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Persistence of *Escherichia coli* O157:H7 in dairy cattle and the dairy farm environment

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SUMMARY

The persistence of *Escherichia coli* O157:H7 in cattle and the farm environment was investigated on eight Ontario dairy farms positive for *E. coli* O157:H7 in a longitudinal study commenced one year previously. Faecal samples from cows, calves, humans, cats, rodents, wild birds, a composite fly sample and numerous composite and individual environmental samples were cultured and tested for verotoxin-producing *E. coli* (VTEC). VTEC isolates were serotyped and *E. coli* O157:H7 isolates were phage typed. *E. coli* O157:H7 phage type 34 was isolated from one calf on each of two farms. The same phage type had been isolated on one of these farms 12 months earlier. Most *E. coli* O157:H7-positive animals and farms became culture-negative within 2 and 3 months, respectively. *E. coli* O157:H7 was not isolated from any environmental samples, although evidence of VTEC was found in composite samples from calf feeders (19·1%), calf barn surfaces (18%), cow feeders (14·9%), flies (12·5%), cow barn surfaces (11·3%), and individual milk filters (12·5%). VTEC belonging to 21 non-O157 serotypes were isolated from 24 cows (8·2%), 21 calves (18·3%), 2 cow feeder samples (3·0%), and 1 calf feeder sample (4·8%). Shedding of *E. coli* O157:H7 by infected dairy cattle appears to be transient and persistence of *E. coli* O157:H7 was not demonstrated from the farm environment sites tested.

INTRODUCTION

Infection with *E. coli* O157:H7 and other serotypes of verotoxin (VT)-producing *E. coli* (VTEC) is a cause of serious human foodborne illness [1, 2]. Cattle are an important reservoir of these organisms [3] and consumption of undercooked beef contaminated by the faeces of infected cattle at slaughter is a frequent source of human VTEC infection [1, 2]. Infection may also occur through consumption of unpasteurized milk and other foods [4, 5], person-to-person transmission [6, 7] and direct contact with infected cattle or their manure [8].

An integrated approach to reducing foodborne transmission of *E. coli* O157:H7 and other VTEC

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potentially includes controlling these organisms in cattle, particularly those destined for meat production. To evaluate the potential success of on-farm control strategies, information is required regarding the epidemiology of these organisms in cattle in the farm environment. Although previous studies of farm husbandry practices have identified a number of risk factors for herd infection with VTEC [9–13], little is known about the persistence of VTEC in naturally-infected animals or in the environment of infected farms. The objective of this study was to examine the duration of faecal shedding of *E. coli* O157:H7 by infected dairy cattle, and to investigate the farm environment as a possible reservoir of *E. coli* O157:H7 and other VTEC.

MATERIALS AND METHODS

Study population

A longitudinal study of VTEC infection on Ontario dairy farms was undertaken between July 1992 and March 1993 [14, 15]. Eighty dairy farms, randomly chosen from a list of all dairy producers in 12 counties in southern Ontario were selected for the study. Rectal swabs from a random sample of 25% of the milking herd (minimum 10 cows) and all calves less than 3 months of age, and a stool sample from family members on each farm were cultured and tested for VTEC. On farms having an *E. coli* O157:H7-infected animal or a family member infected with any serotype of VTEC, the same animals were retested approximately 1 and 3 months after the initial test. As a result of these three samplings (termed the 1992 study), seven farms having one or more *E. coli* O157:H7-infected animals and one farm having an *E. coli* O157:H7-infected person were identified. These eight *E. coli* O157:H7-positive farms formed the study population for the second investigation (1993 study).

Farm visits and sample collection

The eight farms were revisited for a fourth sampling between 1 July and 31 August 1993, creating intervals of 3–15 months since the last isolation of *E. coli* O157:H7 on the farms. Samples for microbiological testing were collected from the animals and environmental sites listed in Table 1. Family members were asked to submit a single faecal sample collected in Cary–Blair medium [16]. The sample was refrigerated until it was collected by a member of the study team, no more than 2 days later.

Table 1. *Sampling for VTEC on eight farms, Ontario, Canada, 1993*

Animals	Environment
Faecal samples from:	Swab samples from:
All milking cows	Cow and calf barns
All calves < 3 months	Manure piles
Family members	Waterbowls, mangers
Cats	Floors, stanchions
Rodents	Sills, walls, fans
Birds	Milk pipelines, filters
Flies (100 whole flies)	Calf hutches

A single rectal swab was obtained from all calves under 3 months of age. A rectal swab was also obtained from all lactating cows on farms having 50 or fewer cattle milking on the day of the visit. For farms having greater than 50 milking cows, rectal swabs were obtained from all cows on the farm that were milking and had been sampled in the original study, and from a formal random sample of the other lactating cows on the farm, to a total of 50 milking cows.

