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Concentration and Prevalence of *Escherichia coli* O157 in Cattle Feces at Slaughter

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The concentration and prevalence of *Escherichia coli* O157 in cattle feces at the time of slaughter was studied over a 9-week period from May to July 2002. Fecal samples ($n = 589$) were collected from the rectums of slaughtered cattle, and the animal-level prevalence rate was estimated to be 7.5% (95% confidence interval [CI], 5.4 to 9.6%) while the group prevalence was 40.4% (95% CI, 27.7 to 53.2%). Of the 44 infected animals detected, 9% were high shedders that contained *E. coli* O157 at concentrations of $>10^4$ CFU g^{-1}. These 9% represented >96% of the total *E. coli* O157 produced by all animals tested. All isolates possessed the vt_2 gene, 39 had the *eaeA* gene, and a further five had the vt_1 gene also. The presence of high-shedding animals at the abattoir increases the potential risk of meat contamination during the slaughtering process and stresses the need for correctly implemented hazard analysis and critical control point procedures.

Escherichia coli O157 was first identified as a food-borne pathogen in 1982 during an outbreak that was traced to contaminated hamburgers (20). The pathogen is associated with a range of symptoms, including watery or bloody diarrhea, vomiting, hemorrhagic colitis, and hemolytic uremic syndrome, which is characterized by acute renal failure affecting mainly children and the immunocompromised (7). While the majority of foods linked to human outbreaks of *E. coli* O157 are not assessed quantitatively, some studies have indicated a low infective dose (1, 26), highlighting the need for stringent control of contamination during food production.

Cattle and other ruminants have been established as major natural reservoirs for *E. coli* O157 (18) and play a significant role in the epidemiology of human infections (7). It has been estimated that 1 to 4% of United Kingdom cattle are infected at slaughter (3, 19), although more recently a prevalence rate of 8.6% has been reported from a farm study in Scotland (25). In the United States, breeding herd prevalences of 1% (21) and 9.3% (6) have been recorded, whereas in feedlot animals, rates have varied between 2.8% (4) and 35.8% (5). Prevalences in the summer months were usually greater than in the winter months. A number of environmental and food-borne sources have caused *E. coli* O157 incidents, with many attributed to the consumption of food of bovine origin (22) or with either direct or indirect contact with cattle and other farm animals (13).

The concentration at which *E. coli* O157 is shed in feces varies from animal to animal as demonstrated in a North American study with calves (29), where a range from 10^2 to 10^5 CFU g^{-1} was observed. High-shedding sheep (excreting $>10^4$ CFU g^{-1}) were responsible for the New Deer *E. coli* O157 outbreak in Scotland (16, 23). High-shedding animals pose an elevated risk of contaminating the food chain if presented to slaughter. However, little published data are available on the concentration of *E. coli* O157 in cattle feces at the time of slaughter.

The health risk from *E. coli* O157 and other pathogens is minimized by abattoir carcass inspection for visible signs of fecal contamination supplemented with appropriate hazard analysis and critical control point systems. Quantitative microbiological risk assessments have been developed for ground beef (2) and direct contact environmental transfer (24) pathways, but these need to be parameterized with prevalence and concentration data. The aim of this study was to determine the individual and group animal prevalence together with the concentration of *E. coli* O157 in cattle at the time of slaughter. The proportion of high-shedding individuals was also investigated.

MATERIALS AND METHODS

Sample collection. Weekly samples were collected from a local abattoir. Approximately 75 g of fecal material was taken from the rectum of each animal after disembowelment. This was done on the process line of the factory by rectum retrieval and manually milking of the fecal contents. Samples were collected in sterile plastic bags, stored in a cool box, and transported to the laboratory within 3 h. All samples were stored at 4°C and processed within 48 h of collection. Cross-contamination was minimized by washing (with water at approximately 50°C) the processing bench and by the use of sterile gloves changed for each animal.

Isolation of *E. coli* O157. Samples were analyzed by enrichment followed by immunomagnetic separation (IMS) (15). Each fecal sample (25 g) was homogenized with 225 ml of buffered peptone water (Oxoid CM509) supplemented with 8 mg of vancomycin $liter^{-1}$ and incubated at 42°C for 6 h. To determine the presence or absence of *E. coli* O157, 1 ml of the enriched sample was analyzed by IMS (KingFisher mL; Thermo Life Sciences, Basingstoke, United Kingdom) with 0.02 ml of Captivate *E. coli* O157 immunomagnetic beads (International Diagnostic Group [IDG], Bury, United Kingdom). After IMS, the beads were washed three times (in buffered saline [PBS] plus Tween 20), resuspended in 0.1 ml of the same buffer, spread equally on two sorbitol MacConkey agar plates (SMAC; Oxoid CM813) supplemented with cefixime (0.05 mg $liter^{-1}$) and potassium telluride (2.5 mg $liter^{-1}$) (28) (CT-SMAC; Mast Diagnostics, Merseyside, United Kingdom), and incubated at 37°C for 18 to 24 h. Presumptive *E. coli* O157 colonies (non-sorbitol fermenting) were confirmed by agglutination with a latex test kit (Oxoid DR620). Positive isolates were further confirmed biochemically, by the production of indole from tryptone water at 44°C, and genotypically (see below). The remainder of each fecal specimen was stored at 4°C for further analysis.

