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# Isolation of Vero cytotoxin-producing *Escherichia coli* O157 from wild birds

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J.S. WALLACE, T. CHEASTY AND K. JONES. 1997. In a survey of wild birds (mainly gulls), 0·9% of the bacterial isolates from faecal samples at an urban landfill site and 2·9% of bacterial isolates from faecal samples on intertidal sediments in Morecambe Bay were Vero cytotoxin-producing *Escherichia coli* O157. Isolation procedures employing commonly used cultural methods were hindered by the selection of a large number of false positives. The only procedure which resulted in the isolation of *E. coli* O157 from bird faecal samples was: enrichment (18 h) in a selective tryptone soya broth followed by filtration using hydrophobic grid membranes and growth on Chromagar® O157. The majority of isolates selected as potential *E. coli* O157 by characteristic growth on Chromagar® O157 could be eliminated by subsequent growth on CT-SMAC or CR-SMAC. This second identification (characterization) stage reduced the number of potential *E. coli* O157 requiring further confirmation by typing methods (serotype and Vero cytotoxin) by more than 70%.

## INTRODUCTION

Vero cytotoxin-producing *Escherichia coli* serotype O157 are recognized as an important cause of diarrhoea, haemorrhagic colitis and haemolytic uraemic syndrome in humans (Anon. 1995). Since its first recognition in 1982 (Riley *et al.* 1983) the number of confirmed cases of Vero cytotoxin-producing *E. coli* O157 in England and Wales has continued to escalate (Anon. 1996). Locally in South Cumbria, an area administered by the Morecambe Bay Health Authority, cases increased from two and three in 1993 and 1994 to 16 in 1995 (Griffiths, personal communication). Beef and dairy cattle have been established as the major reservoirs of *E. coli* O157 (Chapman *et al.* 1993; Hancock *et al.* 1994; Zhao 1995) with outbreaks of infection directly associated with the consumption of foods of bovine origin (Padhye and Doyle 1992; Synge *et al.* 1993; Willshaw *et al.* 1994). However, the distribution of *E. coli* O157 in the environment and the potential routes of transmission to cattle remain largely unknown.

We have investigated wild birds as vectors of *E. coli* O157 because birds have been implicated in the transmission of other bacterial pathogens, for example, *Campylobacter* spp. (Luechtefeld *et al.* 1980; Quessy and Messier 1992), *Salmonella* spp. (Jones *et al.* 1978; Quessy and Messier 1992), *Vibrio cholerae* (Ogg *et al.* 1989; Lee *et al.* 1982) and *Listeria* spp. (Fenlon 1985). The bacteria can be picked up as a result of the feeding habits of birds, e.g. from rubbish tips, sewage outfalls, consumption of contaminated shellfish and foraging on pasture land following the application of farm slurries and sewage sludge to land. The bacteria complete the cycle back to cattle by being deposited onto grazing land and into troughs and streams used by cattle.

The conventional cultural methods for the isolation of *E. coli* O157 continue to be improved (Chapman *et al.* 1991; Zadik *et al.* 1993; Hindle *et al.* 1995) and novel methods, such as immunomagnetic separation have been developed to improve the sensitivity of detection (Chapman *et al.* 1994; Mortlock 1994). However, the presence of colonies with morphology and growth characteristics similar to that of potential *E. coli* O157 continues to be a major hindrance. This problem is exacerbated in environmental samples when the size of the target population is low and that of the competing population high. This paper documents the isolation of *E. coli* O157 from the faeces of wild birds using cultural methods and validates a protocol, developed for the isolation of *E. coli* O157 from cattle faeces (Wallace and Jones 1996), for use with bird samples.

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## MATERIALS AND METHODS

### Sample collection

Freshly voided faecal samples were collected on four occasions from Morecambe Bay, Morecambe, UK, and a waste disposal landfill site, Lancashire Waste Services Ltd, Lancaster, UK.