A single rectal swab was obtained from cats that could be hand-caught on the day of the visit. On farms where four or fewer cats could be caught, all cats were sampled. On farms where more than four cats could be caught, a random sample of four cats was tested.

To collect rodent faeces, three live rodent traps, baited with a mixture of peanut butter, honey and oatmeal, were set in areas within the cow barn showing recent evidence of rodent presence (droppings, burrows, nests, etc.). Traps were checked daily and live-trapped rodents were released unharmed. The traps were taken to the laboratory where faecal matter was transferred into sterile containers until processed.

Fresh faecal matter from wild birds was obtained from sites in areas where cattle were housed. On farms where four or fewer such sites could be identified, all sites were sampled. On farms where greater than four sites could be identified, a random sample of sites was obtained. Sterile applicator sticks were used to scrape bird faecal matter into sterile containers for transportation. On one farm (Farm 114), no bird faecal samples could be found.

A minimum of 100 flies was collected on each farm by sweeping a sterile butterfly net through the air throughout the length of the barns. Care was taken to avoid contact of the net with barn surfaces. Flies were

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transferred to a sterile container for transportation to the laboratory.

Environmental samples were collected with compressed sponge material (Industrial Commercial Supply, Akron, OH), cut into 5×5 cm squares for sampling discrete sites (site swabs), or 5×10 cm rectangular drag swabs [17] for sampling floors. Swabs used to sample dry areas (e.g. walls, dust) were premoistened with Nutrient broth (Difco, Detroit, MI).

The farm environment was sampled as follows. Site swabs from five randomly selected areas of run-off from the manure pile were obtained. Drag swabs were used to sample the length of each alley within the cow barn. Site swabs of dust were collected from the following locations within the cow barn, where present: one swab was obtained from window sills on each side of the barn, one swab was obtained from stanchion tops on each side of the barn, one swab was obtained from the upper surfaces of milk pipelines along each side of the barn and one swab from each of three randomly selected exhaust fans, or all fans in the barn, whichever was the lesser.

Swabs were also obtained from waterbowls and feed mangers in the cow barns. One swab was used to obtain a composite sample from five waterbowls. Five such composite swabs were obtained on each farm where 25 or more waterbowls were present. Otherwise, all waterbowls were swabbed in this manner. Similarly, up to five composite site swabs were obtained from feed mangers on each farm.

If calves were housed in a room or building separate from cows, the above protocol was repeated in the calf areas. If calves were housed in individual hutches, then swabs were obtained from all hutches, or from 10 randomly selected hutches, whichever was the lesser. Where 1–5 hutches were sampled, 3 composite swabs were used: one for sampling the inside roofs of all hutches, one for sampling the inside walls of all hutches, and the third was used to sample the feeding areas of all hutches. If greater than five hutches were present, one set of three swabs was used to sample half of the hutches as described above, and a second set of three swabs was used to sample the other half.

Following sample collection, the sponges and other samples were repackaged individually in sterile containers, packed on ice and transported to the laboratory on the same day. In addition, the farm manager on each farm was asked to collect a milk filter from the most recent milking. The filter was placed in a sterile Whirlpak bag (Nasco, New Hamburg, Ontario) and refrigerated until collected, along with the human faecal samples, for transfer to the laboratory.

Detection and isolation of VTEC

All samples were processed on arrival at the laboratory, or after overnight storage at 4 °C. Identification of VTEC-positive samples and isolation of VTEC were conducted as described previously [18, 19], with some modifications. Faecal samples and rectal swabs were grown overnight in MacConkey broth (Difco) and subcultured overnight in brain heart infusion broth (BHIB, Difco). The resulting cultures were tested by a polymerase chain reaction (PCR) procedure for VT genes [20], and supernatants obtained after centrifugation of the cultures at 10000 ***g*** were tested at fivefold dilutions in a Vero cell cytotoxicity assay (VCA) for evidence of VT production [21].

