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Prevalence of *Escherichia coli* O157:H7 in range beef calves at weaning

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SUMMARY

This study was designed to determine the prevalence of *Escherichia coli* O157:H7 infection of beef calves at weaning, prior to arrival at the feedlot or mixing with cattle from other sources. Fifteen range cow-calf herds, which weaned calves in October and November, were sampled in Kansas, Missouri, Montana, Nebraska and South Dakota. Faecal culture for *E. coli* O157:H7 was performed and anti-O157 serum antibody titres were determined by blocking ELISA. Thirteen of the 15 herds (87%) were found to have at least one positive isolation of *E. coli* O157:H7 in faecal samples. Within positive herds, prevalence ranged from 1·7–20·0%, with an average of 7·4 $\pm$ 6·2% S.D. of individual animals shedding *E. coli* O157:H7 in faeces. All herds had high prevalence of anti-O157 antibodies, ranging 63–100% of individuals within herds seropositive. This study indicates that *E. coli* O157:H7 infection before weaning, prior to entry into feedlots, is widespread. Furthermore, serologic evidence suggests that most calves (83%) and all herds (100%) have been exposed to *E. coli* O157.

INTRODUCTION

Escherichia coli O157:H7 is a recently emerged pathogen which is capable of causing severe disease and death in humans [1]. Serious outbreaks as well as sporadic cases of *Escherichia coli* O157:H7 infection resulting in haemorrhagic colitis, thrombocytopaenia and haemolytic uraemic syndrome have been reported with increasing frequency since 1982 [1–3]. Outbreaks have been associated with ground beef as well as various other foods, such as apple cider, which may have been contaminated with bovine faeces [2, 4–14]. Epidemiologic studies indicate that a significant proportion of cattle herds worldwide contain individuals which shed *E. coli* O157:H7 in their faeces, suggesting a serious potential risk for meat and environmental contamination [1, 15–23]. The gastrointestinal tract of clinically healthy cattle is a major reservoir of *E. coli* O157:H7 [1, 15, 24]. However, since faecal shedding of *E. coli* O157:H7 by cattle is intermittent, the actual prevalence of infection and associated risks, are likely to be higher than that estimated by faecal culture [25–27].

One of the basic principles of a hazard analysis critical control point (HACCP) approach to preharvest control of foodborne pathogens is to identify points in the chain of transmission with the greatest potential for animal infection [28]. Previous studies have demonstrated that the highest prevalence of faecal shedding of *E. coli* O157:H7 in feedlot cattle occurs early in the feeding period, in the weeks immediately following weaning [29, 30]. One possible explanation for this observation is rapid transmission of the bacteria from a small number of patently shedding individual calves to a large number of naive animals, possibly influenced by the stresses of weaning,

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dietary changes, transport, sorting and mixing, similar to bovine respiratory disease complex pathogenesis [29–32]. Alternatively, the high prevalence early in the feeding period may simply reflect infection of a large number of calves prior to weaning. To test this alternative hypothesis, 15 range cow-calf herds were sampled at weaning to estimate the prevalence of faecal shedding of *E. coli* O157:H7 and the seroprevalence of antibodies to O157.

METHODS

Herds and sampling

Fifteen range cow-calf herds, which weaned calves in October and November 1997, were identified. The herds were distributed across five major beef cattle producing states: Kansas, Missouri, Montana, Nebraska and South Dakota. A questionnaire covering ranch resources and herd management was completed by each cooperating producer. Sixty calves from each herd were randomly selected for sampling. As calves were separated from cows at weaning, cooperating veterinarians obtained rectal grab samples of faeces using a freshly gloved hand for each sample. Faecal samples were placed in sterile plastic bags on ice for transport to the laboratory. A 9 ml blood sample was also obtained from each calf sampled by jugular venipuncture using Sarstedt clot tubes (Sarstedt, Inc., Newton, NC). All samples and questionnaires were coded to blind laboratory personnel to the source of the samples. In addition, sequential serum samples, starting within 24 h of birth and then approximately every 6 weeks thereafter until weaning, were available from calves in one of the herds. These samples were used to estimate force of infection.