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Detection limit of IMS technique. Fecal samples found absent from *E. coli* O157 by IMS analysis (see above) were collected and stored at 4°C. A cocktail (5 laboratory strains from cattle, numbers 74, 96, 99, 177, and 308) of *E. coli* O157 was prepared in nutrient broth to contain approximately 10^8 CFU ml^{-1}. Dilutions (10^{-1} to 10^{-10}) of the cocktail in PBS were spiked (0.1 ml) into 25-g portions of the cattle feces in triplicate. IMS assays were performed in triplicate on these samples as described above.

Enumeration of *E. coli* O157. The enumeration of IMS-positive *E. coli* O157 fecal samples was attempted by serially diluting (10^{-1} to 10^{-4}) a further 25 g of feces with PBS. From each dilution, 0.1 ml was spread on duplicate Harlequin SMAC 5-bromo-4-chloro-3-indolyl-β-D-glucuronide (BCIG) (Lab M; IDG) supplemented with cefixime and telluride (Harlequin CT-BCIG) and CT-SMAC. Plates were incubated at 37°C for 18 to 24 h, and presumptive colonies (five were randomly selected when >5 were present on the plate) were confirmed to be *E. coli* O157 biochemically and by latex agglutination, as described above, and enumerated manually.

Calibration of enumeration technique. Experiments were performed to calibrate the direct plate count technique by using the same spiked samples as described above in the section "Detection limit of IMS technique." Each dilution (0.1 ml) was spread onto duplicate CT-SMAC plates and incubated at 37°C for 18 to 24 h, and the number of target colonies was counted (colonies confirmed to be *E. coli* O157 as described above). The whole calibration was performed in triplicate with cocktails comprised of the five *E. coli* O157 strains described above.

Identification of virulence markers. The detection of virulence markers (vt_1, vt_2, and *eaeA* genes) in the positive isolates was determined by PCR (12). The amplification products were separated on a 2% agarose gel in 0.5 M Tris-borate-EDTA buffer and visualized under UV by using a 100-bp ladder as a standard (Amersham Biosciences, Little Chalfont, Bucks, United Kingdom). The expected product sizes were as follows: vt_1, 282 bp; vt_2, 164 bp; and *eaeA*, 410 bp.

Detection limit of IMS technique. We assumed that the *E. coli* O157 organisms were Poisson distributed in the samples, and therefore, an exponential model (8) was used to describe the IMS data. Hence, the probability (π_i) of an IMS sample testing positive can be expressed as:

$$\pi_i = 1 - e^{-rC}$$

where C is the concentration of organisms in the sample and r is a constant. The maximum-likelihood estimate was used to fit the exponential to these data (12). The maximum-likelihood estimate method involves minimizing the deviance (Y) by sampling different values of r:

$$\min Y = -2\sum_{i=1}^{k}\left\{P_1\left[\ln\left(\frac{\pi_i}{\pi_i^0}\right) + (T_i - P_i)\ln\left(\frac{1-\pi_i}{1-\pi_i^0}\right)\right]\right\}$$

where π_i is the predicted probability of an IMS sample being positive estimated from the exponential model and π_i^0 is the observed proportion of samples positive at a particular concentration. P_i and T_i are the number of positive and total samples tested at each concentration. The Microsoft Excel solver function was employed to perform this task. The value of minimized deviance was compared to a χ^2 distribution with $k - m$ degrees of freedom (k is the number of samples tested and m is the number of parameters in the exponential model −1). The fit acceptability is rejected if the deviance is in excess of the 5th percentile of the distribution (8). The detection limit of the assay was defined when the sensitivity was 95%, i.e., the 95% probability that a sample will test positive given that it contains at least one *E. coli* O157 organism (27). This definition was chosen because a method can be claimed, for example, to detect a single organism in a sample, but if it finds this organism in a small proportion of the assays then the test is not reliable at this limit of detection.