Faecal samples from shore birds at Morecambe Bay were predominantly from herring gulls, *Larus argentatus*, black-headed gulls, *L. ridibundus*, common gull, *L. canus*, and lapwings, *Vanellus vanellus*.

The main birds on the landfill site were herring gulls, black-headed gulls and common gulls. A small number of crows, *Corvus corone corone*, and jackdaws, *corvus monedula*, were also present.

One hundred samples were collected from each site on each occasion and transported to the laboratory for immediate analysis. Representative 0·5 g aliquots were taken from each sample, amalgamated and tested for the presence of *E. coli* O157 as described by Wallace and Jones (1996).

### Media

*Enrichment broth.* mTSB-nov-Tryptone Soya Broth (Oxoid) supplemented with bile salts, dipotassium hydrogen orthophosphate and novobiocin (Sigma).

*Differential and selective agars.* Four types of selective agar were used for the isolation of *E. coli* O157 : Chromagar® O157 (Chromagar, Paris, France), SD-39 agar (QA Life Sciences, Inc.), CT-SMAC-Sorbitol MacConkey agar (SMAC) (Oxoid CM813) supplemented with cefixime (Cyanamid GB) and potassium tellurite (Sigma) and Cr-SMAC-SMAC supplemented with cefixime and rhamnose (Sigma).

Two further selective agars were used to help differentiate *E. coli* O157: LMG (Lactose Monensin Glucuronate agar; QA Life Sciences, Inc.) and BMA (buffered MUG agar; QA Life Sciences, Inc.).

### Direct isolation methods

Serial dilutions (1 : 10) of the amalgamated samples were prepared in 0·85% NaCl and 0·1 ml samples from each dilution spread across the surface of CT-SMAC and CR-SMAC plates which were incubated for 18 h at 37°C.

### Filtration method

Aliquots (1 ml) of appropriate serial dilutions were filtered individually through hydrophobic grid membrane filters (Iso-Grid®; QA Laboratories, Inc.) (HGMF) as described by Wallace and Jones (1996). Membranes were placed on to the surface of SD-39 and Chromagar® O157 and incubated, according to the manufacturer's instructions, at 44·5 and 37°C, respectively, for 25 h. This method provided a large number of distinguishable and separated colonies.

### Enrichment procedure

Fifty g of each amalgamated faecal sample was added to 500 ml of mTSB-nov and incubated at 37°C, without agitation, for 18 h. One ml aliquots were then removed and serial dilutions prepared in 0·85% NaCl. Samples from each were examined using the direct isolation methods above.

### Colony selection and characterization

A maximum of 10 colonies were selected from the agars used for isolation. Isolates were purified on nutrient agar (Oxoid CM3) and grown up on nutrient agar for 24 h at 37°C. Two to three colonies of each were transferred to 2 ml of sterile water and inoculated using a multipoint inoculator (Denley® A500) onto the six types of agar, i.e. the four selective/differential agars used in the original isolation methods (Chromagar® O157, CT-SMAC, CR-SMAC and SD-39) and the two additional agar (LMG agar and BMA). Selective and/or differential components of each of the six agars and the culture characteristics indicative of *E. coli* O157 are presented in Table 1.

Isolates which produced growth characteristic of *E. coli* O157 on four or more of the agars, were identified as presumptive *E. coli* O157 by testing for agglutination with an *E. coli* O157 latex kit (Oxoid DR620). Presumptive isolates were confirmed biochemically as *E. coli* by use of API 20E strips (BioMérieux).

All positive isolates were confirmed as *E. coli* O157, phage typed and tested for the presence of Vero cytotoxin genes VT1 and VT2 by the Laboratory of Enteric Pathogens, CPHL, using the methods described by Thomas *et al.* (1993).

