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Studies of the presence of verocytotoxic *Escherichia coli* O157 in bovine faeces submitted for diagnostic purposes in England and Wales and on beef carcases in abattoirs in the United Kingdom

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SUMMARY

A survey of beef carcases in abattoirs in the UK was carried out in order to estimate the prevalence of contamination with verocytotoxin – producing *Escherichia coli* (VTEC) serogroup O157. Contamination with verocytotoxin-producing *E. coli* (VTEC) O157 was confirmed in 0·47% of the 4067 (95% confidence limits 0·22–1·00%) of neck muscle samples. A significant tendency for carcases present in the same abattoir on the same day to have similar results was found, thus suggesting cross contamination.

VTEC O157 was found in 0·83% of 6495 bovine faeces samples routinely submitted for diagnostic purposes to Veterinary Investigation Centres in England and Wales. Of the samples from cattle less than 6 months old, 3·7% of 68 samples from animals without gastrointestinal disease were positive for *E. coli* O157, in contrast to 0·75% of 2321 samples from cases of gastrointestinal disease. No association with season or herd type (beef or dairy) was found.

INTRODUCTION

Verocytotoxin-producing *E. coli* (VTEC) have been recognized increasingly as a cause of haemorrhagic colitis and the life threatening haemolytic uraemic syndrome (HUS) in man [1]. Serotype O157:H7 is the predominant cause of human infection. In the USA and Canada (where VTEC is regarded as the commonest cause of HUS) outbreaks have often been linked to the consumption of undercooked minced beef, and cattle are widely held to be a reservoir of infection. In the UK it has been shown that the organism occurs in cattle without causing clinical disease [2] and that products of bovine origin such as unpasteurized milk and post-pasteurization failure [3] and minced beef [4] have acted as sources of human infection.

Two studies were carried out under the auspices of the former Steering Group on the Microbiological Safety of Food (SGMSF) in order to assess the extent to which this organism can be detected in the faeces of cattle and on their carcases after slaughter and to provide some information to contribute to the understanding of the epidemiology of the infection in both animals and humans.

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MATERIALS AND METHODS

Study design (faeces study)

During the 13-month period from June 1994 to June 1995, as many as conveniently possible of all bovine faeces submitted to MAFF Veterinary Investigation Centres (VICs) in England and Wales for whatever reason were examined for the presence of VTEC O157. Most samples were submitted from cases of gastrointestinal disease. Where it was available, the following information was recorded for each group of samples examined; county of origin, herd type (dairy, beef, other), age of animals (0–6, 6–12, 12–24 and over

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24 months) and the reason for the submission of material to the VIC (clinical gastrointestinal disease, other clinical disease, and other reason). In what follows, a group of one or more samples submitted together is referred to as a submission. To preserve confidentiality samples were examined blind so that results could not be attributed to individual farms or veterinary practices.

Study design (abattoir study)

A list of the 445 abattoirs slaughtering cattle in the UK was obtained together with their latest recorded annual throughput. A total of 900 visits were assigned with each abattoir selected at a rate proportional to its throughput, so that larger ones were assigned more than one visit while smaller ones had a proportional chance of a single visit. Five samples were to be selected at each visit so that 4500 samples in total were expected. The selected abattoirs were asked if they wished to cooperate in the survey, and where any declined or their circumstances had changed replacements, where possible, were selected from the list. Each visit had a month specified between September 1994 and August 1995, so that the total number of visits each month was even and also so that repeat visits to the same abattoir, where appropriate, were evenly spread through the year.

Examining this number of samples would give a 95% chance of detecting at least one contaminated carcase if 0·1% of the 3·1 million cattle carcases produced each year in the UK were contaminated, and the degree of clustering of positives did not exceed 0·125 as measured by the correlation between samples taken from different carcases in the same abattoir at the same time.

The scheduled visits were made by staff of the State Veterinary Service who selected five beef carcases (chilled whenever possible) making every effort to ensure that they were as widely separated as possible from each other in order to minimize correlations due to cross contamination on the slaughter line or animals coming from the same holding. If there were fewer than 5 carcases available at a visit, all were sampled.