Culture methods

To maximize sensitivity of detecting positive animals, two culture methods were used in parallel on each faecal sample: direct plating with immunodetection, and immunomagnetic bead enrichment with plating on selective media. For each faecal sample, a 10% faecal suspension was prepared by homogenizing 10 g of faeces in 90 ml of GN broth (Fisher Scientific, St Louis, MO) containing vancomycin (8 mg/l, Sigma, St Louis, MO), cefixime (0·5 mg/l, Lederle Laboratories, Pearl River, NY) and cefsuludin (10 mg/l, Sigma). For direct plating, 100 μl of this suspension was immediately plated on sorbitol–MacConkey (SMAC) agar plates. After incubation overnight at 37 °C, colony lifts were prepared on nitrocellulose membranes by standard techniques [33]. A minimum of three suspect colonies, detected using two monoclonal antibodies (MAb) with specificity for O157 lipopolysaccharide, MARC 13B3 and MARC 19F8, were picked for further characterization [34]. The remaining suspension was incubated at 37 °C for 6 h followed by anti-O157 immunomagnetic bead enrichment performed according to manufacturer's instructions (Dynal, Inc., Lake Success, NY). The enriched cultures were plated on SMAC plates containing cefixime (0·5 mg/l, Lederle Laboratories, Pearl River, NY) and potassium tellurite (2·5 mg/l, Difco Laboratories, Detroit, MI, SMACct). After 18 h incubation at 37 °C, a minimum of three sorbitol-negative colonies, if present, were picked as suspect O157:H7 isolates for further characterization. Isolates were confirmed as O157:H7 serologically using MAbs to O157 and H7 antigens [34, 35]. Numbers of colony-forming units (c.f.u.) were estimated from the number of O157 positive colonies on plates from calves with confirmed isolates.

Pulsed-field gel electrophoresis (PFGE)

Genetic relatedness of isolates confirmed as O157:H7 was defined by PFGE [36]. Briefly, isolates were grown in LB broth to an optical density of about 0·8 at 600 nm. Chloramphenicol was added to 180 μg/ml, the cells incubated for 30 min, washed and resuspended in buffer (10 mM Tris, 20 mM NaCl, 50 mM EDTA, pH 7·2), mixed with an equal volume of 2% low melting point agarose (Aquapor ES, National Diagnostics, Atlanta, GA) and aliquoted into plug molds. When the plugs were set, they were placed in five volumes of buffer (10 mM Tris, 50 mM NaCl, 0·2% Na deoxycholate, 0·5% Na lauryl sarcosine) containing 1 mg/ml lysozyme and incubated for 1 h at 37 °C. Plugs were washed and incubated with 1 mg/ml proteinase K in 100 mM EDTA, 0·2% Na deoxycholate, and 1·0% Na lauryl sarcosine overnight, followed by four 1 h washes in 20 mM Tris-50 mM EDTA, pH 8·0, the third wash also containing 1 mM phenylmethylsulphonyl fluoride. Plugs were stored at 4 °C until used. Plugs were equilibrated with *Xba*I buffer for 1 h, the buffer replaced and 50 units of *Xba*I added followed by 2–4 h incubation at 37 °C. Plugs were rinsed in 0·5% (TBE) and transferred to a 1% agarose gel. PFGE was

performed using a CHEF-DR II (Bio-Rad Laboratories, Hercules, CA) for 23 h at 6 V/cm using a linear ramped pulse time of 7–52 s with a single step change from 15 to 20 s at 10 h. Lambda phage DNA standards (New England Biolabs, Beverly, MA) as well as digested plugs containing a standard *E. coli* O157:H7 isolate (ATCC 43895) were included in each gel. Gels were stained with ethidium bromide (1 μg/ml) and photographed. Restriction fragment patterns were compared visually, and by molecular weight of fragments determined using SigmaGel software (SPSS Inc., Chicago, IL). Isolates with similar fragment patterns were designated as a group [37].

