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A 1-year study of *Escherichia coli* O157 in cattle, sheep, pigs and poultry

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SUMMARY

Samples of rectal faeces were collected immediately after slaughter from 400 cattle each month for a 1-year period and from 1000 each of sheep, pigs and poultry over the same period. Samples were examined for *Escherichia coli* O157 by enrichment culture in buffered peptone water with vancomycin, cefixime and cefsulodin followed by immunomagnetic separation and culture of magnetic particles onto cefixime tellurite sorbitol MacConkey agar. *E. coli* O157 was isolated from 752 (15·7%) of 4800 cattle, 22 (2·2%) of 1000 sheep and from 4 (0·4%) of 1000 pigs, but not from any of 1000 chickens. Of the cattle sampled, 1840 (38·4%) were prime beef animals, 1661 (34·6%) were dairy animals being culled and the status could not be determined for the other 1299 (27%) animals. *E. coli* O157 was found in 246 (13·4%) of the 1840 beef cattle and 268 (16·1%) of the 1661 dairy cattle. The monthly prevalence of *E. coli* O157 in cattle was 4·8–36·8% and was at its highest in spring and late summer. Seventeen of the 22 isolates from sheep were also made over the summer period. All *E. coli* O157 isolates from sheep and 749 (99·6%) of the 752 *E. coli* O157 isolates from cattle were verocytotoxigenic as determined by Vero cell assay and DNA hybridization, *eaeA* gene positive, contained a 92 kb plasmid and were thus typical of strains causing infections in man. In contrast isolates from pigs were non-toxigenic, *eaeA* gene negative and did not contain a 92 kb plasmid and would, therefore, be unlikely to be a source of infection for man.

INTRODUCTION

Verocytotoxin-producing (VT^+) *E. coli* (VTEC) cause haemorrhagic colitis (HC), the haemolytic-uraemic syndrome (HUS) and occasionally mild non-bloody diarrhoea in man, although some infections may be asymptomatic. In the UK, VT^+ *E. coli* O157, the most common serogroup associated with illness in man, has been isolated from cattle [1–4] and beef, beef products, milk and milk products have been identified as sources of human infection [2, 3, 5, 6]. A seasonal prevalence of *E. coli* O157 has been reported in dairy herds in the USA [7] and in one study of a dairy herd in the UK the seasonal prevalence was 1–11% [8]; it is not known whether there is a seasonal prevalence of *E. coli* O157 in cattle at slaughter. At present there is very limited information on the prevalence of *E. coli* O157 in food animals other than cattle.

The aims of this study were to examine samples of rectal faeces taken immediately after slaughter from sufficient cattle to determine any monthly variation in prevalence and from sufficient pigs, sheep and poultry to detect a prevalence rate as low as 0·25%.

MATERIALS AND METHODS

Sample collection

Swabs (Transwabs, Medical Wire Co.) of rectal faeces were taken from cattle, pigs, sheep and chickens

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immediately after slaughter, placed in transport medium supplied by the swab manufacturer, and were stored at 4 °C and transported to the laboratory the same day. All samples from cattle, sheep and pigs were collected at the same abattoir and all samples from chicken from the same poultry processing plant. Samples were collected from April 1995 to March 1996. Each month for a year, 400 samples were collected from cattle to allow detection of monthly variation in prevalence from 1% (95% confidence limits, 0·28–2·6%) to 5% (95% confidence limits, 3·1–7·6%). Ear tag numbers of cattle were recorded in case they were needed to trace *E. coli* O157-positive animals. Whether the cattle were prime beef animals or cull dairy cows was also recorded whenever possible. One thousand samples were collected evenly over the same 1-year period from each of pigs, sheep and poultry; this number was insufficient to detect monthly variation in prevalence within statistically acceptable confidence limits, but would detect a minimum prevalence rate of 0·25% (95% confidence 0·01–0·25%). Samples were collected on 4 days per week (Monday to Thursday) as examination of recent records at the abattoir suggested that this would give the widest possible geographical source of animals.

Isolation of *E. coli* O157

E. coli O157 was isolated by an immunomagnetic separation technique [9, 10] and culture of magnetic beads on to cefixime tellurite sorbitol MacConkey agar [11]. Swabs were placed in 5 ml of buffered peptone water (Oxoid – CM509) supplemented with vancomycin 8 mg l^{-1}, cefixime 0·05 mg l^{-1}, and cefsulodin 10 mg l^{-1} (BPW–VCC) [2] and faecal material was suspended in the medium by vigorous vortex mixing for 20–30 sec. Suspensions were incubated at 37 °C for 6 h and 1 ml of broth was added to 20 μl 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, mixed, separated in a magnetic particle concentrator (MPC-10, Dynal, Oslo) and washed as described previously [9]. After the final wash and separation the beads were resuspended in *c.* 25 μl of nutrient broth, inoculated on to CT-SMAC medium and incubated overnight at 37 °C. Colonies not fermenting sorbitol from CT-SMAC were tested for agglutination with a latex test kit (Oxoid – DR622) for detecting *E. coli* O157. Isolates that gave positive results were confirmed as *E. coli* by biochemical tests and as serogroup O157 or serotype O157:H7 by agglutination to titre with antiserum to *E. coli* O157 (Laboratory for Microbiological Reagents, Central Public Health Laboratory, Colindale, London) [1] or antiserum to *E. coli* H7 (Difco) [8].

