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A fifteen month study of *Escherichia coli* O157:H7 in a dairy herd

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SUMMARY

A dairy herd associated with *Escherichia coli* O157 infection in humans was studied for the 15 months following the outbreak to examine seasonal, age and management factors affecting faecal excretion of the organism and to determine the mode and frequency of milk contamination with the organism. Between May 1993 and July 1994, 28 visits were made to the farm to collect a total of 3593 rectal swabs from cows, heifers and calves and 329 milk samples. *E. coli* O157:H7 was isolated from 153 (4·3%) of 3593 bovine rectal swabs. The maximum prevalence at any one visit was 14% in lactating cows, 40% in non-lactating cows, 56% in calves and 68% in heifers. The prevalence in lactating cows, which was significantly lower than in the other groups, peaked during May–July 1993 and again briefly after the cattle were housed during November 1993 and then again during May 1994. Excretion rates of *E. coli* O157:H7 in lactating cows were highest during the first month after calving, falling during lactation and rising to another peak at 7 months postpartum. Between November 1993 and May 1994 there was no evidence of excretion in any group. Eighty-seven (74%) of the animals which excreted *E. coli* O157:H7 did so on only one occasion but 23 (32%) of 73 cows and heifers and 7 (16%) of 44 calves which excreted the organism did so on more than one occasion. *E. coli* O157:H7 was not isolated from milk taken from the bulk tank but it was isolated from individual milk samples (one milk jar and one fore-milk) from two animals previously shown to be faecal excretors of the organism. All isolates of *E. coli* O157:H7 obtained were of the same phage type, toxin genotype and plasmid profile.

INTRODUCTION

Verocytotoxin-producing (VT^+) *E. coli* (VTEC) cause haemorrhagic colitis (HC), the haemolytic-uraemic syndrome (HUS) and occasionally mild non-bloody diarrhoea in man; 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]. At present there is limited information on the epidemiology of *E. coli* O157:H7 in cattle in the UK.

Six cases of human infection with *E. coli* O157:H7 occurred in Sheffield during May 1993 and unpasteurized milk from a local farm was identified as the source of infection. The isolates of *E. coli* O157:H7 from the patients, the farm cattle and untreated milk were all phage type 2 which harboured a single 92 Kb plasmid and produced verotoxin 2 only [3]. This paper presents the results of a joint MAFF/PHLS study of this dairy herd conducted

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Table 1. *Isolation of* E. coli *O157:H7 from rectal swabs and milk samples from different groups of cattle in the dairy herd over a 15 month period. Between 28 October 1993 and 9 May 1994 lactating cows were housed continuously; at other times they were at pasture during the day and housed at night.*

	1993																		
	27/5	4/6	11/6	6/7	14/7	21/7	28/7	2/8	9/8	16/8	23/8	6/9	20/9	4/10	18/10	1/11	15/11	29/11	13/12
Lactating cows																			
Tested	106	—	—	111	15	112	114	115	114	118	120	113	109	110	106	105	114	118	117
Positive	10	—	—	15	6	6	1	0	2	2	1	0	2	0	0	2	0	0	0
%	9·4	—	—	13·5	40	5·4	0·9	0	1·8	1·7	0·8	0	1·8	0	0	1·9	0	0	0
Non-lactating cows																			
Tested	—	—	—	—	—	—	—	—	10	—	5	12	12	11	19	18	18	13	17
Positive	—	—	—	—	—	—	—	—	4	—	1	0	0	0	0	6	0	0	0
%	—	—	—	—	—	—	—	—	40	—	20	0	0	0	0	33	0	0	0
Heifers																			
Tested	—	—	—	—	—	—	—	—	25	—	22	22	20	19	19	12	10	10	5
Positive	—	—	—	—	—	—	—	—	17	—	4	0	0	0	0	3	0	0	0
%	—	—	—	—	—	—	—	—	68	—	18	0	0	0	0	25	0	0	0
Calves																			
Tested	—	—	—	—	—	—	—	—	16	—	19	17	17	17	15	15	17	22	19
Positive	—	—	—	—	—	—	—	—	9	—	4	0	0	0	0	1	2	0	0
%	—	—	—	—	—	—	—	—	56·3	—	21	0	0	0	0	6·7	11·8	0	0
Complete herd (Rectal swabs)																			
Tested	106	—	—	111	15	112	114	115	165	118	166	164	158	157	159	150	159	163	158
Positive	10	—	—	15	6	6	1	0	32	2	10	0	2	0	0	12	2	0	0
%	9·4	—	—	13·5	40	5·4	0·9	0	19·4	1·7	6	0	1·3	0	0	8	1·3	0	0
Bulk milk																			
Tested	2	5	—	1	5	—	—	1	1	—	—	—	—	—	—	—	—	—	—
Positive	0	0	—	0	0	—	—	0	0	—	—	—	—	—	—	—	—	—	—
Milk jar																			
Tested	—	—	20	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Positive	—	—	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Forestream milk																			
Tested	—	—	—	20	15	—	—	114	114	—	—	—	—	—	—	—	—	—	—
Positive	—	—	—	0	1	—	—	0	0	—	—	—	—	—	—	—	—	—	—
Midstream milk																			
Tested	—	—	—	20	15	—	—	10	10	—	—	—	—	—	—	—	—	—	—
Positive	—	—	—	0	0	—	—	0	0	—	—	—	—	—	—	—	—	—	—
Milk filters																			
Tested	—	2	2	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
Positive	—	0	0	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—