Environmental samples were processed similarly after pre-enrichment in nutrient broth, as follows. Site swabs, milk filters or drag swabs were placed in individual stomacher bags containing 100, 200 or 300 ml, respectively, of nutrient broth. Flies were asphyxiated with carbon dioxide and combined into one composite sample per farm in a stomacher bag containing 100 ml of nutrient broth. The bags were pummelled in a stomacher laboratory blender (Seward Laboratory, London, UK) for 1 min and incubated overnight at 37 °C. A 1 ml volume of each resulting culture was subcultured in 10 ml of MacConkey broth, then in BHIB, as for faecal samples, for testing by the PCR procedure and the VCA.

To obtain VTEC isolates from non-human samples, MacConkey broth cultures corresponding to BHIB cultures with VCA titres of 5 or greater were plated onto sorbitol-MacConkey agar. After overnight incubation, five individual colonies, including non-sorbitol fermenting colonies, and two mixed colony samples (sweeps) from each plate were subcultured overnight in BHIB and were tested by the VCA. BHIB cultures of individual colonies that were positive in the VCA were tested by PCR for the presence of VT genes, and if positive, were identified and serotyped. Where sweeps, but not individual colonies, were positive in the VCA, five additional colonies from the same plate were selected and tested in the VCA. Isolation of VTEC from human samples was attempted in the same way, except that samples were

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selected for further testing if their BHIB cultures showed any evidence of VTs either by VCA or PCR, and the numbers of individual colonies and sweeps tested in the VCA were increased to 20 and 3, respectively. *E. coli* O157:H7 isolates were phage typed [22] at the National Laboratory for Enteric Pathogens, Laboratory Centre for Disease Control, Ottawa, Ontario.

Classification of VTEC status

A sample was classified as VTEC-positive if the initial culture was positive for VT genes by PCR amplification or if a VTEC was isolated from the sample. Samples were classified as *E. coli* O157:H7-positive if they yielded an *E. coli* O157:H7 isolate.

Statistical analysis

All data were entered into a computer data base (Dbase IV, Ashton Tate, Torrance CA) and analysed using Epi Info (Centres for Disease Control and Prevention, Atlanta, GA). Simple contingency table analysis was used to test the following: (1) the difference between the rate of *E. coli* O157:H7 isolation from cattle in the current study, and in the first visit of the 1992 study of 80 dairy farms and (2) the difference in the rate of *E. coli* O157:H7 isolation in cattle and environmental swabs, in the present investigation.

RESULTS

Compliance

Each of the eight farms positive for *E. coli* O157:H7 in the 1992 study participated in this investigation. Each family provided stool samples from a minimum of two family members; 41 (80·4%) family members provided stool samples.

Rectal swabs were obtained from cows and calves on each farm, for a total of 291 cows and 115 calves. Only 50 of the cows were among the 90 cows tested on these farms in the 1992 study. This reflects culling practices within the dairy herds over the previous year, the fact that some of the cows were not lactating at the time of sampling, and that all calves sampled in the 1992 study were more than 3 months old, but were not yet old enough to have entered the milking herd. Calves sampled in the current study, being less than 3 months old, had not been born at the time of the 1992 study.

Flies, milk filters, environmental swabs and rectal swabs from cats were obtained on each of the eight farms. Rat droppings were obtained on four farms (50%), and faecal material from wild birds was obtained on seven farms (88%).

Isolation of *E. coli* O157:H7

E. coli O157:H7 phage type 34 was isolated from one calf on each of two farms (19 and 29). The same phage type of *E. coli* O157:H7 had been isolated from a calf on one of these two farms (Farm 19) 12 months earlier. All other samples were negative for *E. coli* O157:H7. The rate of isolation of *E. coli* O157:H7 from individual cattle on the eight farms (2/406, 0·50%) was lower than on the first visit to the same farms in the 1992 study (10/183, 5·5%, $P = 0{\cdot}001$), and was not significantly different from the prevalence of *E. coli* O157:H7 on the first visit to the 80 randomly selected farms in the 1992 study [14, 15] (7/1477, 0·47%, $P = 0{\cdot}96$). There was no significant difference between the rate of isolation of *E. coli* O157:H7 from cattle (2/406, 0·5%) and either environmental samples (0/241, 0%, $P = 0{\cdot}27$) or flies, cats, rodents and birds (0/58, 0%, $P = 0{\cdot}59$).

Results of sequential culture of *E. coli* O157:H7 from cattle in the present investigation and the 1992 study [14, 15] are shown at the farm level in Table 2 and for individual cattle in Table 3. Four of the eight farms (50%) had at least one animal culture-positive for *E. coli* O157:H7 on more than one of the four visits (Table 2), although no one animal was culture-positive on more than one occasion (Table 3). The 10 *E. coli* O157:H7-positive animals that were retested during the 1992 study became culture-negative within 1–2 months of the initial positive test.