Characterization of isolates

Verocytotoxin production

Verocytotoxigenicity was determined by Vero cell culture assay [1] and toxin type by specific hybridization with DNA probes for the VT_1 and VT_2 genes. Presence of the *eaeA* gene was also determined by DNA hybridization. From published sequence data [12, 13] DNA probes specific for the A cistrons of the VT_1 and VT_2 genes, and for the *eaeA* gene, were prepared and labelled with digoxigenin-11-dUTP by the polymerase chain reaction and used in colony hybridization reactions [2, 14]. Known VT_1^+, VT_2^+, VT^-, $eaeA^+$ and $eaeA^-$ strains were included as controls in each batch of tests.

Plasmid analysis

Plasmids were extracted by an alkaline detergent method [15], 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) carrying plasmids of 148, 63·4, 36 and 6·9 kb was included with each batch of tests so the size (kb) of the plasmid could be estimated.

Phage typing

All *E. coli* O157 isolates were phage typed by the Laboratory for Enteric Pathogens, Central Public Health Laboratory, 61 Colindale Avenue, London.

RESULTS

Of the 4800 cattle sampled, 1840 (38·4%) were prime beef animals, 1661 (34·6%) were cull dairy animals and the status could not be determined for the other 1299 (27%) animals. *E. coli* O157 was found in 246 (13·4%) of the 1840 beef cattle and 268 (16·1%) of the 1661 dairy cattle. Overall, *E. coli* O157 was isolated from 752 (15·7%) of the 4800 cattle with a monthly

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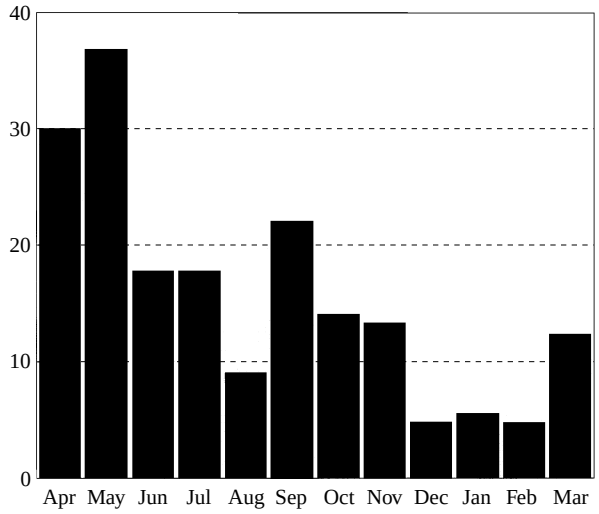

Fig. 1. Seasonal distributon of *E. coli* O157 isolates from cattle, 1995–6. ■, % isolation.

Table 1. *Phage types of 752 isolates of* E. coli *O157 from cattle*

Phage type	*n*	(%)
2	237	(31·6)
4	137	(18·2)
34	85	(11·3)
49	56	(7·4)
RDNC*	48	(6·4)
14	45	(6·0)
8	44	(5·9)
32	36	(4·8)
1	20	(2·7)
21	16	(2·1)
39	13	(1·7)
31	6	(0·8)
54	5	(0·7)
23	1	(0·1)
47	1	(0·1)
51	1	(0·1)
72	1	(0·1)

* RDNC, reacted with the phage set but did not conform to a recognized phage type.

prevalence which varied from 4·8–36·8% (Fig. 1). On initial testing, 604 (86·3%) of these isolates were serotype O157:H7 and the remaining 148 (19·7%) were non-motile; on repeat testing, 23 (3·8%) of 604 isolates previously recorded as H7 were non-motile. Isolates belonged to 17 different phage types (Table 1), with almost half the isolates being phage type 2 ($n = 237$, 32%) or phage type 4 ($n = 137$, 18%). Differences in seasonality were not observed between the main phage types. In tests for verocytotoxigenicity, 573 (76·2%) of isolates produced VT_2 only, 172 (22·9%) produced both VT_1 and VT_2, 4 (0·5%) produced VT_1 only, and 3 (0·4%) were non-verocytotoxigenic; all these isolates were positive for the *eaeA* gene (Table 2). The three non-verocytotoxigenic isolates were biochemically typical of *E. coli* O157. There was complete agreement between the vero cell assay for toxin and DNA probing for the presence of toxin genes. All isolates harboured the large 92 kb plasmid and in 367 (48·8%) isolates this was the only plasmid present (Table 3). The remaining 385 (51·2%)

Table 2. *Toxin genotype of 752 isolates of* E. coli *O157 from cattle*

VCA	VT_1	VT_2	*eaeA*	*n*	%
+	−	+	+	573	76·2
+	+	+	+	172	22·9
+	+	−	+	4	0·5
−	−	−	+	3	0·4

VCA, vero cell assay for verocytotoxin; VT_1, VT_2 and *eaeA* determined by DNA hybridization.

