*Epidemiol. Infect.* (2001), **127**, 555–560. © 2001 Cambridge University Press
DOI: 10.1017/S095026880100615X Printed in the United Kingdom

# *Escherichia coli* O157:H7 infection of calves: infectious dose and direct contact transmission

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(*Accepted 1 July 2001*)

## SUMMARY

Cattle are considered to be a reservoir host of *Escherichia coli* O157:H7 and contaminated foods of bovine origin are important vehicles of human infection. In this study, the susceptibility of calves to experimental *E. coli* O157:H7 infection following low oral exposures was determined. Two of 17 calves exposed to very low (<300 c.f.u.) doses, and 3 of 4 calves exposed to low (<10000 c.f.u.) doses, subsequently excreted the challenge strains in their faeces. All calves ($n = 12$) sharing isolation rooms with calves that excreted the challenge strain in their faeces similarly began faecal excretion of the same strains within 21 days or less. The identity between the challenge strains and the strains excreted in calf faeces was confirmed by restriction digestion electrophoretic patterns using pulsed field gel electrophoresis. Calves shed *E. coli* O157:H7 in their faeces after very low dose exposures at concentrations ranging from $< 30$ to $> 10^7$ c.f.u./g, and for durations similar to the values previously reported for calves challenged by larger doses. The susceptibility of calves to infection following very low exposures or direct contact with infected calves has important implications for programmes for pre-harvest control of this agent.

## INTRODUCTION

*Escherichia coli* O157:H7 infection may result from ingestion of foods of bovine origin contaminated at slaughter or processing [1]. Cattle excreting *E. coli* O157:H7 are typically clinically normal [2–4] with no significant intestinal lesions or identifiable foci of colonization within the gastrointestinal tract [2–5]. With longitudinal sampling, *E. coli* O157:H7 is found on most cattle farms, although the prevalence of infected cattle varies widely both between herds and within herds over time [2, 3]. Peaks of prevalence of infected cattle occur seasonally during the summer and early autumn [3, 6, 7], but winter months are characterized by very low prevalence of infection. Excretion of *E. coli* O157:H7 by cattle following natural and experimental exposure has been reported to persist for periods of weeks to months [4, 5, 8, 9].

Within herds, periods of high prevalence infection of cattle with *E. coli* O157:H7 could plausibly be explained by general exposures such as contaminated feeds or water sources, or by limited exposures initially infecting only a small number of animals that then amplify the agent for epidemic transmission. This study was designed to assess the dose of orally administered *E. coli* O157:H7 required to infect calves.

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## METHODS

### Bovine isolates of *E. coli* O157:H7 for challenge

Nine isolates of bovine-origin *E. coli* O157:H7 expressing Shiga-like toxins I and/or II [3] with known, easily distinguishable restriction digestion electrophoretic patterns (RDEP) after *Xba*I digestion and pulsed field gel electrophoresis [11] were subjected to selection for nalidixic acid resistance (20 mg/l [12]) prior to use for calf challenges in this study. Within each challenge experiment, each calf received a different challenge strain. In re-challenge experiments, each challenge was made with a different strain for each calf. Challenge doses were prepared by estimation of bacterial numbers by spectrophotometry and confirmed by subsequent plate counts. At each calf challenge, triplicate doses were prepared including one dose for the actual challenge and the other two for colony count plating. In the initial round of challenges, three calves each were challenged by direct inoculation of a 1 ml dose into the caudal oral cavity, by absorption of the dose into 100 g of the calves' grain mix, or by addition of the dose to 1 l of water administered by oral drench. All subsequent challenges were administered by direct inoculation of 1 ml doses directly into the caudal oral cavity.