While wearing a separate pair of disposable gloves and using a sterilized knife and forceps for each carcase, a single sample of tissue 10 cm × 15 cm and about 3 mm deep was excised from the outside surface of the neck muscle. Samples were folded in half so that the external surface was in the centre and placed into a new plastic bag which was sealed and identified. Samples were placed in cool boxes with chill packs and delivered to the nearest laboratory of the Veterinary Investigation Service in England and Wales, or the Scottish Agricultural Colleges in Scotland, or the Department of Agriculture in Northern Ireland, within 24 h for examination. Samples were delivered without any identification of their source abattoir, in order that confidentiality could be preserved.

Bacteriological examination

Samples were held overnight at 0–5 °C at the laboratory and examined by immuno-magnetic separation the following day, using the method of Chapman and colleagues [5]. In brief, after the muscle sample had been unfolded, a portion of the external surface approximately 10 × 10 cm was removed aseptically, and added to 225 ml buffered peptone water containing cefixime (0·05 μg ml^{-1}), cefsoludin (10 μg ml^{-1}) and vancomycin (8 μg ml^{-1})(BPW-VCC) or 1 g faeces was added to 9 ml BPW-VCC. Following incubation at 37 °C for 6 h, 1 ml BPW-VCC was added to 20 μl magnetic particles coated with *E. coli* O157 antibody (Dynal, 710.03) in a microtube and mixed for 30 min. The particles were separated and washed 3 times in PBS containing Tween 20 (0·05% v/v) and resuspended in PBS before transfer to Sorbitol MacConkey agar containing cefixime (0·05 μg ml^{-1}) and potassium tellurite (2·5 μg ml^{-1}). After overnight incubation at 37 °C non-sorbitol-fermenting colonies were tested for agglutination with latex particles sensitized with *E. coli* O157 antibodies (Oxoid). Positive colonies were subcultured onto Dorset's egg medium slopes and sent to the Central Veterinary Laboratory for confirmation and H7 antigens detection, motility in the case of non-H7 agglutination and detection of verocytotoxin by verocell assay and gene probes. Confirmed VTEC O157 cultures were submitted to the Laboratory of Enteric Pathogens of the Central Public Health Laboratory (CPHL) for phage typing.

Data recording and statistical analysis

The information recorded for each bovine faeces submission and the results of the bacteriological examinations were entered into a data base using the software package Epi Info. Checks were made on the data to ensure consistency both within the data base

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Table 1. E. coli *O157 in bovine faeces submitted for diagnostic purposes* Characteristics of isolates

Culture type	Number of samples	VT1	VT2	VT1 and 2	VT neg
O157:H7	38	0	34	3	1
Phage types	—	—	2(16)* 8, 14, 31, 32, 34(4), 49(3), 51(4), 68, RDNC(2)	1(2), 2	1 RDNC†
O157:NM	18	0	14	3	1
Phage types	—	—	2(5), 4(4), 14(3), 34, RDNC	8(2), 32	(Not typed)

* Figure in parenthesis indicates the number of isolates.
† RDNC, Reacts but does not conform to a phage type.

Table 2. *Confirmed VT producing* E. coli *O157 infections by herd type*

	Dairy	Beef	Calf rearer	Other	Not known
Number of herds examined	933	423	246	44	3391
Confirmed (%)	0·64	1·20	0·00	0·00	0·65

Table 3. *Confirmed* E. coli *O157 infections by age class and reason for submission*

	Evidence of clinical gastrointestinal disease			Evidence of other clinical disease or other reason			All		
Age class	Number	Percent	S.E.	Number	Percent	S.E.	Number	Percent	S.E.
⩽ 6 months	2321	0·75	0·18	68	3·69	2·17	2389	0·90	0·21
> 6 months	1537	0·54	0·19	150	0·00	0·00	1687	0·51	0·18
All	3858	0·66	0·13	218	2·16	1·28	4076	0·74	0·14

and with the manual monthly summaries made direct from the survey forms.

For statistical analysis the months of submission were grouped into four seasons December–February, March–May, June–August and September–November. Age was grouped into two classes, up to and greater than 6 months and the reason for submission was categorized as either 'evidence of clinical gastrointestinal disease' or a combined category of 'evidence of other clinical disease' with 'other reason'. Records with any missing or unknown information were not used for fitting the statistical models but all available data have been used to construct the summary Tables 1–3 below.

The overall animal prevalence and its confidence interval were calculated according to cluster sampling methods [6]. A score test [7] was used to test for the presence of clustering of cases within herds.