Table 3. *Plasmid profiles of 752 isolates of* E. coli *O157 from cattle*

Plasmid profile	*n*	%
92 kb only	367	48·8
92 kb + one smaller	310	41·2
92 kb + two smaller	65	8·7
92 kb + three smaller	10	1·3

kb, plasmid size in kilobase pairs.

Table 4. *Plasmid profile, toxin genotype and prevalence of* eaeA *gene within 45 strains of* E. coli *O157 isolated from cattle and belonging to phage type 14*

Plasmid profile (kb)	VCA	VT_1	VT_2	*eaeA*	*n*
92	+	−	+	+	11
	+	+	+	+	3
	−	−	−	+	2
92, 63	+	−	+	+	18
	−	−	−	+	1
92, 6·9	+	−	+	+	6
	+	+	+	+	2
92, 63, 2	+	−	+	+	1
92, 8·3, 6·9	+	+	−	+	1

kb, plasmid size in kilobase pairs; VCA, vero cell assay for verocytotoxin; VT_1, VT_2 and *eaeA* determined by DNA hybridization.

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Table 5. *Characteristics of* (*a*) *22 isolates of* E. coli *O157 from 1000 samples of sheep rectal faeces and of* (*b*) *4 isolates from 1000 samples of pig rectal faeces*

Phage type	Plasmid profile	VCA	VT_1	VT_2	*eaeA*	*n*
(*a*) Sheep isolates						
2	92	+	−	+	+	5
4	92	+	−	+	+	1
4	92, 63	+	−	+	+	4
8	92	+	+	+	+	2
14	92	+	−	+	+	2
14	92, 63	+	−	+	+	1
31	92	+	−	+	+	1
54	92	+	−	+	+	1
82	92, 6·3	+	−	+	+	1
RDNC	92, 63	+	−	+	+	3
RDNC	92, 2	+	−	+	+	1
(*b*) Pig isolates						
31	104, 10, 6·3, 2	−	−	−	−	3
RDNC	104	−	−	−	−	1

kb, plasmid size in kilobase pairs; VCA, vero cell assay for verocytotoxin; VT_1, VT_2 and *eaeA* determined by DNA hybridization.

isolates gave 38 different plasmid profiles (Table 3). Based on a combination of phage type, plasmid profile, toxin genotype and presence of the *eaeA* gene, the *E. coli* O157 isolates from cattle belonged to 99 subtypes. Space does not permit showing all data but an example of the variation found within a single phage type (14) is shown in Table 4. Fourteen (1·9%) isolates were urease positive, one did not ferment lactose, and 737 (98%) were biochemically typical of *E. coli* O157 [1].

E. coli O157 was isolated from 22 (2·2%) of 1000 sheep and from 4 (0·4%) of 1000 pigs. All the isolates from sheep were biochemically typical [1] and appeared similar to isolates from cattle and previously from man (Table 5). The isolates from pigs were non-verocytotoxigenic, *eaeA* gene negative and did not contain the 92 kb plasmid (Table 5); three of the isolates were non-motile and one was motile but not H7. *E. coli* O157 was not isolated from 1000 chickens.

DISCUSSION

The overall prevalence of *E. coli* O157 in cattle, in this study 15·7%, is far higher than reported in previous studies from the UK and USA [1, 2, 4, 7]. There may be several reasons for this. There may have been a genuine increase in prevalence of *E. coli* O157 in the cattle population, a hypothesis possibly supported by the rising incidence of *E. coli* O157 infections in man in the UK and USA over the same period. The season during which studies were conducted may also have had an effect on prevalence figures. However, it is also likely that the higher isolation rates achieved could have been a result of the isolation method used: enrichment in BPW–VCC followed by immunomagnetic separation of *E. coli* O157 and culture to CT–SMAC markedly increases the isolation of *E. coli* O157, when compared with direct culture, from bovine and human faecal samples and from food [9, 10, 16]. In a study of a dairy herd in 1993–4 [8, 9], *E. coli* O157 was isolated from 23 of 1024 bovine rectal swabs by direct culture, but was isolated from 84 of these animals by IMS; this fourfold increase in sensitivity could largely explain the increase from the 4% prevalence, as detected by direct culture, in cattle at slaughter in 1992 [2] to the 15·7% prevalence reported from the same abattoir in the present study.

