0
O2	0	1	0
O3	0	0	1
O7	1	1	0
O8	1	0	0
O9a	0	0	1
O15	1	2	1
O21	1	0	0
O26	–	1	2
O31	0	1	0
O35	1	0	0
O38	0	1	0
O39	0	1	0
O46	0	1	0
O53	1	1	1
O60	0	3	3
O77	0	1	2
O80	0	2	0
O88	1	2	0
O98	3	1	0
O100	0	1	0
O101	0	2	0
O103	0	–	0
O108	1	0	0
O112ab	0	1	0
O113	0	1	0
O118	0	1	0
O126	0	3	0
O139	0	1	0
O145	0	0	–
O150	0	8	0
O162	0	2	0
O166	0	0	1
†E40874/85	1	1	1
O?	6	36	4
Total	18	77	17

None of the isolates of *E. coli* detected using IMS O111 beads agglutinated with O111 sera.
†Provisional new serogroups, formal 'O' group pending.
Strains that could not be serogrouped as O1–O173 were designated 'O?'.

O26, and IMS O26 beads identified 2·5 times more strains of VTEC O26 than the PCR/DNA probe method. Nine strains of VTEC O26 were detected by PCR/DNA probes but not by IMS-SA and this may be due to the uneven distribution of bacteria in the faecal sample. The methods for the other VTEC serogroups could not be compared as there were only four strains of VTEC O103, and VTEC O111 and O145 were not identified. Safarikova and Safarik

(2001) found that IMS increased the isolation of *E. coli* O26, O103 and O111 in vegetables with 93–100% of samples positive by IMS compared to 36–93% using direct culture. Urdahl *et al.* (2002) compared automated IMS (AIMS) with an AIMS-ELISA method and showed that AIMS-ELISA was more sensitive detecting *E. coli* O103 in 52·1% of sheep faecal samples compared to 36·5% using AIMS alone. Studies of the detection of *E. coli* O157 using cefixime tellurite sorbitol MacConkey (CT-SMAC) agar showed that IMS was approximately 100-fold more sensitive than direct culture (Chapman *et al.* 1994).

Of the strains of *E. coli* isolated using IMS-SA during this study, only 64% had the VT genes. Certain strains of VT-negative *E. coli*, including the serogroups examined here, harbour the genes required to express the attaching and effacing phenotype and are potentially pathogenic to humans and cattle (Scotland *et al.* 1990, 1993; Pearson *et al.* 1999).

The IMS-SA test for detecting *E. coli* O26 appeared to be more specific than the IMS-SA tests used to detect *E. coli* O103 and O145. However, the efficacy of the IMS-SA test for serogroup O26 may be due to the high number of *E. coli* O26 in the faecal samples, making this serogroup easier to detect on the non-selective media. Isolation of *E. coli* other than serogroups O26, O103, O111 and O145 may be due to either carry-over or non-specific binding to the IMS beads. The experiments were not designed to be quantitative and the exact number of colonies of non-*E. coli* on the Chromocult TBX agar plates (colonies that were not blue to violet in colour) were not recorded, although it was noted that numbers varied significantly between samples. It was not possible to determine the number of non-target *E. coli* colonies on each agar plate because only a maximum of 10 colonies of *E. coli* (blue to violet in colour) were tested with the specific antisera.

The detection of *E. coli* O157 using IMS is assisted by the use of selective, discriminatory media, such as CT-SMAC. Strains of VTEC O157 are characteristically non-sorbitol fermenters and the majority of other *E. coli* are sorbitol-positive (March and Ratnam 1986; Chapman *et al.* 1991). Cefixime is active against *Proteus sp*. and VTEC O157 has a higher tolerance to tellurite than most other *E. coli*. The use of Chromocult TBX agar allows basic differentiation of *E. coli* colonies, facilitating the selection of suitable colonies for SA but does not discriminate between target and non-target *E. coli*. More discriminatory media are needed for the detection of *E. coli* O26, O103, O111 and O145. For example, many strains of *E. coli* O26, including VT+ve strains, do not ferment rhamnose and it has been suggested that MacConkey agar containing rhamnose may be used to enhance the detection of this serogroup from faecal and food samples (Hiramatsu *et al.* 2002).

In the absence of suitable, discriminatory media for non-O157 *E. coli*, alternative methods to the SA test could be introduced. Tube agglutination tests, which involve adding a suspension of the 'O' antigen to the 'O' test antiserum in a Dreyer's tube and incubating overnight at 50°C, are more specific than SAs (Gross and Rowe 1985). The use of more specific agglutination tests would reduce the number of *E. coli* serogroups that need to be sent to the reference laboratory for confirmation of serogroup.

In this study, IMS-SA was a more sensitive test for the detection of VTEC O26 than PCR/DNA probes. However, IMS-SA is not specific for VTEC and other tests, such as PCR or DNA probes, must be used to determine whether VT genes are present. The specificity of IMS-SA for *E. coli* O26, O103, O111 and O145 would be enhanced by the introduction of selective media and the use of tube agglutination tests for confirmation of the SA result. IMS is restricted to the detection of certain serogroups, but this can be an advantage in certain studies. For example, IMS beads coated with antibodies to the LPS of *E. coli* O111 were used during a large Australian outbreak to detect VTEC O111 from contaminated food products linked to the outbreak (Paton *et al.* 1996). In contrast, the PCR/DNA probe method detects all VTEC serogroups, but is not as sensitive as IMS-SA.

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