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The prevalence of *Escherichia coli* O157.H7 in dairy and beef cattle in Washington State

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SUMMARY

Escherichia coli O157.H7 was found in 10 of 3570 (0·28%) faecal samples from dairy cattle in 5 of 60 herds (8·3%). Several tentative associations with manure handling and feeding management practices on dairy farms were identified. Faecal/urine slurry samples, bulk milk samples, and milk filters from dairy herds were negative for *E. coli* O157.H7. *E. coli* O157.H7 was also isolated from 10 of 1412 (0·71%) faecal samples from pastured beef cattle in 4 of 25 (16%) herds. The prevalence of *E. coli* O157.H7 excretion in feedlot beef cattle was 2 of 600 (0·33%). The identification of cattle management practices associated with colonization of cattle by *E. coli* O157.H7 suggests the possibility that human *E. coli* O157.H7 exposure may be reduced by cattle management procedures.

INTRODUCTION

Haemorrhagic colitis, haemolytic uraemic syndrome, and other illnesses associated with *Escherichia coli* O157.H7 have been reported with increasing frequency during the past decade [1–6]. Cattle, especially dairy cattle, have been implicated as the principal reservoir of this organism, and foods of bovine origin have been incriminated in most traceable outbreaks of disease associated with *E. coli* O157.H7 [3, 7]. The nature of the interaction of cattle populations with *E. coli* O157.H7, the means by which the organism is perpetuated, and the role of environmental and nutritional factors in creating an ecologic niche for the organism are unknown [3].

The purpose of the study was to establish the prevalence of *E. coli* O157.H7 in cattle in Washington state. The aims were to: (1) define the proportion of cattle herds and individual cattle colonized with *E. coli* O157.H7; (2) determine if naturally pooled samples from dairy herds, such as manure slurries and bulk milk, could be used to establish a herd's status with regard to *E. coli* O157.H7; (3) determine associations between the existence of *E. coli* O157.H7 in dairy herds and specific management practices; (4) determine the age distribution of cattle colonized with *E. coli* O157.H7 in endemic herds; and (5) compare the prevalence

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of *E. coli* O157.H7 in beef cow/calf herds, feedlot beef cattle, and in dairy cattle in Washington state.

MATERIAL AND METHODS

E. coli *O157.H7 in breeding and fattening cattle herds*

The first study focused on cattle bred and fattened for meat production. Swabs of rectal faeces were obtained from 1412 cattle in 25 pastured breeder herds, including 50–60 samples each from 21 herds, and 26–42 samples each from 4 herds. Samples were obtained from individually restrained cattle, placed in styrofoam shipping containers on ice and transported to the laboratory for bacteriological culture.

At four fattening cattle operations (feedlots), swab samples from 30 different fresh faecal pats were collected from newly arrived cattle, and pens of cattle which had been on the farm for 30–60 days, 60–90 days, and 90–120 days. One of the feedlots positive for *E. coli* O157.H7 on initial sampling was re-sampled 1 year later (after approximately three population turnovers).

E. coli *O157.H7 in dairy herds*

On each of 58 dairy farms, 60 different faecal samples were obtained. These included rectal swabs from 10 unweaned calves, swabs from 20 fresh faecal pats from post-weaned heifers, 10 fresh faecal pats from non-lactating cows, and 20 fresh faecal pats from milking cows. If < 10 unweaned calves were present, additional faecal pats from older animals were obtained so that a total of 60 faecal samples were tested from each farm. On 2 farms, 40 and 50 swabs only were obtained. The sampling of fresh faecal pats minimized the risk of the same animal being sampled twice. Five herds initially negative for *E. coli* O157.H7, and three herds in which the organism was found, were re-sampled on one or more occasions.

Detection of dairy herds positive for E. coli *O157.H7 using pooled faecal or milk samples*

Three types of pooled samples were evaluated: individual faecal swabs from unweaned calves, weaned heifers, non-lactating cows, and lactating cows pooled prior to culture (60 herds), filters from the milk pipeline (49 herds), and samples of faecal/urine slurry (47 herds).