Each submission was categorized as VTEC O157 positive if at least one sample was positive, otherwise as negative to give a single binary variable. This was used as the dependent variable in logistic linear regression models fitted by the statistical package GENSTAT 5 to test for possible effects on the probability of being positive due to season, reason for submission, age-class, herd type, VI centre and the number of samples in the submission. A backward elimination procedure was used to eliminate non-significant ($P > 0{\cdot}05$) terms (other than number of samples) in order to arrive at a final model from which

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the predicted (adjusted) means and standard errors were estimated.

In the case of the carcase survey no information was available on the origin of each sample other than the time and place of examination. Clustering of positive results within the groups of 5 samples taken at each visit was examined by fitting a beta binomial model [8] to the results.

RESULTS

Faeces study

Altogether 14401 submissions, comprising 25891 samples, were received in VICs in England and Wales during the period of the survey. Of these, 6495 samples from 5037 herds were examined for the presence of *E. coli* O157.

A summary of the bacteriological results is given in Table 1. Sixty-nine out of 6495 samples (1·06%) were positive by the IMS methods when tested by latex agglutination but the presence of VT *E. coli* O157 was confirmed by the CVL in only 54 samples (0·83%) representing 50 herds (0·99%). Four cultures were not sent for confirmation, two were not *E. coli*, four were untypable serotypes and three were serotypes other than O157 (O2, O11 and O35). Of the confirmed isolates, 38 of the 56 strains of the O157 serotype (68%) were positive for the H7 antigen and 18 were non-motile. Only one strain O157 H7, the phage type which was designated RDNC (reacts but *does not* conform to a designated type) and one O157:H-were verocytotoxin negative and none of the strains was positive for VT1 alone. Eleven phage types were reported, the most common of which was PT2.

Herd type was recorded for only about one third of the submissions. A preliminary analysis did not show any significant effect of herd type (Table 2) and therefore this variable was omitted from all subsequent analyses in order to maximize the number of records available. After the elimination of records with missing or unknown information on the other variables 4076 submissions remained for use in the logistic regression model.

The final regression model included terms for the main effects of VI centre ($P = 0{\cdot}032$) and for age and reason for submission and their interaction. Season was not significant ($P = 0{\cdot}237$) and there was no evidence of geographical clustering, with *E. coli* O157 being isolated at 11 of the 13 VI Centres. Age and reason for submission were significant through their interaction ($P = 0{\cdot}014$) with the highest prevalence by far in young calves without clinical gastrointestinal disease. Table 3 shows the proportions positive in each category as predicted by the final model.

Estimated from all the submissions the overall prevalence among submissions of *E. coli* O157:H7 was 0·66% with 95% confidence interval from 0·43–0·88%, and the prevalence on a per animal basis was 0·59% with 95% CI from 0·38–0·79%. The evidence for clustering of cases within herds was significant with $P < 0{\cdot}001$.

Carcase study

There were 210 abattoirs in the final list of visits to be made, all of which had agreed to take part or were replacements for other abattoirs. Some of these dropped out during the course of the survey; no further replacements were made. At the end of testing 186 abattoirs were left in the study.

Altogether 4092 samples were collected during 827 visits. However, for 25 samples taken at 5 visits the test controls set up during bacteriological examination failed and the results were considered to be invalid, and are not shown in the tables. Five samples were positive by IMS but although confirmed as being *E. coli* they were not serotype O157, and were not examined for verocytotoxin or phage typed. These samples were considered as negative.

Positive results were obtained from tissue samples taken at 10 of 822 visits (1·22%) and 19 out of 4067 (0·47%) samples were contaminated with *E. coli* O157. The 95% confidence limits for the proportion of carcases contaminated, allowing for the correlation within visits, were from 0·22–1·0%.

Two isolates were non-motile but all were positive for VT2. Although 5 phage types were identified, phage types 4, 21, 49 and 51 only occurred once each while PT2 was found in 15 isolates (Table 4).

The number of positives was greater in summer than in winter but the difference in numbers of visits giving a positive result between the 4 seasons (September–November, December–February, March–May and June–August) was not significant.

The laboratories making positive isolations were geographically widespread.

There was a significant degree of correlation between carcases sampled at the same visit. The estimated coefficient of correlation is 0·44 with 95% confidence limits from 0·21–0·71.