All VTEC

Evidence of VTEC was found in faecal samples from cattle on seven of the eight farms (87·5%). Overall, more calves were VTEC-positive than cows (56/115, 48·7% *vs.* 49/291, 16·8%, $P < 0{\cdot}001$). Among the 50 cows tested in both the 1992 and 1993 studies, six were VTEC-positive in both studies, however, the three serotypes isolated from these animals in the 1993 study were not among those detected on the same

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Table 2. *Farm level results of sequential culture of* E. coli *O157:H7 and other VTEC, Ontario, Canada, 1992–3*

Farm	First visit Month 0	Second visit Month 1	Third visit Month 3	Fourth visit Interval* (months)	Fourth visit Result
			Type of isolate		
19	O157:H7 PT34†	VTEC‡	VTEC	12	O157:H7 PT34
29	O157:H7 PT67	VTEC	VTEC	12	O157:H7 PT34
79	VTEC	VTEC	O157:H7 PT23	3	VTEC
85	O157:H7 PT23	VTEC	VTEC	15	VTEC
99	VTEC	O157:H7 PT14	O157:H7 PT14	8	VTEC
113	O157:H7 PT23, 32	O157:H7 PT23	VTEC	10	VTEC
114	VTEC	O157:H7 PT14	VTEC	11	VTEC
115§	VTEC	O157:H7 PT14	VTEC	8	None

* Interval between last isolation of *E. coli* O157:H7 on the same farm in 1992 study and the fourth visit.
† PT, phage type of *E. coli* O157:H7.
‡ VTEC, VTEC of a serotype other than O157:H7.
§ Farm 115 was classified as *E. coli* O157:H7-positive on the basis of an *E. coli* O157:H7-infected person. All other farms were classified as *E. coli* O157:H7-positive on the basis of bovine culture results.

Table 3. *Results of sequential culture of* E. coli *O157:H7 from individual cattle, Ontario, Canada, 1992–3*

Farm	Animal	First visit Month 0	Second visit Month 1	Third visit Month 3	Fourth visit Interval* (months)	Fourth visit Result	Interval from positive to negative test (months)
19	Cow	O157:H7 PT34†	Neg‡	Neg	12	NT§	1
	Calf	O157:H7 PT34	Neg	Neg	12	NT	1
	Calf	NT	NT	NT		O157:H7 PT34	UND‖
29	Cow	O157:H7 PT67	Neg	Neg	12	NT	1
	Calf	NT	NT	NT		O157:H7 PT34	UND
79	Calf	Neg	VTEC¶	O157:H7 PT23	3	NT	UND
85	Cow	O157:H7 PT23	Neg	Neg	15	Neg	1
99	Cow	Neg	O157:H7 PT14	Neg	10	NT	2
	Calf	Neg	Neg	O157:H7 PT14	8	NT	UND
113	Calf	O157:H7 PT32	VTEC	Neg	11	NT	1
	Calf	O157:H7 PT23	VTEC	Neg	11	NT	1
	Calf	O157:H7 PT23	VTEC	VTEC	11	NT	1
	Calf	Neg	O157:H7 PT23	Neg	10	NT	2
114	Calf	Neg	O157:H7 PT67	Neg	11	NT	2

* Interval between last isolation of *E. coli* O157:H7 from the animal and the fourth visit.
† PT, phage type of *E. coli* O157:H7.
‡ Neg, VTEC not isolated.
§ NT, not tested.
‖ UND, undetermined since animal was not retested after isolation of *E. coli* O157:H7.
¶ VTEC, VTEC of a serotype other than O157:H7 isolated.

farms in the 1992 study [15]. Two of 41 human faecal samples (4·9%) had PCR evidence of VTEC, but VTEC were not isolated from either sample. Among 58 samples from other animals on the farms (16 from cats, 7 from rodents, 27 from birds and 8 composite fly samples), only the composite fly sample from 1 of 8 farms (12·5%) was VTEC-positive, but an isolate was not obtained. Overall, 34 (14·1%) of 241 environmental samples were VTEC-positive, the most frequent being calf mangers, calf waterbowls and calf barn surfaces (Table 4).