	1994								
	4/1	7/2	8/3	5/4	3/5	17/5	31/5	14/6	6/7
Lactating cows									
Tested	120	109	104	101	106	110	104	113	102
Positive	0	0	0	0	5	5	6	0	0
%	0	0	0	0	4·7	4·5	5·8	0	0
Non-lactating cows									
Tested	15	11	17	18	16	17	—	—	10
Positive	0	0	0	0	2	2	—	—	0
%	0	0	0	0	12·5	11·7	—	—	0
Heifers									
Tested	4	4	3	—	—	2	—	—	—
Positive	0	0	0	—	—	1	—	—	—
%	0	0	0	—	—	50	—	—	—
Calves									
Tested	9	17	23	15	18	23	20	16	21
Positive	0	0	0	0	5	7	14	2	0
%	0	0	0	0	27·8	30·4	70	12·5	0
Complete herd (Rectal swabs)									
Tested	148	141	147	134	140	152	124	129	133
Positive	0	0	0	0	12	15	20	2	0
%	0	0	0	0	8·6	9·9	16	1·6	0
Bulk milk									
Tested	—	—	—	—	—	—	—	—	—
Positive	—	—	—	—	—	—	—	—	—
Milk jar									
Tested	—	—	—	—	—	—	—	—	—
Positive	—	—	—	—	—	—	—	—	—
Forestream milk									
Tested	—	—	—	—	—	—	—	—	—
Positive	—	—	—	—	—	—	—	—	—
Midstream milk									
Tested	—	—	—	—	—	—	—	—	—
Positive	—	—	—	—	—	—	—	—	—
Milk filters									
Tested	—	—	—	—	—	—	—	—	—
Positive	—	—	—	—	—	—	—	—	—

during the 15 months after the outbreak. The aim of this study was to determine if seasonal, age and management factors affected faecal excretion of *E. coli* O157:H7 and to determine the prevalence of contamination of milk.

MATERIALS AND METHODS

The dairy herd

The farm was situated on the outskirts of Sheffield and the herd comprised between 110 and 140 Holstein/Friesian cows and young stock during the 15 months of the study (27 May 1993–6 July 1994). The majority of the stock were home bred with replacement heifers bred using artificial insemination. No other stock had joined the herd since 1990.

The lactating cows were housed continuously in a cubicle building between 28 October 1993 and 9 May 1994; at other times they were at pasture during the day and housed at night. Their diet consisted of grass, silage and compound dairy concentrates, the latter being fed to individual cows according to milk yield. Between 24 November 1993 and 9 May 1994 non-lactating cows and heifers were housed in straw yards and fed on silage and straw; at other times they were at pasture. Compound dairy concentrates were given only to dry cows in apparently poor condition.