In addition, samples of pooled bulk unpasteurized milk from 603 individual Washington dairy farms were obtained from a laboratory which conducted routine bacteriological monitoring of milk for quality control, and cultured for *E. coli* O157.H7.

Culture for E. coli *O157.H7 in faecal samples*

In the initial studies of breeding and fattening cattle herds, swabs of faeces were used to inoculate MacConkey's agar in which lactose was replaced by sorbitol (SMac, Baxter Healthcare Corp., Hayward, CA). Up to 10 sorbitol non-fermenting colonies from each were subcultured onto MacConkey's agar (Mac, Difco, Detroit, MI), and lactose fermenting colonies tested for typical *E. coli* reactions on TSI agar and for indole production. Suspect colonies were then tested for the O157 antigen using a latex agglutination test kit (Oxoid Ltd, Basingstoke, England), probed to determine the presence of verotoxin genes [10], and demonstrated to belong to typical human/bovine *E. coli* O157.H7 clones by multilocus enzyme genotype

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(courtesy of T. E. Whittam, Department of Biology, The Pennsylvania State University, University Park, PA) [9].

Prior to the beginning of the dairy cattle study, it was determined that the addition of a broth enrichment step provided additional sensitivity for the detection of *E. coli* O157.H7 (data not shown), and the following modified procedure was used for all the dairy herd studies as well as for the repeat culture of the initially positive feedlot. Faecal swabs were placed into tubes with 3 ml trypticase soy broth containing 40 μg/ml of vancomycin (TSB-V) (Vancocin HCl, Eli Lilly Co., Indianapolis, IN). These were placed on ice for transport to the laboratory and stored at 4 °C for < 5 days prior to incubation, after it was determined that this storage period did not reduce the sensitivity with which *E. coli* O157.H7 could be detected in experimentally inoculated faecal samples (data not shown). Tubes containing the faecal swabs were then incubated at 37 °C for 24 h, after which the contents were serially diluted to 10^{-6} in sterile distilled water containing 0·9% NaCl. One-tenth ml of the 10^{-6} dilution was plated evenly onto SMac, using a glass spreader. Up to 10 sorbitol non-fermenting colonies were transferred to Mac. Lactose fermenting, sorbitol non-fermenting colonies were tested for β-glucuronidase activity using a 4-methylumbelliferyl-β-D-glucuronide (MUG) [7]. MUG negative colonies were then tested for O157 antigen, and positive isolates were evaluated for verotoxin gene sequences and clonal relationship to other *E. coli* O157.H7 isolates as described previously for the beef cattle isolates.

Culture methods for pooled samples from dairy farms

Milk filters were placed in a sterile plastic bag with 100 ml of TSB-V and transported on ice directly to the laboratory and stored at 4 °C until processing. Filters were incubated in TSB-V for 24 h at 37 °C and then treated as the dairy cattle faecal cultures. Faecal/urine slurry samples from manure handling facilities were collected in 50 ml quantities and transported to the laboratory on ice. A cotton-tipped swab was used to mix the slurry and this swab was placed in a tube containing TSB-V. These broth cultures were then treated as the dairy cattle faecal cultures. Bulk milk samples (50–100 ml) were received frozen and maintained at −20 °C prior to culture. After thawing, vancomycin was added to give a final concentration of 40 μg/ml. The samples were incubated for 24 h at 37 °C, after which 0·1 ml of a 10^{-2} dilution was inoculated onto SMac, and treated as the beef cattle samples.

Data analysis

A management questionnaire was completed at the time of farm visit for each of the 60 dairy farms. The association of specific management practices with the *E. coli* O157.H7 status of the herds was evaluated by determination of odds ratios for dichotomous variables and by the Kruskal–Wallis one-way analysis of variance procedure for continuous variables (Statistix, Analytical Software, St Paul, MN).