E. coli O157 was found in a slightly higher percentage of dairy cattle (16·1%) than beef cattle (13·4%), in contrast to findings in Washington State, USA [7]. There was marked month-to-month variation in the prevalence of *E. coli* O157 in cattle, with rates being highest in spring and late summer. We have previously reported a seasonal prevalence of faecal excretion of *E. coli* O157 in spring in a Sheffield dairy herd [8] and Hancock and colleagues [7] reported their highest prevalence in dairy herds in late summer. During the period of this study, clusters of human cases of *E. coli* O157 infections were observed in May–June, as in previous years [1, 2, 3, 15], and also in September (Sheffield PHL, unpublished data).

The majority of the isolates were biochemically typical, verocytotoxigenic, harboured the 92 kb plasmid and were *eaeA* gene positive and were therefore typical of strains causing infection in man. Phage typing and plasmid analysis have both been used previously for typing strains of *E. coli* O157 isolated in epidemiological studies [15, 17–19]. In this study, both had drawbacks. Although isolates belonged to 17 phage types, 2 types (2 and 4) accounted for almost half of the isolates. In studies of human isolates from England and Wales during 1992–4, Thomas and colleagues [20] also reported that although isolates belonged to 19 phage types, 84% of isolates belonged to only 5 phage types. Others have suggested that phage typing is of very limited value due to this lack of discriminatory power [21]. Similarly, with plasmid analysis, there were 39 different plasmid profiles but almost half of the isolates had only the single 92 kb

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plasmid, thus lessening the value of this technique used alone for typing *E. coli* O157. With a combination of phage type, plasmid profile, toxin genotype and presence of the *eaeA* gene, it was possible to subdivide isolates into 99 subtypes. Thus a combination of typing techniques may provide better discrimination between isolates than any of the techniques used singly, as has been suggested in studies of human and bovine strains of *E. coli* O157 isolated in the Sheffield area during 1987–93 [22]. Inclusion of the H type in this may have increased discrimination between strains yet further, but as the H type appeared to be a less stable character, with 23 isolates apparently changing from H7 to non-motile on repeat testing, this was not included in the subtyping scheme in this study.

E. coli O157 was also isolated from 2·2% of 1000 sheep. Although insufficient numbers were isolated to determine any variation in seasonal prevalence, 17 were isolated in the period June–September. Since the commencement of this study the isolation of *E. coli* O157 from sheep in Idaho has also been reported and in this study all were isolated in the summer months [23]. Isolates from sheep in our study and in Idaho appear typical of those causing infections in man and further studies of the prevalence of *E. coli* O157 in sheep are needed.

Strains of *E. coli* O157 which may or may not ferment sorbitol and which may be enterotoxigenic but not verocytotoxigenic have been isolated frequently from pigs [24, 25]. However 4 out of 6 such strains, kindly provided by Dr Clifford Wray, Central Veterinary Laboratory, Weybridge, failed to grow on the CT–SMAC medium used in this study, probably due to their susceptibility to the concentration of tellurite used; of the two strains which did grow on CT–SMAC, one fermented sorbitol (Sheffield PHL, unpublished data). The four isolates from pigs in this study are therefore probably atypical, but nevertheless were non-verocytotoxigenic, did not have the 92 kb plasmid and were *eaeA* gene negative.

E. coli O157 was not isolated from 1000 chickens, indicating a prevalence of $< 0{\cdot}25\%$. Two case-control studies [26, 27] have statistically indicated poultry meat as a possible risk factor for infection by *E. coli* O157, although neither was confirmed microbiologically. In feeding studies performed on young chickens, three groups in the USA [28–30] have shown that even very low inocula of *E. coli* O157 lead to rapid colonization of the caecum and excretion of large numbers of the organism in the faeces for up to 3 months. A possible criticism of these experimental colonization studies is that all three groups used the same highly atypical strain of *E. coli* O157 which was resistant to nalidixic acid 200 mg/l. Whilst the use of such a strain may facilitate subsequent laboratory detection of the organism, the results obtained should be interpreted with caution and further studies with typical strains of *E. coli* O157 are probably needed. However, despite the limited experimental evidence suggesting that chickens may act as a reservoir of the organism, there have not been, to date, any microbiologically confirmed cases of *E. coli* O157 infection from a poultry source.

This study indicates that cattle remain a major reservoir of *E. coli* O157 and that the seasonal prevalence observed in cattle is similar to that observed in human infections in our area. It is not possible to determine whether the prevalence may have increased over recent years. *E. coli* O157 was also isolated from sheep and has also been isolated from lamb and lamb products [31]; although lamb and lamb products have not been documented as causes of infection in man, they should not be overlooked as possible vehicles of infection.

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