Calving took place throughout the year either in straw yards or at pasture. Calves were allowed to suck for 12–24 h before being separated from their dams and moved to a house where they were reared in single pens. They were given colostrum for 4–5 days and then milk replacer and calf concentrate. After weaning at 6 weeks of age, calves were grouped in batches of 5 or 6 in straw bedded pens. They were moved to another farm in the same ownership when 3 months of age to be reared as heifer replacements or fattened for beef.

Slurry and manure was spread on land intended for silage or re-seeding and occasionally during winter it was spread on land intended for grazing.

The cows were milked in a conventional herringbone parlour. The cows' teats were washed with water, dried with individual paper towels and foremilk was removed prior to milking. After milking the teats were dipped in or sprayed with an iodophore antiseptic solution. Most of the milk was pasteurized on the farm or sold to the Milk Marketing Board. Approximately 30 gallons per week of unpasteurized milk was sold locally.

Farm investigations

Between 27 May 1993 and 6 July 1994, 28 visits were made to the farm and 3538 swabs of rectal faeces, 338 milk samples and 4 samples of milk filter washings were collected (Table 1). The time between visits varied from 1–4 weeks. These were delivered immediately to Sheffield PHL for microbiological examination.

Between 27 May 1993 and 8 August 1993 rectal swabs were taken only from lactating cows but at other times rectal swabs were collected from other cattle in the herd. Whenever a group was included in the study all cattle in the group were sampled. Individual calves were sampled for the 2–3 months before they were moved to the rearing farm. Sampling of non-lactating cows, heifers and calves commenced on the 9 August, after the period of peak excretion in lactating cows. The number of heifers tested gradually decreased with time as they calved. Several heifers joined the lactating cows late in the study from a group which had not previously been sampled. Animals were culled at intervals throughout the study for a variety of reasons unrelated to this investigation.

Milk samples were collected from the bulk tank on five occasions during May, June, July and August 1993 (Table 1). Milk from the milking jars were also collected during June 1993 from 20 individual cows, 10 of which had previously excreted *E. coli* O157:H7 in their faeces and from 10 which had not. On four occasions fore and mid stream milk samples were collected from cows previously identified as faecal excreters of *E. coli* O157:H7 and fore stream milk samples were collected from the entire milking herd on two occasions. Fore milk samples were collected by pooling milk from each quarter before the teats were washed and dried. Mid stream milk samples were collected by pooling milk from each quarter after the foremilk had been removed and the teats sanitised with cotton wool soaked in alcohol. Milk filters were sampled on two occasions in June by rinsing material directly from the filter into 250 ml of buffered peptone water (Oxoid-CM509). At the time of the visits information including freeze brand number, age, calving date, housing, culling, and evidence of ill health e.g. mastitis, diarrhoea and lameness was recorded.

Microbiology

Isolation of E. coli *O157:H7*

E. coli O157:H7 was isolated by an immunomagnetic separation technique [7, 8] and culture of magnetic

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beads on to cefixime tellurite sorbitol MacConkey agar [9]. 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 suspended in the medium by vigorous vortex mixing for 20–30 s. Milk samples (25 ml) were added to 225 ml of BPW-VCC and mixed. Vancomycin, cefixime and cefsulodin were added to the filter washings to give the above concentrations. All broths 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:H7 (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 [7]. 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:H7. Isolates that gave positive results were confirmed as *E. coli* using biochemical tests and as serogroup O157 by agglutination to titre with antiserum to *E. coli* O157 (Laboratory for Microbiological Reagents, Central Public Health Laboratory, Colindale, London) [1].

Characterization of isolates

Verocytotoxin production

Toxigenicity 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. DNA specific for the A cistrons of the VT_1 and VT_2 genes was prepared by the polymerase chain reaction, random-prime labelled with digoxigenin-11-dUTP, and used in colony hybridization reactions [2, 10]. Known VT_1^+, VT_2^+ and VT^- strains were included as controls in each batch of tests.

Plasmid analysis

Plasmids were extracted by an alkaline detergent method [11], 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 test so the size (kb) of the plasmid could be estimated.