Blocking ELISA (bELISA)

Serum anti-O157 antibody titres were determined by bELISA as previously described [38]. Briefly, optimal concentrations of O157 lipopolysaccharide (LPS) and MARC 13B3 MAb were determined by checkerboard titration to provide near maximal binding of MARC 13B3. Plates were coated with highly purified LPS diluted in 0·5 mM carbonate buffer, pH 9·6 for 1·5 h at 37 °C, then washed 5× with phosphate buffered saline–0·1% Tween 80-0·5% horse serum (PBS-T-HS). Sample sera were diluted twofold in PBS-T-HS and 100 μl added to each well, incubated for 45 min at 37 °C then plates were washed 6× with PBS-T-HS. Ascites containing MARC 13B3 (100 μl of a 1:7000 dilution) was added to each well and incubated for 15 min at 37 °C, the plates washed 6× with PBS-T-HS and bound MARC 13B3 detected by addition of 100 μl of peroxidase labeled rabbit anti-mouse IgG diluted 1:1500 (Kirkegaard & Perry Laboratories, Gaithersburg, MD), incubating for 15 min, washing 8× with PBS-T-HS then adding 100 μl ABTS for 15 min. Reaction was stopped by adding 50 μl of 1% SDS. Results were expressed as percent inhibition of MARC 13B3 binding relative to foetal calf serum (FCS) according to the following formula:

$$\frac{(OD_{405/490}\ [FCS] - OD_{405/490}\ [sample])}{OD_{405/490}\ [FCS]} \times 100 = \%\ \text{inhibition}.$$

Cut-off values were determined on a subset of experimentally infected cattle sera by non-parametric Receiver-Operator Characteristic (ROC) analysis [39]. Titres were defined as the reciprocal of the highest twofold dilution of test serum resulting in percent inhibition greater than 50% of that of FCS alone. Animals were considered seropositive if they had bELISA titre ($-1/\log_2$) greater than or equal to 2. All sera were tested in duplicate.

Statistical analysis

Descriptive statistics and correlations were determined using the Astute software module (University of Leeds, UK) for Excel 5.0 (Microsoft, Bellevue, WA). Force of infection was determined from serum antibody titres [40, 41].

RESULTS

Cooperating herds ranged in size from 100 to > 500 cow-calf pairs. All of these herds were on open range grazing systems, principally on grass with alfalfa supplementation in some herds. All but one herd were on dryland grass pastures, the remaining herd grazed irrigated pastures. Only one herd grazed pastures on which manure spreading was practiced. Most herds had multiple types of water supplies, with 73% of herds primarily using watering tanks and pumped well water, with the remainder of the herds using surface water (ponds and/or creeks). Peak calving in all herds was in March (12 herds) and April (3 herds). Introduction of new animals into herds was uncommon in this sample, with 11/15 of herds having purchased no new animals in the past 4 years or more. Calves were weaned in October and November 1997.

A total of 22 out of 900 samples were not analysed. Of 878 samples cultured, 61 were positive for *E. coli* O157:H7 (6·9% Table 1). Faecal shedding of *E. coli* O157:H7 was detected in 13 of the 15 herds sampled for an 87% herd prevalence (Table 1). Prevalence of faecal shedding in positive herds ranged from 1·7 to 20%, with a median of 6·8% of calves shedding. Average numbers of *E. coli* O157:H7 in faecal samples, estimated by direct plating–immunostaining, ranged from 4×10^3 to $1{\cdot}3 \times 10^7$ c.f.u./g (Table 1). Sixteen PFGE patterns were identified, with most herds having one or two closely related patterns, but five herds had isolates with two distinct patterns (Table 1). Two herds had isolates with identical PFGE patterns. Five individual animals shedding *E. coli* O157:H7 of more than one PFGE group were identified from four herds.