## RESULTS

Three hundred and forty-nine isolates from birds from the landfill site and 342 from Morecambe Bay sediments were selected as potential *E. coli* O157 on the basis of characteristic growth on Chromagar® O157, CT-SMAC, CR-SMAC and SD-39 agar. The isolates were purified and further characterized by inoculation onto six agars (Chromagar® O157, CT-SMAC, CR-SMAC, SD-39 together with LMG agar and BMA). The results for this second selection stage are shown in Fig. 1. At least 85% of the isolates from the landfill site and the Bay were β-glucuronidase negative irrespective of the initial isolation media. A higher number of presumptive coliforms were isolated using Chromagar® O157 than with the other three solid media (Fig. 1a). The most discriminatory

**Table 1** Selective and differential components of agars producing culture characteristics indicative of *Escherichia coli* O157

| Solid media used | Selective and differential agents | Culture characteristic selected |
|---|---|---|
| CT-SMAC | Cefixime<br>Potassium tellurite<br>Sorbitol | Cream colour |
| CR-SMAC | Cefixime<br>Rhamnose<br>Sorbitol | Cream colour |
| SD-39 agar | X-gluc<br>Monensin<br>Phenol red | Pink colour |
| Chromagar | ? | Pink colour |
| BMA agar (buffered MUG agar) | MUG (4-methylumbelliferyl, β-D-glucuronide) | Non-fluorescent under 365 nm light |
| LMG agar (lactose monensin glucuronate agar) | Lactose<br>Monensin<br>Glucuronic acid<br>Aniline Blue | Blue colour presumptive for coliforms |

?, Information withheld as commercially sensitive.

of the agars used for isolate characterization following initial isolation on Chromagar® O157 were CT-SMAC and CR-SMAC reducing the number of false positives by more than 70% (Fig. 1a). Following initial isolation on SD-39 (Fig. 1b), CT-SMAC (Fig. 1c) and CR-SMAC (Fig. 1d), the most discriminatory agar was Chromagar® O157 reducing the number of potential false positives by more than 80% in all cases.

The percentage of isolates with one or more of the growth characteristics typical of *E. coli* O157 is shown in Fig. 2. From the landfill site 82·6% of cultures isolated by direct culture and 77·4% isolated following enrichment were positive for three of the characters (Fig. 2a). Corresponding figures for the isolates from Morecambe Bay sediments were 76·7% and 81·8% (Fig. 2b). None of the isolates selected following direct culture from either the landfill site or Morecambe Bay sediments possessed all six of the growth characteristics. In contrast following enrichment 4·0% of the isolates selected from the landfill site (Fig. 2a) and 5·9% of the samples selected from Morecambe Bay sediments (Fig. 2b) possessed all six.

All isolates which possessed four or more of the diagnostic characteristics (119 from the landfill site and 84 from the Bay) were tested by latex agglutination. All isolates with only four or five of the growth characteristics of *E. coli* O157 were eliminated by latex agglutination. Only 13 out of 17 isolates with all six of the growth characteristics were presumptively identified as *E. coli* O157 by latex agglutination. These presumptive isolates were later confirmed as *E. coli* O157. All the confirmed isolates were phage type 4 and all possessed the Vero cytotoxin genes VT1 and VT2.

Out of the 691 strains originally isolated as potential *E. coli* O157 using the criteria of characteristic growth on specific agars, only 13 (1·9%) were confirmed as *E. coli* O157. Three of the confirmed isolates came from birds at the landfill site and 10 from birds on Morecambe Bay sediments. *Escherichia coli* O157 was isolated from Morecambe Bay bird samples on three out of the four sampling occasions but from landfill site birds on only one occasion.

Of the methods assessed *E. coli* O157 was only isolated using the following procedure: enrichment in mTSB-nov for 18 h at 37°C, followed by filtering using HGMF and growth on Chromagar® 157. All of the strains initially isolated as potential *E. coli* O157 from CT-SMAC, CR-SMAC and SD-39 were false positives.