RESULTS

E. coli *O157.H7 prevalence: breeding and fattening cattle herds*

Rectal swab samples were obtained from 1412 beef cows located on 25 farms in the major cattle grazing areas of Washington state (Fig. 1). *E. coli* O157.H7 was isolated from 10 (0·71%) individual cows from 4 (16%) herds.

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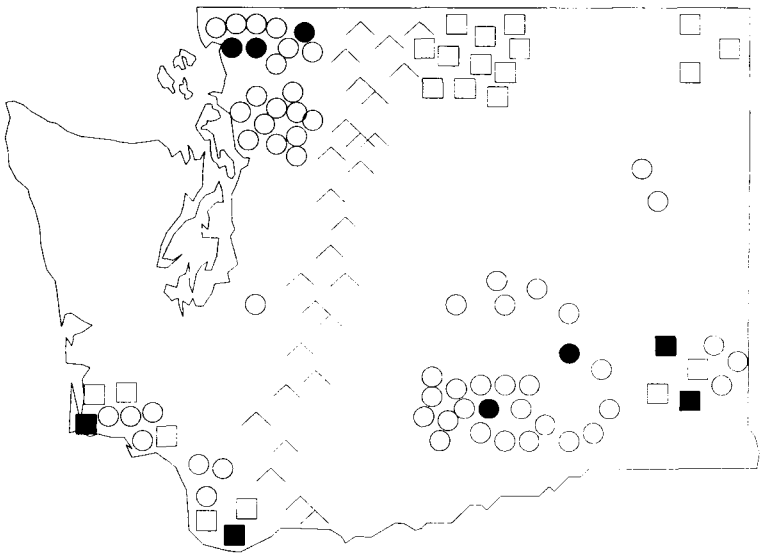

Fig. 1. The distribution of dairy and beef cattle herds in Washington state which were sampled for *E. coli* O157.H7. The herds containing at least one animal positive for *E. coli* O157.H7 are indicated in black symbols. ●, *E. coli* O157+dairy herds; ○, *E. coli* O157−dairy herds; ■, *E. coli* O157+beef herds; □, *E. coli* O157−beef herds.

Faecal pat samples were obtained from 480 cattle located in 5 feedlots in Washington state, and *E. coli* O157.H7 was isolated from 2 cattle. The positive cattle had been on the farm for 30–60 days and for 90–120 days, respectively. On re-visiting one of the positive feedlots 12 months later, faecal samples from an additional 120 cattle were cultured using the enrichment method developed for the dairy herd samples and no additional *E. coli* O157.H7 isolates were obtained. The overall prevalence of *E. coli* O157.H7 determined in feedlot cattle was 2 of 600 (0·33%) from 2 of 20 (10%) pens.

E. coli *O157.H7 prevalence: dairy farms*

E. coli O157.H7 was isolated from 10 of 3570 (0·28%) individual animal faecal samples obtained from 5 of 60 (8·3%) dairy herds sampled. Weaned calves had the highest prevalence of *E. coli* O157.H7 (7/1083, 0·65%). *E. coli* O157.H7 were also isolated from lactating cows (2/1273, 0·16%), and non-lactating cows (1/477, 0·21%), but not from unweaned calves (0/649). Within positive herds, *E. coli* O157.H7 was cultured from 7/73 (9·6%) weaned calves, 1/39 (2·6%) non-lactating cows, and 2/120 (1·7%) lactating cows. On each of the 5 positive farms, at least 1 of the positive samples was obtained from a weaned calf.

Five dairy herds negative on the initial sampling were re-sampled, and no additional *E. coli* O157.H7 isolates were obtained from 226 (4 visits), 191 (3 visits), 127 (2 visits), 127 (2 visits), and 132 (2 visits) samples, respectively. Three positive herds from the initial sampling were re-sampled, and *E. coli* O157.H7 was isolated from a weaned heifer in one of these herds at 48 days after initial sampling; the other two herds were negative on re-sampling at 22 and 91 days after the initial sampling, respectively.