VTEC belonging to 19 non-O157:H7 serotypes

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Table 4. *Environmental samples with evidence of VTEC contamination, Ontario, Canada, 1993*

Site tested	No. VTEC-positive*/ no. tested	Percent positive	No. of isolates
Calf mangers, water bowls	4/21	19·1	1
Calf barn surfaces, manure pile	7/39	18·0	0
Cow mangers, water bowls	10/67	14·9	2
Cow barn surfaces, manure pile	12/106	11·3	0
Milk filters	1/8	12·5	0
Total	34/241	14·1	3/241

* Positive by PCR for VT genes or by isolation of VTEC.

Table 5. *Serotypes of VTEC isolated on seven dairy farms in 1993, Ontario, Canada, 1993*

Farm	Serotype*	Source	Isolated on same farm in 1992
19	**O26:H11**	Calf	No
	O98:H25	Cow	No
	O103:H$^-$	Calf	No
	O119:H$^-$	Cow, environment	No
	O156:H$^-$	Calf	Yes
	O157:H7 PT34	Calf	Yes
	O?:H21	Calf	Yes
	O?:H25	Cow	No
	O?:H$^-$	Cow, calf	Yes
29	**O113:H4**	Calf	Yes
	O157:H7 PT34	Calf	No (PT67)
79	**O111:H8**	Cow	No
	O153:H25	Cow, calf	No
	O?:H21	Cow, calf	Yes
85	**O7:H4**	Calf	No
	O15:H7	Cow	No
	O26:H11	Calf	No
	O45:H2	Calf	No
	O?:H2	Calf	No
	O?:H4	Calf	No
99	**O2:H29**	Cow	Yes
	O8:H9	Calf	No
	O156:H$^-$	Cow	Yes
	O?:H21	Cow, calf	Yes
113	**O26:H11**	Calf	No
	O153:H25	Environment	No
	O?:H21	Calf	Yes
114	**O113:H21**	Cow	No
	O?:H$^-$	Calf	Yes

* Serotypes shown as O? may belong to different O serogroups. Those shown in bold type have been isolated previously from humans [23–25].

were isolated from 16·5% (19/115) of calves, 8·9% (26/291) of cows and from 1·3% (3/241) of environmental samples (Table 5). One environmental isolate from a calf manger was of the same serotype (O119:H$^-$) as an isolate from a cow on the farm (Table 5). Several of these serotypes had been present in cattle on the same farm on one or more of the three samplings in the 1992 study (Table 5). Fifteen of the 20 serotypes isolated from cattle have also been isolated from humans [23–25].

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DISCUSSION

The results of this study suggest that the occurrence of *E. coli* O157:H7 on dairy farms is relatively transient. Six of eight farms previously *E. coli* O157:H7-positive reverted to negative status within 3 months of a positive test. Furthermore, the proportion of *E. coli* O157:H7 culture-positive cattle on these farms decreased within 15 months from 5·5% to 0·5%, which was not significantly different from the prevalence of this serotype in the initial sampling of cattle in 80 randomly selected herds (0·47%) in the 1992 study [14]. Similar prevalence rates of *E. coli* O157:H7 in cattle have been reported in other regions [12, 26–29].

There appears to be a seasonal influence on the prevalence of *E. coli* O157:H7 in cattle, with highest rates of infection in the summer months [12]. Since testing in the 1993 study was conducted in July and August, it is unlikely that a seasonal effect accounted for the reduced prevalence on the eight farms in 1993. However year-to-year variations in climatic or related factors associated with this apparent seasonal effect may have contributed to the differences noted on these farms in 1992 and 1993.

Our findings suggest that *E. coli* O157:H7 may not persist, or may persist at below detectable limits in the dairy farm environment, since none of the 241 environmental samples, most of which were composite samples, from the eight farms was positive for this organism. Similarly, none of the samples from cats, rodents, wild birds or flies was positive for *E. coli* O157:H7. These results are consistent with those of others who failed to isolate *E. coli* O157:H7 from manure slurries [12, 29], environmental samples and feeds [29], or numerous samples from birds, cats, dogs and other non-bovine species [28, 29]. However, Hancock and colleagues [12] identified spreading of manure as a risk factor for infection of cattle with *E. coli* O157:H7. Among the environmental sites tested in the present study, feed mangers and water bowls had the highest rates of positivity for VTEC, suggesting they may play a role in animal-to-animal transmission. Recently, water has been identified as a possible reservoir of *E. coli* O157:H7 on farms [29] and contaminated well water has been linked to bovine and human *E. coli* O157:H7 infections (Jackson and colleagues, manuscript submitted).