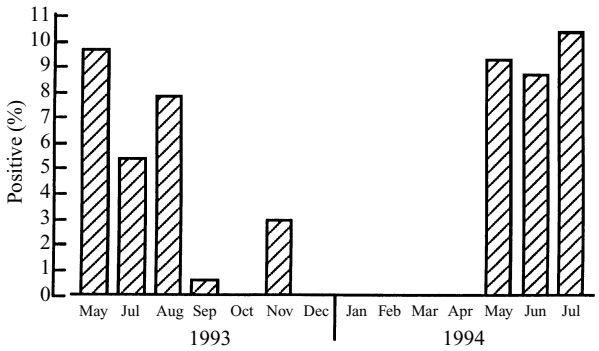

Fig. 1. Seasonal excretion of *E. coli O157:H7 by cattle in the dairy herd.*

Phage typing

All *E. coli* O157:H7 were phage typed by the Laboratory for Enteric Pathogens Central Public Health Laboratory, Colindale, London.

RESULTS

Faecal excretion of *E. coli* O157:H7

E. coli O157:H7 was isolated from 147 (4·2%) of 3538 bovine rectal swabs. Table 1 gives the percentage of animals positive for *E. coli* O157:H7 in each group. At individual visits the prevalence varied from none detected to a maximum of 14% in lactating cows, 40% in non-lactating cows, 68% in heifers and 56% in calves. The highest prevalence was recorded in heifers on 9 August 1993 while the prevalence in lactating cows was highest during the early part of the survey (May–July 1993) when no other cattle were sampled. On occasions when excretion was detected in other groups the excretion rate was always lower in lactating cows. In lactating cows excretion rates peaked in May–July 1993 and then again in May 1994. Between November 1993 and May 1994 there was no evidence of excretion in any group (Fig. 1).

Overall, *E. coli* O157:H7 was isolated from 73 (44%) of 167 cows and heifers and from 44 (38%) of 117 calves sampled during the survey. 87 (74%) of the animals were positive on only 1 sampling occasion. However, 23 (32%) of 73 cows and heifers and 7 (16%) of 44 calves which excreted the organism did so on more than one occasion (Table 2). Seven cows/heifers and six calves excreted the organism at two consecutive tests at weekly intervals. One cow excreted

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Table 2. *Animals in the herd excreting* E. coli *O157:H7 in their faeces on more than one occasion. Sampling dates on which all the animals were negative are not shown. L/N = lactating or non-lactating cows, H = heifers, C = calves, n = not tested on that date.*

Animal/type number	Date of sample												
	1993									1994			
	27/5	6/7	14/7	21/7	9/8	16/8	23/8	20/9	1/11	3/5	17/5	31/5	14/6
L/N													
1	+	−	n	−	+	+	n	n	n	n	n	n	n
19	−	+	+	−	−	−	−	−	−	n	n	n	n
158	−	+	+	−	−	−	−	−	−	−	+	−	−
164	−	+	+	−	−	−	−	−	+	−	−	−	−
170	n	+	+	−	−	−	−	−	−	−	−	−	−
182	−	+	−	+	−	−	−	−	n	n	n	n	n
211	−	+	−	+	−	−	−	−	+	−	−	−	−
229	−	−	n	−	−	+	+	−	−	−	−	n	n
238	−	−	n	−	+	n	−	−	−	+	−	+	−
240	−	−	n	+	−	n	−	−	+	−	−	−	−
249	n	+	+	−	−	−	−	−	−	−	−	−	−
272	n	−	n	−	−	−	−	+	−	−	+	n	n
575	+	−	n	−	−	−	−	−	−	−	+	n	−
638	+	−	n	+	−	−	−	−	−	n	n	n	n
662	−	+	−	−	−	−	−	−	−	+	−	n	−
732	−	+	+	+	−	−	−	−	−	n	n	n	n
H													
21	n	n	n	n	+	n	+	−	−	−	−	−	−
22	n	n	n	n	+	n	−	−	+	−	−	−	−
228	n	n	n	n	+	n	−	−	−	−	+	−	−
299	n	n	n	n	+	n	+	−	−	−	−	+	−
301	n	n	n	n	+	n	−	−	+	−	−	−	−
303	n	n	n	n	+	n	+	−	+	−	−	−	−
305	n	n	n	n	+	n	−	+	−	−	−	−	−
C													
1919	n	n	n	n	+	n	+	n	n	n	n	n	n
1998	n	n	n	n	n	n	n	n	n	−	+	+	n
2000	n	n	n	n	n	n	n	n	n	+	+	+	n
2002	n	n	n	n	n	n	n	n	n	−	+	+	n
2004	n	n	n	n	n	n	n	n	n	−	+	+	n
2007	n	n	n	n	n	n	n	n	n	−	+	+	−
2019	n	n	n	n	n	n	n	n	n	n	n	+	+