In total 99·1% of the isolates from birds at the landfill site and 97·1% of the isolates from birds from Morecambe Bay were false positives.

## DISCUSSION

The results show that a proportion of shore birds in Morecambe Bay and an urban landfill site in Lancaster carry *E. coli* O157. To the authors' knowledge this is the first report of the isolation of Vero cytotoxin-producing *E. coli* O157 from the faeces of wild birds.

As other workers have suggested, enrichment was found to be an essential step in the isolation of *E. coli* O157 (San-

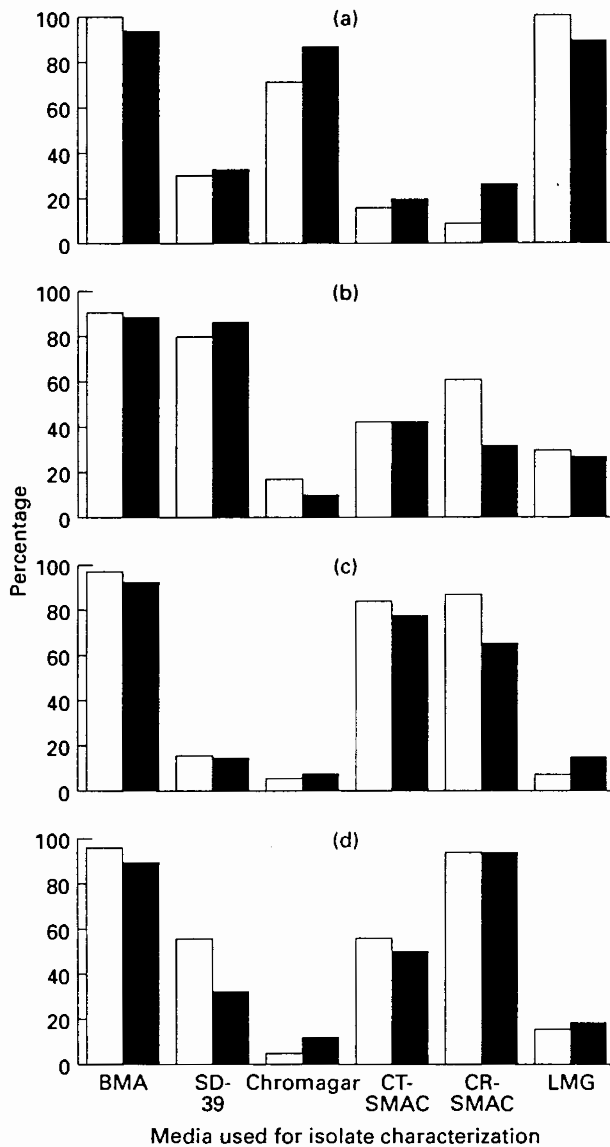


**Fig. 1** The percentage of strains isolated from avian faecal samples giving growth characteristics indicative of *Escherichia coli* O157. Strains were isolated before and after enrichment using four types of agar. (a) Chromagar® O157; (b) SD-39 agar; (c) CT-SMAC; (d) CR-SMAC. □, Landfill site; ■, Morecambe Bay