Prevalence data. Microsoft Excel was used to determine the 95% binomial confidence intervals of the prevalence of fecal carriage of *E. coli* O157 at the individual and group level of the infected animals for all cattle at slaughter and all finishing groups of animals in Scotland, respectively.

Calibration of enumeration technique. The calibration of the enumeration technique was performed with Microsoft Excel by regressing the log actual *E. coli* O157 plate count against the log expected plate count.

RESULTS

Sample collection. Cattle (n = 721) were examined in the study over a period of 9 visits to the abattoir (May to July 2002), and of these, 18% were found to have rectums devoid of fecal material and, hence, no sample could be obtained. The 589 fecal samples tested represented cattle from five of the six Animal Health Divisions in Scotland and from 56 individual farms.

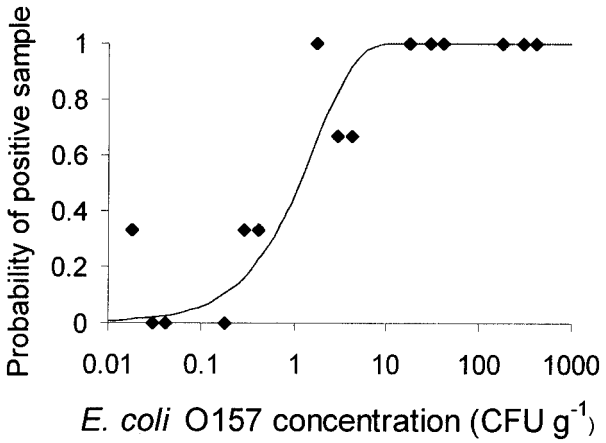

FIG. 1. Exponential fit of the IMS method applied to fecal samples.

Detection limit of IMS method. Fig. 1 shows the best fit of the exponential model to the experimental data. The best fit value of r (0.596) occurred at a deviance of 11.4. This fit is acceptable, being less than the critical chi-squared value for 15 − 1 = 14 degrees of freedom (χ^2 = 23.7). The limit of detection (when 95% of the spiked samples at a given concentration are detected as positive) occurred at a concentration of 5 CFU g^{-1}. With the assumption that the organisms are Poisson distributed, there is a negligible probability (≪0.1%) of a 25-g sample at this concentration not having at least one organism contained within it.

Prevalence of *E. coli* O157. The prevalence of *E. coli* O157 carriage in the feces of individual animals tested was 7.5% (95% confidence interval [CI], 5.4 to 9.6%). These consisted of 25% young bulls, 29.5% heifers, and 45.5% steers. The prevalence of farms having at least one positive animal in the group sent for slaughter was 40.4% (23 of 57) (95% CI, 27.7 to 53.2%).

Calibration of enumeration technique. The calibration of the enumeration technique (Fig. 2) gave a highly significant (R^2 = 0.9173 and $P < 0.001$) linear log-log relationship for the recovery of *E. coli* O157. The slope (0.9 ± 0.1) was not statistically significantly ($P > 0.05$) different from unity and the x-axis intercept (2.26, equivalent to 180 organisms), demonstrating that the technique has a limit of detection of *E. coli* O157 from feces of approximately 10^2 CFU g^{-1}.

Enumeration of *E. coli* O157. The concentration of *E. coli* O157 in cattle feces varied from $<10^2$ to 10^5 CFU g^{-1} (Table 1). The proportion of cattle which were low shedders ($<10^2$ CFU g^{-1}) was 61%, the majority of which were not enumerated by the direct plating method because they were below the detection threshold. High shedders totaled 4 of 44 (9%) of the infected cattle and carried >96% of the total *E. coli* O157 contained within all animals tested. There was no significant difference between the counts of target colonies on CT-SMAC and Harlequin CT-BCIG (data not presented). The ease of

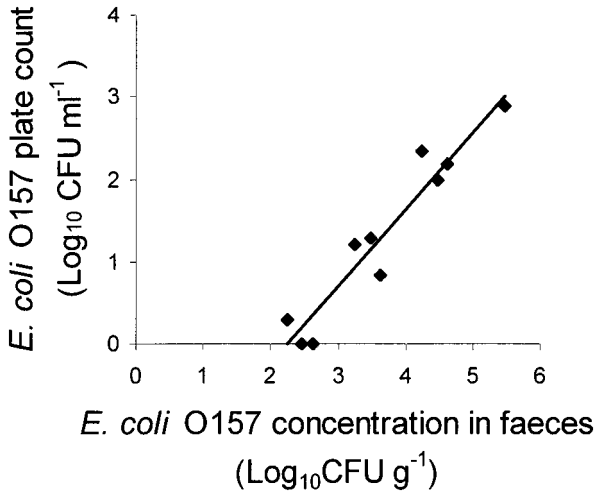

FIG. 2. Calibration of the direct plating enumeration method for the recovery of *E. coli* O157 in fecal samples.

target colony identification varied between samples on the two selective agars for reasons which were unclear.