### Detection of *E. coli* O157:H7 in calf faeces

Four different methods were used to isolate *E. coli* O157:H7 from calf faecal samples, including two methods utilizing the nalidixic acid resistance of the challenge strains and two methods suitable for detecting either wild type or nalidixic acid resistant strains. Method 1: Faecal samples (1·0 g) were serially decimally diluted with sterile water, and 300 $\mu$l of each dilution was plated on tryptic soy agar plates (TSA, Difco 0370-17-3, Detroit, MI) containing 20 mg/l nalidixic acid (US Biochemical Corp., Cleveland, OH) (TSA–NAL). Method 2: Faecal samples (1·0 g) were incubated (37 °C) overnight in 9 ml tryptic soya broth (TSB, Difco) containing 20 mg/l nalidixic acid, followed by plating on TSA–NAL. Method 3: Faecal samples (swabs, approximately 0·1 g) were incubated (37 °C) overnight in modified TSB followed by plating on sorbitol MacConkey agar containing cefixime and tellurite, as described [4]. Method 4: Faecal samples (swabs, approximately 0·1 g) were subjected to immunomagnetic separation using a commercial system (Dynabeads, Dynal, Oslo, Norway) as described [8, 13]. Methods 3 and 4 were used to screen faecal samples from newly purchased calves prior to challenge to detect possible pre-existing infections with *E. coli* O157:H7, as well as on all post-challenge calf samples to determine analytic sensitivity. The proportions of positive tests by methods 3 and 4 on calf faecal samples obtained during the period of excretion (define by the results of methods 1 and 2) were compared by $\chi^2$ test. Methods 3 and 4 would also have served to identify infection with wild-type (nalidixic acid susceptible) *E. coli* O157:H7 occurring during the study, but none was isolated.

### Experimental calves

Ten-week-old weaned Holstein calves were purchased, grouped under isolation, and fed a mixed grain ration with free choice access to cubed alfalfa and water. Faecal samples were obtained from each calf (1) on 3 occasions at intervals of 3 days or longer during the 2-week period prior to experimental inoculation, (2) at 3, 6, and 10 days post-inoculation, and (3) at intervals thereafter (as described for each challenge experiment) until 3 sequential negative samples were obtained or until the calf was euthanized. All pre-inoculation faecal samples were culture negative for *E. coli* O157:H7 isolation by all methods, confirming the absence of pre-existing wild-type *E. coli* O157:H7 infections as well as the lack of a nalidixic acid resistant flora that might interfere with quantitation of the challenge strains.

### Low dose calf challenge experiments

Calf challenge experiments were performed in isolation rooms that were divided into two pens by a solid steel fence panel that prevented direct calf-to-calf contact but allowed passage of aerosols and small amounts of urine and faecal matter from pen to pen via the spaces around the fence panel. Each isolation room was cleaned and disinfected prior to each experiment.

In the first set of challenges, 9 calves were placed in groups of 3 into both pens of 1 isolation room and 1 pen of another isolation room. In the second set of challenges, 8 calves were placed in groups of 2 into both pens of both isolation rooms. Challenges were performed by diluting the challenge doses into 1 ml volumes, which were administered either by syringe directly into the caudal oral cavity (23 calf challenges), or by absorption into 100 g boluses of grain feed (5

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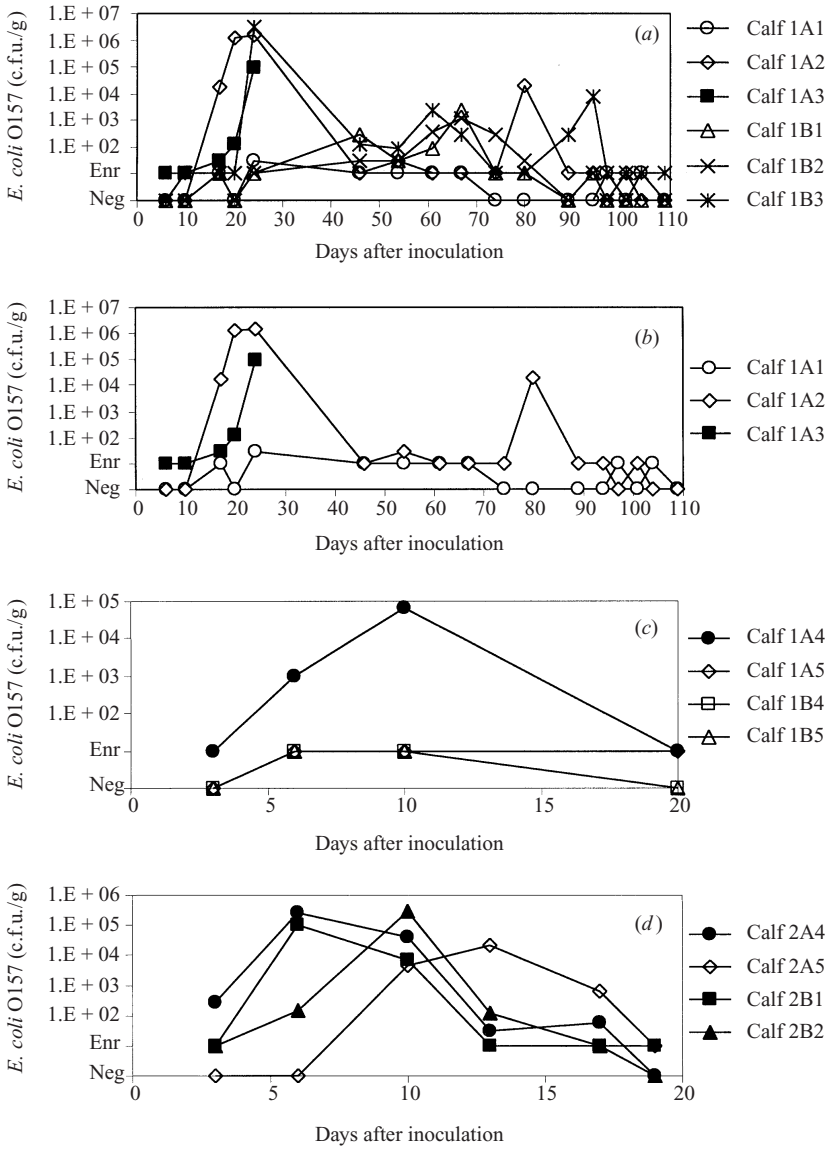