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Table 4. E. coli *O157 in bovine carcases in UK abattoirs* Characteristics of isolates

Month	Laboratory	H7	VT	Phage type
September	Thirsk	+	2	51
December	Sutton Bonington	+	2	49
December	Bury St Edmunds	+	2	2
April	Bury St Edmunds	+	2	2
		+	2	2
May	Aberdeen	+	2	21
May	Preston	+	2	2
		+	2	2
		+	2	2
		+	2	2
July	Preston	+	2	2
		+	2	2
		+	2	2
		+	2	2
		+	2	2
August	Starcross	−	2	2
		+	2	2
August	Aberdeen	−	2	4
August	Sutton Bonington	+	2	2

Note: Where more than one positive occurred in a laboratory in one month, all the positives were invariably from the same abattoir collection.

DISCUSSION

The faeces survey indicated that the prevalence of cattle excreting VTEC O157, among those from which material was submitted to a Veterinary Investigation Centre in England and Wales is about 0·83%. This is higher than the 0·28% (14/5237 faeces samples) recorded in Scotland [9] and 0·35% (25/7000) recorded in the USA [10]. A possible explanation for the difference between our results and those from Scotland may relate to the isolation techniques used. In our study immuno-magnetic separation was used which is more sensitive than direct plating. Both surveys are liable to a degree of unquantified bias due to the fact that samples came only from herds submitting samples to VI Centres, most samples coming from animals with gastrointestinal disease, and the possibility that antibiotic therapy may have preceded the submission of samples, while the USA study was a randomized survey, and studied only unweaned calves.

The prevalence among entire herds is doubtless greater since single samples were obtained in most cases. During a 15-month study of a dairy herd [2], it was found that 74% of the animals excreted O157:H7 on only one occasion, and the remainder on more than one occasion. In the present study the herd prevalence was about 1%, lower than the 1·9% recorded in the USA survey described above of about 7000 samples from more than 1000 farms [10]. Still higher figures, ranging from 7·1–100%, have been recorded in the USA in studies where more samples were taken from each herd included [11–14], and it would seem possible that most herds in the USA could be shown to be infected if sufficient samples were examined.

No link could be established between infection and season or herd type and perhaps, surprisingly none was detected in samples from calf rearers. However, the biasses in using diagnostic samples could mask any real seasonal effect, while herd type could only be tested using one third of the submissions because of missing information. The significant effect of VI centre suggests a non-uniform geographical occurrence of the infection but no particular regional pattern is apparent, and differences could be ascribed to other factors such as differing propensities to submit material to VI Centres. Few (1·9%) of the submissions were not associated with clinical gastrointestinal disease, which limits the power of any comparison between the infection status of these and the others, but a significant difference in this association between calves and older cattle was nevertheless detected. The much higher prevalence (3·8%) among groups of samples from calves without clinical gastrointestinal disease may be due to the reduced chance of antibiotic treatment in such cases, and hence be closer to the true prevalence in the region.

The abattoir study has shown that VTEC O157 are present on 0·47% of the beef carcases but because the testing of the samples was 'blind', no analysis of herd or abattoir type is possible. The distribution of the laboratories isolating the organism, however, indicates that it is not confined to any particular part of the UK, although it must be remembered that substantial movement of animals from farm to abattoir can occur. The contamination rate of 0·47% of carcases examined is slightly higher than the 0·2% of 2081 carcases examined in the USA [1]. A higher prevalence was recorded in an abattoir in South Yorkshire [15] where O157 was isolated from seven of 23 (30·4%) carcases from which the organism had previously been isolated from rectal contents and from two of 25 carcases adjacent on the production line.

The degree of clustering of positives was higher than expected when planning the survey although since the proportion of positives found was well above

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the minimum envisaged, the exercise was not invalidated. This should be borne in mind, however, when planning similar surveys. Altogether there were 4 occasions when 2 or more samples collected at a visit were contaminated. Although in three instances the characteristics of each isolate were indistinguishable by phage type, verotoxin type and H type, it is not possible to determine whether the contaminated animals came from the same holding or whether cross contamination had occurred at the abattoir. In the fourth instance, the 2 isolates made on the same occasion showed different flagellar characteristics suggesting different origins of the infection.

Thus it would appear that VTEC O157 infected cattle are likely to be presented to abattoirs, and that contamination and quite likely cross contamination of carcases occurs. Reducing the frequency of any of these will help to reduce the occurrence of human disease especially as the distribution of the phage types, VT types are, in general, similar to those detected in VTEC O157 isolated from human infections [16].

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