The transient occurrence of *E. coli* O157:H7 on the farms seems logical, since individual cattle remained culture-positive for *E. coli* O157:H7 for relatively short periods. As noted in other studies [12, 27], none of the 10 animals identified as *E. coli* O157:H7-positive on one of the first two visits was culture-positive for more than 2 months, although longer periods of faecal shedding were noted in some animals following experimental infection with *E. coli* O157:H7 [30]. *E. coli* O157:H7 has been re-isolated from some herds after long periods [28, 29, 31]. However, the strains isolated over time were often genetically different [29, 31], or of different phage types [28], indicating infection with different strains rather than persistence of the original strains. In the present study, phage typing provided similar evidence that the *E. coli* O157:H7 strain isolated on one of two farms after an interval of 12 months was different from the previous isolate from the same farm.

The apparent persistence of *E. coli* O157:H7 in cattle and the farm environment may be influenced by the methods used for its detection. Although the techniques chosen for this study enabled detection of VTEC of any serotype and were not specific for *E. coli* O157:H7, the prevalence of *E. coli* O157:H7 in cattle on the initial sampling of the 80 farms in the 1992 study was consistent with the 0–2·5% range reported from other surveys using methods specific for *E. coli* O157:H7 [3, 12, 13, 27, 28]. Higher prevalence rates have been reported following the use of newer, more sensitive methods for detection of *E. coli* O157:H7 [29, 31, 32]. For example, in follow-up studies of dairy herds, an improved method detected *E. coli* O157:H7 in 50% of previously culture-positive herds and 22% of previously culture-negative herds [31]. While the use of a more sensitive method in the present follow-up study may have revealed higher rates of *E. coli* O157:H7 infection, a change in methodology would have confounded comparison with our previous results.

It has been noted [33] that the results of faecal culture in on-farm studies may not reflect the true infection status of the tested animals. Recent evidence suggests that previously infected but culture-negative cattle may harbour *E. coli* O157:H7 and re-excrete high numbers of the organism following dietary change or food deprivation and partial re-feeding [34, 35]. Like Salmonella and Type I *E. coli* [36], low numbers of *E. coli* O157:H7 may persist in the rumen and replicate to high numbers when the suppressive effects of high volatile fatty acid concentration and low pH in the rumen are abrogated by dietary changes [37]. Relatively few animals, responding to these conditions by shedding high numbers of *E. coli*

O157:H7 and other VTEC, may therefore be important reservoirs of infection on farms.

Many cattle harboured VTEC belonging to serotypes other than O157:H7, as noted in earlier investigations [19, 27] and the 1992 study [15]. Several of the non-O157 VTEC serotypes isolated in the 1993 study were present on the same farms 3–15 months previously. Whether these were persistent strains, or new strains on the farms requires more detailed characterization of isolates, such as that applied to *E. coli* O157:H7 isolates. Many of these serotypes, like *E. coli* O157:H7, are not bovine pathogens and their ecological relationship with cattle is probably similar to that of *E. coli* O157:H7. Infection of cattle with *E. coli* O157:H7 does not confer protection against re-infection with the homologous strain [30, 38]. Should the same hold true for other VTEC, animals may be re-infected with the same strains of non-O157:H7 VTEC over time.

Based on the environment sites tested, and given the inherent limitations of the testing methodology, our results suggest that dairy cattle are a more frequent reservoir of *E. coli* O157:H7 than the dairy farm environment. *E. coli* O157:H7 was not isolated from environmental samples, however, this does not preclude the need for further studies in the farm environment to investigate other niches for *E. coli* O157:H7. Therefore, efforts to reduce the prevalence of this organism might best be focused initially on controlling infection in the bovine reservoir. Single test and cull programmes are unlikely to be effective in view of the relatively frequent and mostly transient nature of infection in cattle, the limited efficacy of faecal culture and the possible persistence of the organism in some culture-negative cattle. Repeat testing however, may identify chronic or recurrent shedders that contribute to maintenance of infection in some herds.

Since open herds have a higher risk for VTEC infection [9, 10], preventing the introduction of infected cattle may reduce the prevalence of *E. coli* O157:H7 on dairy farms. Replacement calves and heifers may pose a particular risk because of the higher rate of infection in younger cattle [9, 10, 12, 13, 27, 31]. Within dairy herds, control of factors, such as contact between calves and cows, communal housing, feeding and watering of calves [9–11, 29] and manure management [12], together with prevention of stress and/or dietary changes that induce recrudescence of faecal shedding of *E. coli* O157:H7 [34, 35] may reduce spread of infection. Further investigation is required to characterize and control the seasonal factors [12] influencing spread and survival of *E. coli* O157:H7 on farms where infection has occurred.

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