**Fig. 1(a–d).** Faecal excretion of *E. coli* O157:H7 by experimentally challenged calves orally inoculated with 100–400 c.f.u. (*a–c*) or 6000–11000 c.f.u. (*d*). Calves that excreted the same *E. coli* O157 RDEP strain with which they had been challenged are depicted in solid symbols. (*a*) All calves in both pens A and B excreted only the strain used to challenge calf 1A3. (*b*) After calf 1A3 was moved into pen B of isolation room 2 on day 24, the strain it was excreting was subsequently isolated from faeces of all other calves in isolation room 2. (*c*) All calves in both pens A and B excreted only the strain used to challenge calf 1A4. (*d*) Calves 2A4, 2B1 and 2B2 excreted their respective challenge strains beginning on day 3, and calf 2A5 excreted the strain used to challenge (and infect) calf 2A4 beginning on day 10. Enr indicates samples that were only culture positive by Method 2. Neg indicates samples that tested negative by all culture methods.

calf challenges) or diluted into 1 l water drenches (5 calf challenges).

### Restriction digestion electrophoretic patterns (RDEP)

RDEP determination of isolates using pulsed gel electrophoresis, using routine methods [3, 8], enabled unambiguous determination of the source of infection for each calf including calves exposed by oral challenge inoculations and calves exposed by sharing rooms with calves excreting *E. coli* O157 in their faeces.

## RESULTS

### Infection resulting from low dose challenge

In the first set of experimental challenges, NalR *E. coli* O157 was detected in the faeces of calf 1A3 (challenge dose, 260 c.f.u.) at 6 days after challenge, and from the other 5 calves sharing isolation room 1 with calf

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Table 1. *Detection of* E. coli *O157 in bovine faeces by different enrichment culture and isolation techniques*

| *E. coli* O157:H7* (c.f.u./g) | No. | Method 2† No. (%) | Method 3† No. (%) | Method 4† No. (%) |
|---|---|---|---|---|
| < 30 | 54 | 43 (80) | 10 (19) | 38 (70)‡ |
| 30–3000 | 32 | 31 (97) | 14 (44) | 25 (78)‡ |
| > 3000 | 32 | 32 (100) | 31 (97) | 31 (97) |
| All | 118 | 106 (89·8) | 55 (46·6) | 94 (79·7)‡ |

* *E. coli* O157:H7 concentration in faeces as determined by direct plating enumeration (30 or more c.f.u./g) or only after broth enrichment (< 30 c.f.u./g). The latter includes results from all samples taken during the period of infection, including some samples negative by all methods.
† Number (percentage) of samples yielding *E. coli* O157 isolates using broth enrichment in nalidixic acid (Method 2), overnight broth enrichment cultures plated on SMAC-CT (Method 3) and IMS plated on SMAC-CT (Method 4).
‡ Method 4 significantly different from Method 3 ($\chi^2 = 26{\cdot}3$, $P < 0{\cdot}01$).