A majority of animals in all herds had serum antibodies to O157 antigen (Table 1). Seroprevalence ranged from 63·3 to 100%. The proportion of animals

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Table 1. *Prevalence of* E. coli *O157:H7 in faecal samples, and antibodies to O157 antigen of calves at weaning* (*titers* $\geqslant$ *1:8 were considered high titres*)

Herd	*n*	% Seropositive	% High titre	% Culture positive	Mean c.f.u./g	PFGE groups
A	60	75·0	41·7	18·3	$2{\cdot}8 \times 10^4$	I
B	40	95·0	57·5	12·5	$1{\cdot}3 \times 10^7$	II, III
C	60	83·3	55·0	13·3	$4{\cdot}2 \times 10^4$	IV
D	60	86·7	43·3	20·0	$3{\cdot}4 \times 10^4$	I
E	60	80·0	35·0	0	NA	NA
F	60	63·3	28·3	1·7	$4{\cdot}0 \times 10^3$	V
G	60	76·7	18·3	1·7	$6{\cdot}7 \times 10^4$	VI
H	60	85·0	36·7	0	NA	NA
I	59	84·7	30·5	6·8	$3{\cdot}1 \times 10^4$	VI, VII
J	60	100·0	55·0	8·3	$4{\cdot}3 \times 10^4$	VIII
K	60	93·3	56·7	8·3	$1{\cdot}9 \times 10^4$	IX, X
L	60	90·0	33·3	5·0	$1{\cdot}8 \times 10^4$	XI, XII
M	60	91·7	21·7	5·0	$7{\cdot}3 \times 10^4$	XIII
N	60	75·0	28·3	6·7	$2{\cdot}3 \times 10^4$	XIV, XV
P	59	76·3	30·5	3·4	$4{\cdot}8 \times 10^4$	XVI
Mean		83·7	38·1	7·4	$9{\cdot}9 \times 10^5$	
S.D.		9·6	12·9	6·2	$3{\cdot}5 \times 10^6$	
Median		84·7	35·0	6·7	$3{\cdot}1 \times 10^4$	

NA, no isolates to test.

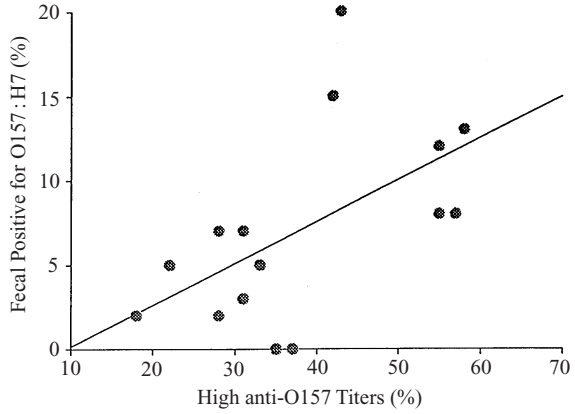

Fig. 1. Spearman rank correlation between herd prevalence of faecal shedding of *E. coli* O157:H7 and herd prevalence of high anti-O157 titres ($\geqslant$ 1:8) ($P = 0{\cdot}015$, $r_s = 0{\cdot}613$).

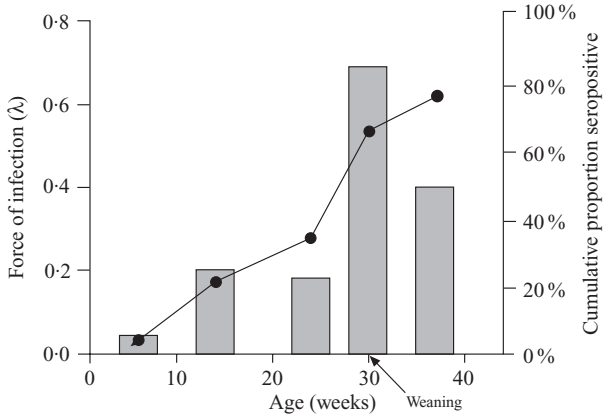

Fig. 2. Cumulative incidence of seroconversion to O157 antigen and force of infection in a cohort of calves from an *E. coli* O157:H7 positive herd ($n = 46$).

with high titres ($\geqslant$ 8) ranged from 18·3 to 56·7%. There was a significant positive correlation between prevalence of faecal shedding within a herd and prevalence of high anti-O157 antibody titres in serum ($P = 0{\cdot}026$, Fig. 1). Initial seroconversion to O157 was used to estimate force of infection and cumulative incidence on the herd for which serial serum samples were available (Fig. 2). Force of infection was highest late in the summer, prior to weaning, and declined during 6 weeks on feed. The cumulative incidence of seroconversion indicates that, in this herd, the majority of calves had been exposed to O157 prior to weaning (Fig. 2).