the organism at three consecutive tests at weekly intervals and one calf excreted the organism at three consecutive test at fortnightly intervals. The maximum period over which consecutive tests were positive was therefore 4 weeks. The remaining animals showed intermittent excretion, sometimes on more than one occasion, with the intervals between excretion ranging from 2–51 weeks (Table 2). Excretion of *E. coli* O157:H7 relative to age and calving date are shown in Figures 2 and 3. The highest prevalence was in animals of two years of age. It peaked during the first month after calving, was lower during lactation and then peaked again at seven months postpartum (Fig. 3).

Milk contamination

E. coli O157:H7 was not isolated from any of the milk samples taken from the bulk tank but was isolated from a milk jar sample from an animal previously shown to be a faecal excretor of the organism. *E. coli* O157:H7 was also isolated from one sample of fore stream milk but not mid stream milk from the same

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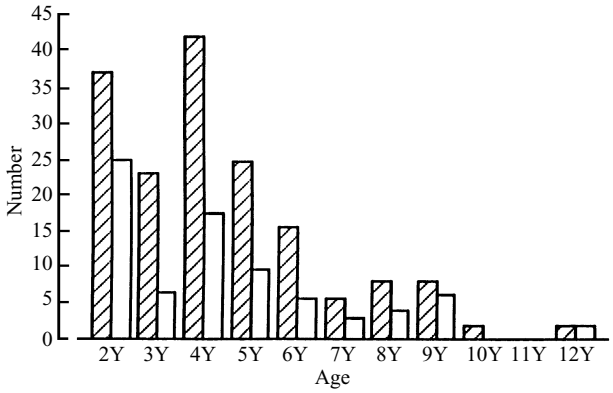

Fig. 2. Faecal excretion of *E. coli* O157:H7 relative to age of animal. ▨, number of animals in the age group; □, number of animals positive for *E. coli* O157:H7 in the age group.

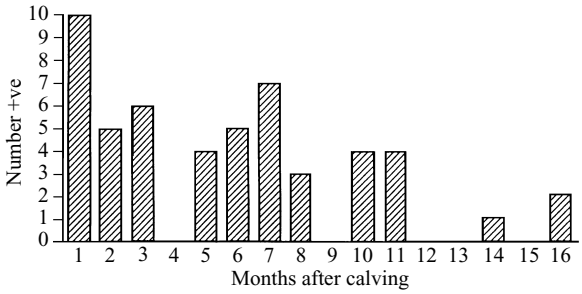

Fig. 3. Faecal excretion of *E.coli* O157:H7 relative to calving date.

animal. All other fore and mid-stream samples of milk and the washings from milk filters were negative for *E. coli* O157:H7.

Microbiology

All isolates of *E. coli* O157:H7 obtained in the study agglutinated to the expected titre of the antiserum with both O157 and H7 antisera, produced vero-cytotoxin, as determined by cell culture assay, hybridized with a DNA probe to VT_2 but not with one to VT_1, harboured a single 92 kbp plasmid and were of phage type 2.

DISCUSSION

Over the 12 month period from August 1993 at visits where all groups were sampled the excretion rate was lowest in lactating cows (0·9%) and highest in heifers (14·2%) ($P < 0{\cdot}01$), with the incidence in non-lactating cows and calves being 6·3% and 9·3% respectively. Other studies have shown similar differences in prevalence [12–14]. The reasons for the differences in excretion rates in this study are not known but may reflect differences in ruminal development, diet, specific immunity to infection or other factors. Excretion rates for all groups varied during the survey with a similar seasonal pattern. This perhaps is to be expected due to the movement of non-lactating cows and heifers into the lactating herd at calving and the close contact between calving cows and their calves.The seasonal peak in late spring and early summer, as observed in human infections in previous years in the Sheffield area [1, 2, 9], coincided with an outbreak of human infection linked to consumption of unpasteurized milk produced from the herd [3]. Between November 1993 and May 1994 there was no evidence of excretion of *E. coli* O157 in any group of animals despite close confinement within the housing. Hancock and colleagues [15] identified previous irrigation of grazing land with faecal slurry as a positive risk factor for carriage of *E. coli* O157 in a dairy herd. Although this was the practice on this farm, the slurry was spread occasionally only in mid-winter and it was therefore thought that contaminated pasture was unlikely to re-infect animals grazing some months later. However, it was not possible to test this hypothesis as excreters of *E. coli* O157 were identified in lactating cows, non-lactating cows and calves on 3 May 1994, 6 days prior to turnout to pasture. This is difficult to explain as there had been no changes in farming practices, water supply or feedstuffs. After this time the prevalence in the herd again rose steadily in May and June until the study terminated in early July.