1A3 between 10 and 17 days after challenge. The 3 calves in isolation room 2 remained negative on faecal culture by all test methods through 6 sequential faecal samples over 24 days.

Following 4 sequential positive faecal samples, calf 1A3 was moved, at 24 days after challenge, to the empty isolation room 2 pen adjacent to that containing the 3 calves that had remained culture negative after challenge. REDP analysis of the isolates from the first isolation room demonstrated that all calves were excreting the REDP strain used to challenge calf A3. At this point, faecal sampling of the calves in both isolation rooms was resumed, and the 3 calves in the second isolation room were also found to be excreting the REDP strain used to challenge calf 1A3.

In the second set of experimental challenges, the 4 calves in the first isolation room began excreting NalR *E. coli* O157 by day 3 (calf 1A4, challenged with 210 c.f.u.) or day 6 (the other 3 calves) after challenge. The REDP of the *E. coli* O157 isolates from all 4 of these calves matched that used to challenge calf 1A4. No faecal excretion of NalR *E. coli* O157 was detected in samples from the 4 calves in the second isolation room.

After the uninfected calves in the second isolation room remained uninfected through three additional rounds of challenge with very low (< 500 c.f.u.) doses, a fifth round of challenges with doses approximating 10000 c.f.u. was then conducted. NalR *E. coli* O157 was detected in the faeces of calves 2A4 (challenge dose, 8000 c.f.u.), 2B1 (5000 c.f.u.), and 2B2 (9000 c.f.u.) when sampled on day 3 after challenge, and in the faeces of calf 2A5 sampled on day 10 after challenge. REDP analysis demonstrated that calves 2A4, 2B1, and 2B2 each excreted the strains with which they had been challenged, while calf 2A5 excreted the strain used to challenge calf 2A4.

### Duration of infection

The calves in the first set of challenges excreted NalR *E. coli* O157 consistently through the first approximately 70 days after challenge, and intermittently thereafter. At necropsy on day 118 after challenge, NalR *E. coli* O157 was isolated from faeces and colonic and caecal contents of 3 (1A3, 2A1 and 2A2) calves, while the other 6 calves were culture negative. In the second round of challenges in isolation room 1, NalR *E. coli* O157 was isolated from faeces and colonic and caecal contents of calf 1A4 at necropsy, while the other 3 calves were culture negative. In the second set of challenges in isolation room 2, NalR *E. coli* O157 was isolated from faeces and colonic and caecal contents of calves 2A4, 2A5, and 2B1 at necropsy on day 22 after challenge, while all samples from calf 2B2 were culture negative.

### Concentration of *E. coli* O157:H7 in faeces of infected calves

Calves infected by either low dose exposures or by calf-to-calf transmission excreted *E. coli* O157:H7 at levels ranging from < 30 to > $10^6$ c.f.u./g (Fig. 1). Using quantitative plating on nalidixic acid containing plates, infected calves were determined to excrete the organism at < 30 c.f.u./g in 45·8% of samples, 30–300 c.f.u./g in 27·1% of samples, and > 300 c.f.u./g in 27·1% of samples (Table 1).

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### Sensitivity of detection of *E. coli* O157:H7 faecal excretion

One hundred and eighteen faecal samples from calves during their infected period were tested by broth enrichment culture with (Method 4) and without (Method 3) immunomagnetic separation (IMS, [4, 8]) prior to plating on CT-SMAC agar. IMS (Method 4) increased the proportion of successful isolation overall ($\chi^2 = 26{\cdot}3$). However, the increased sensitivity of IMS appeared to be limited to samples in which NalR *E. coli* O157 was present at 3000 c.f.u. or less (Table 1). Overall, 51 samples were culture positive by both Methods 3 and 4, 43 were positive by Method 4 but negative by Method 3, 4 were positive by Method 3 but negative by Method 4, and 20 were negative by both Methods 3 and 4.