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Table 1. *Comparison of dichotomous management factors between* E. coli *O157.H7-positive and -negative farms*

Management variable	O157 Neg* (%) ($N = 55$)	O157 Pos* (%) ($N = 5$)	Odds ratio	90% confidence interval
Alfalfa hay	89†	80	0·50	0·07–3·55
Alfalfa haylage	23	20	0·81	0·12–5·43
Brewer's grains	21	0	ND‡	—
Beet pulp	65	40	0·35	0·07–1·69
Bakery waste	6	0	ND	—
Cannery waste	20	0	ND	—
Canola meal	44	60	1·93	0·41–9·19
Computerized feeding	14	60	8·81§	1·74–23·57
Corn silage	66	60	0·79	0·16–3·84
Cottonseed meal	2	0	ND	—
Delinted cottonseed	9	20	2·51	0·35–17·96
Grain fed in parlour	44	40	0·86	0·18–4·11
Wheat fines	13	40	4·57	0·89–23·57
Whole cottonseed	87	40	0·10§	0·02–0·50
Urea	20	20	1·00	0·15–6·81
Soybean products	53	40	0·60	0·13–2·85
Grass hay	14	20	1·46	0·07–28·93
Grass haylage	31	60	3·32	0·69–16·03
Green chop	22	20	0·90	0·13–6·05
Lactating cows in drylot	66	60	0·79	0·16–3·80
Lactating cows on pasture	26	40	1·95	0·40–9·50
Dry cows in drylot	74	40	0·23	0·05–1·10
Dry cows on pasture	53	60	1·34	0·28–6·41
Sawdust bedding	40	40	1·00	0·21–4·77
Wood shavings bedding	36	40	1·17	0·24–5·59
Manure solids bedding	11	0	ND	—
Flush system	11	0	ND	—
Manure on crops	75	80	1·36	0·20–9·14
Manure on pasture	36	80	7·00‖	1·06–46·15
Standing water observed	26	20	0·73	0·11–4·92
Purchased animals/1 yr	55	60	1·25	0·26–5·96
Purchased animals/3 yr	67	80	1·95	0·30–12·88
Heifers raised off-farm	46	40	0·80	0·17–3·81

* Herd status: Pos. ≥ 1 cow(s) detected shedding *E. coli* O157.H7; Neg. no shedding detected in the herd.

† %. Percentage of herds in that category with the management variable positive.

‡ ND. Odds ratio undefined due to lack of variation in a group; no evidence of significant association ($P > 0{\cdot}10$).

§, ‖ Positive herds different from negative herds (§$P < 0{\cdot}05$, ‖$P < 0{\cdot}10$).

Pooled samples from dairy farms

E. coli O157.H7 was isolated from the pooled faecal samples from weaned calves on two of the farms positive by individual animal sampling. All other pooled faecal samples were negative. All 47 faecal/urine slurry samples and 49 milk filters cultured were negative for *E. coli* O157.H7, including the 3 filters and 3 slurries that were obtained from farms on which *E. coli* O157.H7 was isolated from individual faecal samples. *E. coli* O157.H7 was not isolated from any of the 603 bulk milk samples tested.

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Table 2. *Comparison of continuous management factors between* E. coli *O157.H7-positive and -negative farms*

Variable	O157 Negative* $N = 55$ Median	O157 Negative* $N = 55$ Range	O157 Positive $N = 5$ Median	O157 Positive $N = 5$ Range	P†
Number of cows	230	60–3000	123	72–460	0·08
Milk production (kg)	9625	6136–11500	9955	7486–10910	0·59
Somatic cell count in bulk milk (× 1000/ml)	260	55–700	250	53–550	0·84
Days between manure application and grazing or harvesting	21	0–300	10	0–30	0·08

* Positive, ⩾ 1 cow(s) detected shedding *E. coli* O157.H7; Negative, no shedding detected in the herd.