The highest excretion rate (68%) was recorded in heifers on 9 August 1993; 3 days before this, the heifers broke into a silage field recently treated with slurry. This supports the suggestion of Hancock and colleagues [15] that grassland and slurry management may be important in the spread of this organism in cattle by the faecal-oral route.

One calf excreted the organism at three consecutive tests over a 4-week period, the longest period of excretion identified during the survey. Despite the calves being sampled for a period of only 2–3 months, 16% excreted *E. coli* O157:H7 on more than one occasion. This would agree with the findings of experimental studies published since the completion of this survey: Cray and Moon [16] found that when feeding 10^{10} CFU of *E. coli* O157:H7 to calves and adults that at 7 weeks after inoculation all calves remained positive whilst only 22% of adult animals were excreting the organism.

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The excretion rate of *E. coli* O157:H7 relative to age and calving dates of animals is shown in Figures 2 and 3 respectively; other studies have found a higher prevalence of *E. coli* O157 in weaned calves and heifers than in unweaned calves or adult animals [17, 18]. It is possible that the increase in excretion of *E. coli* O157:H7 during the first month after calving was due to dietary changes at this time. Whilst non-lactating animals were fed on a high roughage diet, mostly of grass and silage, after calving they fed on a diet supplemented with compound dairy concentrates according to individual milk yield. Since the completion of this study, such dietary change has been shown to alter excretion patterns of *E. coli* O157:H7 in sheep [19]. The incidence fell during lactation and then rose to a peak again at 7 months postpartum. At this time milk yield would normally be declining and the level of concentrate fed to the cattle would also be reduced. Again, it is possible that this dietary change could have altered the excretion rate at this time.

Throughout the investigation there was no evidence of clinical disease in any of the cattle which may have been associated with *E. coli* O157:H7 infection. Synge and Hopkins suggested that the organism may be associated with diarrhoea in calves [4] but feeding experiments have not supported this hypothesis [16].

E. coli O157:H7 was isolated from fore milk samples but not from mid stream milk samples suggesting that the organism is not secreted by the udder. Thorough teat sanitization and removal of foremilk, though laborious, may thus prevent contamination of the milk. *E. coli* O157:H7 was not isolated from the milk filters or from any of the samples of milk taken from the bulk tank but the latter was probably due to dilution of contaminated samples to a level of infection undetectable by the microbiological method used. Although they used a less sensitive method than used in the present study, Hancock and colleagues [15] also found that screening of milk filters or bulk milk samples was unsatisfactory as an indicator of herd carriage status.

All strains of *E. coli* O157:H7 isolated throughout the study were indistinguishable with regard to phenotypic properties, plasmid profile, toxin genotype and phage type. This is in contrast to a more recent study [20] in which strains of more than one type of *E. coli* O157, as determined by analysis of genomic DNA by pulsed field gel electrophoresis (PFGE), usually occurred in any given herd over an 8-month period. Our results may suggest clonal spread of the organism within the herd. However, it is also possible that the strain had varied during the study but that this variation was not detected by phage and plasmid typing; others have suggested that phage typing is less useful than PFGE for the subtyping of *E. coli* O157 in epidemiological studies [22].

Hygienic milking practices may reduce the level of contamination of milk but effective pasteurization is necessary to ensure that this pathogen is destroyed. Although unpasteurized milk is sold in England and Wales, the governments Chief Medical Officer has recently advised that it should not be consumed by vulnerable groups [22].

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