Virulence markers. Isolates from all 44 *E. coli* O157-positive specimens contained the vt_2 gene, and 39 (89%) had the intimin-encoding *eaeA* gene. Five samples (11%), including three from animals at the same farm slaughtered on the same day, also carried the vt_1 gene.

DISCUSSION

The prevalence rate of individual animals tested here (7.5%) is similar, within the statistical variation, to that of a previous farm-based study in Scotland (25) which gave a value of 8.6%. However, a 12-month abattoir-based study (17) in the United Kingdom showed a lower prevalence of 4.7%. The group prevalence of 40.4% in the study reported here represents the batch of animals sent from a farm to the abattoir and may not be a true prevalence rate for the entire herd. The United Kingdom abattoir study (17) observed a similar group prevalence of 44%. However, the Scottish farm-based study (25) covered a period of 2 years and showed a group-level prevalence of 23.7% (95% CI, 21.0 to 26.5%), which is significantly lower than that reported here. The reasons for this difference may be because the present study was performed in the summer months, which is defined as the period of high prevalence by Hancock et al. (9), because the animals may have shed increasing loads of *E. coli* O157 from stress due to transport prior to slaughter (14), or because larger sample volumes were assayed in the present project (25 g compared to 1 g).

The majority of *E. coli* O157 organisms isolated were potentially pathogenic to humans, with all of them having the verotoxin gene vt_2 and 89% having the attaching and effacing gene *eaeA*. Most (89%) were vt_1 negative and vt_2 positive, which is comparable to the ratios of clinical *E. coli* O157 isolates in Scotland, where in 2002, 81% (Scottish *E. coli* O157 Reference Laboratory, personal communication) have been vt_1 negative and vt_2 positive. This is further evidence that cattle are a source of human *E. coli* O157 infections.

The detection limit of the method used to estimate the prevalence of *E. coli* O157 in feces influences the isolation rate. This study demonstrated for the IMS technique that there was a 95% likelihood of detecting an animal shedding 5 CFU g^{-1} and a 5% likelihood of detecting an animal shedding 0.09 CFU g^{-1}. The direct plating technique demonstrated a linear relationship (Fig. 2) which was used for enumerating the shedding concentrations of animals excreting $>10^2$ CFU g^{-1}. However, one problem associated with the direct plating technique was that some positive samples with a relatively low concentration of *E. coli* O157 may have been underestimated in samples with a high background flora, which makes target recognition difficult. Biochemical characterization of selected nontarget colonies identified *Aeromonas hydrophila* which suggests further method improvement to eradicate such organisms. No correlation was observed between numbers of *E. coli* O157 and background flora.

TABLE 1. Range of concentrations of *E. coli* O157 in abattoir cattle fecal samples

E. coli CFU g^{-1}	No. of cattle
$<10^2$	27
10^2–10^3	6
10^3–10^4	7
10^4–10^5	2
10^5–10^6	2
Total	44

Risk factors which have been strongly associated with human infection include the likelihood of contact with farm animals or their feces (13) and the consumption of ground beef (22). The abattoir is a major link in the transmission of *E. coli* O157 to the food chain, and cross-contamination of the carcass with feces (19) and the return of waste to the fields (10, 11) are a major concern. This study determined that 1 in 11 cattle positive for *E. coli* O157 in the abattoir is potentially a high-shedding animal which may produce over 96% of the total *E. coli* O157 shed in feces by all the infected animals slaughtered at a particular time period.

This implies that the high concentration being shed by a few of the infected animals may be of much greater importance than the prevalence rate.

The differences in the concentration of *E. coli* O157 shed in the feces of infected animals within the same group varied, and the reason for this could not be identified during the course of this work. Further work is required to understand why a range of concentrations exist. The presence of high concentrations of pathogens in some animals at the time of slaughter highlights the need for risk mitigation strategies to screen for high-shedding animals prior to slaughter (2). However, there are practical issues which must be addressed here associated with time, place, and cost of screening as well as availability of facilities to enable safe depuration of high-shedding animals.

This study has established the presence of high-shedding animals at abattoirs, providing an increased risk of contamination to both the food chain and the environment. The need for suitable control measures for such animals cannot be underestimated, and future research is needed to devise mitigation strategies that will reduce the risk of gross or major contamination of the food chain or the environment.

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