## DISCUSSION

The most significant result of this study is the clear demonstration that low dose exposures have the potential to result in faecal excretion of the exposure strain in high numbers for prolonged periods. The limited numbers of calves preclude using these data to estimate the percentage of calves susceptible to infection from low dose exposures. However, these results provide a reasonable basis for expecting that at least some calves susceptible to low dose infection with *E. coli* O157:H7 are to be expected on cattle farms with hundreds or more animals. Cray et al. showed that *E. coli* O157:H7 challenge doses of $10^7$ c.f.u. are insufficient to infect all adult cattle, and that doses of $10^4$ c.f.u. did not infect four adult cattle [5]. Similarly, in previous studies in our laboratory, not all calves challenged with a dose of $10^8$ c.f.u. became infected (data not shown). These previous results are not inconsistent with the findings reported here, in that both dose- and age-related effects on the probability of bovine infection after challenge are likely. For example, calves have higher prevalence of natural *E. coli* O157:H7 infection and excrete the agent at higher levels following infection [2, 3, 5] than older cattle. Additional factors that may affect *E. coli* O157:H7 infectivity include strain differences, dietary effects and seasonal effects [3, 9].

A second important finding of this study was that calves housed in the same room with calves excreting NalR *E. coli* O157 subsequently began faecal excretion of the same strain, in some cases in similarly high numbers or for similarly long durations. This finding was interpreted as calf-to-calf transmission of *E. coli* O157. In all cases, the first calves within each room to shed NalR *E. coli* O157 shed the strain with which they had been challenged, and the other calves in the room began excreting that strain in their faeces only subsequently. In the case of the second isolation room in the second set of challenges, where a somewhat higher ($\sim$ 10000 c.f.u.) challenge dose was used, calf (2A5) began shedding the strain used to challenge 2A4 (with which it shared the same pen, and which had been excreting its challenge strain in higher numbers in the samples taken prior to detection of *E. coli* O157 in the faeces of 2A5). Apparent transmission of *E. coli* O157:H7 infection from challenged lambs to their dams has been shown previously [10], but the source of infection in that experiment was less clearly demonstrated than in this study. The relatively efficient transmission of *E. coli* O157 from calf to calf demonstrated here strongly suggests that such transmission could play an important role in increasing the prevalence of cattle excreting this agent on farms.

The duration of faecal excretion of the exposure strains of NalR *E. coli* O157 following very low dose exposures falls within the range of those previously reported for calves in the same age range infected by higher doses [5], and those reported for naturally infected calves on farms [6, 8]. Calves exposed by sharing rooms with calves excreting NalR *E. coli* O157 also excreted the organism for at least 6 weeks, and three of the calves were still infected at necropsy 10–16 weeks after colonization (Fig. 1).

The specific exposures that resulted in this apparent calf-to-calf transmission of infection within isolation rooms were not identified. The transmission occurred both within ($n = 6$) and between ($n = 6$) pens within isolation rooms. The solid pen dividers prevented nose-to-nose contact or mutual grooming, and feed trays and water bowls were not shared between pens. Potential routes of transmission across pens include aerosols as well as small amounts of faeces and urine that may have passed through the small (1/2 inch) spaces under the pen dividers. We interpret these observations as supporting the result of the direct challenge experiments: that low exposure doses of *E. coli* O157 are sufficient to allow this organism to establish itself in the calf gastrointestinal tract and be excreted in the faeces.

Considerable variability was observed in the numbers and durations of *E. coli* O157 excretion in calf faeces in these experiments. In particular, although calves 1A1, 1A5, 1B4 and 1B5 were detected

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excreting *E. coli* O157 on numerous occasions, the bacterium was never detected in numbers of 100 c.f.u./g or higher. In contrast, all other calves in these experiments were detected excreting *E. coli* O157 on one or more occasions at 1000 c.f.u./g or higher. The low numbers excreted by these calves raise the possibility that gastrointestinal colonization either did not occur or was more limited in extent in these calves than in most of their cohorts. If this phenomenon also occurs in groups of cattle on farms, it could account in part for the higher prevalence of faecal excretion detected by more sensitive methods such as IMS. In this study, culture methods without IMS were markedly inferior at detecting *E. coli* O157 when it was present at $< 30$ c.f.u. (Table 1).

The key findings of this study are that some calves may begin prolonged, high level faecal shedding of *E. coli* O157:H7 after only very low exposure doses, and that other calves exposed to calves excreting *E. coli* O157 in their faeces are at high risk of acquiring this agent. These findings suggest that temporal clustering of bovine *E. coli* O157:H7 excretion observed in on-farm studies [3] could result from an initial infection of a small number of animals susceptible to low-dose infection, followed by large scale transmission.

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