† P-values from Kruskal–Wallis non-parametric test.

Management variables associated with positive dairy herds

Comparison of management characteristics in herds in which *E. coli* O157.H7 was found with other herds revealed several associations (Tables 1 and 2). Positive herds tended to be smaller ($P = 0{\cdot}08$), were less likely to feed whole cottonseed ($P < 0{\cdot}05$), were more likely to use an automated computer feeder ($P < 0{\cdot}05$), and tended to irrigate grazing land with slurry ($P < 0{\cdot}10$). Among the 52 herds that placed slurry onto crops or pasture, positive herds tended to have a shorter interval between the application of slurry and grazing ($P = 0{\cdot}08$).

Sorbitol fermentation as a screening method to deter E. coli *O157.H7*

Lack of sorbitol fermentation has been found to be a useful initial screening method to identify presumptive *E. coli* O157.H7 colonies [8, 11]. The average number of sorbitol non-fermenting colonies screened per faecal sample varied among the herds in this study, from less than 1 to more than 9 (Fig. 2). The 5 herds found to contain *E. coli* O157.H7 were in the middle of the distribution with an average of 2–5 sorbitol non-fermenting colonies per sample. No *E. coli* O157.H7 were detected in any of the 19 herds averaging more than 5 sorbitol non-fermenting colonies per sample.

Seasonal variation in the prevalence of sorbitol non-fermenting bacteria in cattle faecal samples was observed, with the lowest average number of sorbitol non-fermenting colonies (1·6 colonies per sample) in December and January and the highest (6·6 per sample) in June (Fig. 3). The five positive herds were identified in June (1), July (3), and September (1). Because of this seasonal variation, herds positive and negative for *E. coli* O157.H7 were compared for average numbers of sorbitol non-fermenting bacteria. Herds positive for *E. coli* O157.H7 during June to September had significantly fewer sorbitol non-fermenting colonies per faecal sample (3·9 versus 5·0, $P < 0{\cdot}001$) than did negative herds; this difference was greatest among the weaned calves (2·6 versus 4·6, $P < 0{\cdot}001$) where most of the *E. coli* O157.H7 were found. These differences in sorbitol non-fermenting bacterial counts between positive and negative herds were obscured by the variation due to season when data from the full year of sampling were combined (3·9 versus 4·0, NS).

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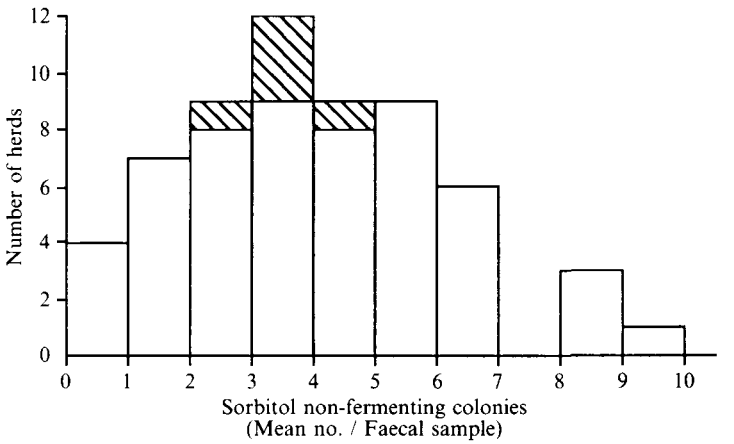

Fig. 2. The distribution of dairy herds in this survey by the average number of sorbitol non-fermenting colonies present on platings of diluted enriched faecal samples, which were then screened for *E. coli* O157.H7 identification. ▨, O157.H7 positive herds: □, O157.H7 negative herds.