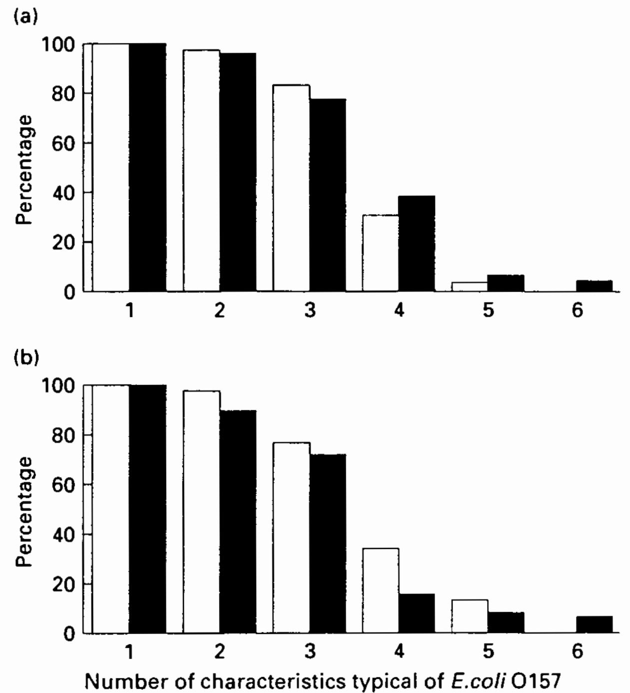


**Fig. 2** The percentage of bacterial isolates from bird faecal samples from (a) a landfill site (Lancaster, UK) and (b) intertidal sediments in Morecambe Bay (Morecambe, UK) with growth characteristics typical of *Escherichia coli* O157. □ Direct; ■, enriched

derson *et al.* 1995; Bolton *et al.* 1995; Zhao *et al.* 1995). In the present study the only procedure which resulted in the isolation of *E. coli* O157 from bird faecal samples was: enrichment in mTSB-nov for 18 h followed by filtration and growth on Chromagar® O157. Similar results were found by Wallace and Jones (1996) working with cattle faeces. None of the other selective and differential agars either with or without enrichment resulted in the isolation of *E. coli* O157.

The present study confirms the inability of selective and differential agars to be truly specific for the isolation of *E. coli* O157 in environmental samples where the numbers of the target organism are low in comparison to other bacteria. The same was found by Wallace and Jones (1996). The isolation of *E. coli* O157 from bird faeces was intermittent and only very low numbers of the bacteria isolated with growth characteristics indicative of *E. coli* O157 were confirmed as *E. coli* O157.

The use of an appropriate second agar during identification stages markedly reduced the number of isolates requiring further confirmation by serotyping and Vero cytotoxin typing. Testing for β-glucuronidase has been proposed as an aid in the identification of *E. coli* O157 (Thompson *et al.* 1990). In the present study this was found to be the least discriminatory of the agars used to differentiate between false positives and the target organism, as more than 90% of the isolates examined lacked β-glucuronidase activity. Growth on either CT-SMAC or CR-SMAC following initial isolation of strains on Chromagar® O157 after enrichment reduced the number of isolates requiring further confirmation by more than 70%.

Unlike other procedures which have been described for the detection of *E. coli* O157, for example, the immunoblot technique (Doyle and Schoeni 1987; Szabo *et al.* 1990),

immunoassay (Johnson *et al.* 1995; Dylla *et al.* 1995) and DNA probes (Gunzer *et al.* 1992), the methods described here are within the financial and technical scope of routine microbiology laboratories.

Although carriage appears to be low the potential for birds such as gulls to spread *E. coli* O157 to cattle and other animals is considerable. In Morecambe Bay there are tens of thousands of gulls (Wilson 1988) and the landfill site in Lancaster has a huge population of gulls. Many of the gulls roost on nearby farmland and fells and have been implicated in contaminating water sources with *Salmonella* spp., so much so that a culling programme was implemented (Jones *et al.* 1978).

This is the first report concerning the isolation of *E. coli* O157 from wild birds and is the first to implicate wild birds as potential vectors for the dissemination of *E. coli* O157 throughout the environment. Only one method was found to be useful in the isolation of *E. coli* O157 from avian faecal samples, i.e. enrichment in mTSB-nov followed by filtration and subsequent growth on Chromagar® O157. Of the isolates selected as potential *E. coli* O157, more than 70% could be eliminated by the use of CT-SMAC or CR-SMAC as a second identification (characterization) stage. However, typing (serotype and Vero cytotoxin) remain essential confirmatory stages in such studies before it can be concluded that isolates are Vero cytotoxin-producing *E. coli* O157.

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