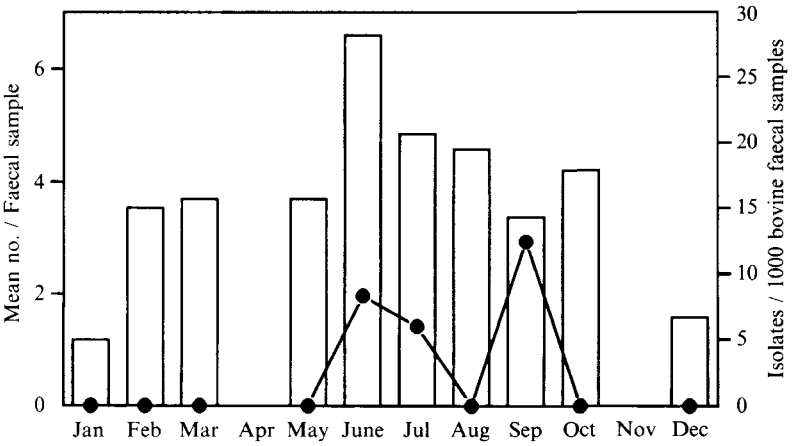

Fig. 3. Seasonal variation in the average number of sorbitol non-fermenting colonies present on platings of diluted, enriched faecal samples. ●, *E. coli* O157.H7: □, sorbitol non-fermenting colonies.

DISCUSSION

The study was designed with the estimation of herd prevalence of *E. coli* O157.H7 as a central goal. Sixty herds in Washington state were sampled in order to provide a reasonably precise and unbiased estimate of herd prevalence and sufficient samples within each herd ($n = 60$) were taken to identify accurately herds containing $\geqslant 5\%$ colonized animals. In spite of these measures, the 8·3% estimate probably underestimates the true herd prevalence. First, the number of samples collected per herd was probably inadequate, since the prevalence within positive herds was only 3·3%. The failure to detect *E. coli* O157.H7 on re-sampling in 2 of 3 initially positive herds provides additional evidence that the sample size was inadequate. Second, the failure to find *E. coli* O157.H7 in any

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herd in which the average number of sorbitol non-fermenting colonies per faecal sample exceeded 5, and the significantly lower numbers of sorbitol non-fermenting colonies per faecal sample in herds positive for *E. coli* O157.H7, suggests that *E. coli* O157.H7 was missed in some herds because of large numbers of sorbitol non-fermenting bacteria which were not *E. coli* O157.H7. Third, if the tendency towards seasonal variation in *E. coli* O157.H7 shedding observed is real, some herds which were sampled during the cooler months were probably misclassified as negative. The tendency for seasonal variation in *E. coli* O157.H7 excreting by cattle, if confirmed, would parallel the seasonal variation in human sporadic cases of *E. coli* O157.H7 disease [2].

A screening method for naturally pooled samples would permit more efficient detection of positive herds. However, none of the pooled samples evaluated in the present study (bulk milk, milk filters, slurry) resulted in the identification of *E. coli* O157.H7 positive herds. This failure may be due to the low prevalence of the organism and the predominance of the organism in heifers which do not generally contribute to the samples tested. More promising results on the use of bulk milk screening to identify *E. coli* O157.H7 positive herds have recently been reported in a study in which a different isolation method was used, suggesting that the results of the present study may have been due to inadequate sensitivity of the culture method [15].

A tentative association between slurry application to pasture and herd *E. coli* O157.H7 status was found, indicating that this may be a risk factor. A tendency for shorter average waiting periods between slurry application and grazing or harvesting in positive herds also supports this hypothesis. Other management variables significantly associated with herd *E. coli* O157.H7 infection status were dietary factors. Correlation among ration components creates special difficulties in observational studies of diet, however, the positive association with computer feeding and the negative association with the feeding of whole cottonseed could be related to their effects on the gastrointestinal flora. Though tentative, the associations observed in the present study merit further exploration, since definition of the risk factors for the presence or absence of *E. coli* O157.H7 on farms would be useful for control programmes aimed at reducing the bovine reservoir for that agent.

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