DISCUSSION

The results of this study indicate that a significant proportion of calves from range beef herds are shedding *E. coli* O157:H7 in faeces at weaning. Furthermore, most herds have at least one faecal-positive animal and all herds had serologic evidence of exposure. It is difficult to compare studies on faecal

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shedding of *E. coli* O157:H7 due to variability in populations, sampling and culture techniques. The variability of populations relative to faecal shedding of *E. coli* O157:H7 even within a study is emphasized by the range of numbers of these bacteria in faeces in this study (Table 1). However, it appears that a significant proportion of *E. coli* O157:H7 faecal shedding occurs during the early feeding period and may reflect extension of infections acquired on range, rather than new infections acquired at the feedlot or the effects of transport, sorting, feed change or other stressors inherent in placing cattle on feed [30]. A decrease in force of infection in the period immediately following placement on feed, indicating proportionately fewer new cases amongst susceptibles, further supports this conclusion.

The majority of calves in this study were seropositive to O157 antigen. Detection of high levels of *E. coli* O157:H7 exposure in range beef calves prior to weaning provides some insight into the ecology of this infection. Most calves in this study were raised on dryland grass pasturage, without manure spreading or irrigation. Calves were not confined to dry lots or otherwise confined to high density areas which might enhance transmission of *E. coli* O157:H7. Two sources of primary transmission appear likely. First, direct or indirect maternal transmission is possible. Calves are in close contact with cows and, in one small study, nearly 100% of cows were seropositive for *E. coli* O157 (data not shown). While it is not clear that cows routinely experience recrudescence of faecal shedding, it is known that intermittent shedding can occur, even under controlled conditions, over prolonged periods of time [42]. The factors which initiate intermittent shedding are not known but dietary stress has been implicated [32]. The effect of perinatal events, such as parturition and lactation, on shedding of *E. coli* O157:H7 has not been determined. *E. coli* O157:H7 can persist in faeces for up to 70 days [43]. Indirect transmission through contamination of teats with faecal material from the dam, other cattle in the herd or non-bovine sources such as wildlife would appear to be an efficient means of transmission to nursing calves. A second potential means of transmission is via water, either through contaminated water tanks or surface water. Prolonged recovery of *E. coli* O157:H7 from experimentally inoculated water tanks and environmental water samples has been reported [44, 45]. Stock tanks and surface water sources may represent efficient means for spread of bacteria to naive calves, although the importance of this mode of transmission between calves is undetermined as yet.

Results of serologic testing indicate that exposure of calves to *E. coli* O157:H7 is far greater than estimates derived from studies based on bacterial isolation suggest. A similar result was obtained in a study which found prevalence of neutralizing antibodies against shiga-like toxins in cattle to be higher than the prevalence of shiga-like toxin-producing bacteria isolation from faeces [46]. The serologic test used in this study is highly specific for O157 antigen, lacking the cross-reactivity inherent in other serologic tests for O157 [38, 47]. However, *E. coli* isolates other than *E. coli* O157:H7, as well as other bacterial species such as *E. hermanii* and group N *Salmonella* species, may bear the O157 antigen [48, 49]. Thus, it is conceivable that the serologic responses observed in these calves resulted from exposure to bacteria other than *E. coli* O157:H7. Colony lift immunoblots of direct faecal cultures on MacConkey agar, which should support the growth of most of the known bacterial species which bear the O157 antigen, only detected three O157 positive non-O157:H7 isolates out of 878 samples tested, far fewer than the 61 isolates confirmed as *E. coli* O157:H7. Furthermore, there was a significant association between prevalence of high anti-O157 serum antibody titres and herd prevalence of *E. coli* O157:H7 shedding in faeces (Fig. 1). While it is possible that bacteria other than *E. coli* O157:H7 contributed to the seroprevalence of anti-O157 serum antibodies, the evidence described above, along with the high herd prevalence of *E. coli* O157:H7 isolation, strongly suggest that the observed seroprevalence is reflective of actual exposure to *E. coli* O157:H7.

E. coli O157:H7 appears to be ubiquitous in those dairy cattle herds and feedlots which have been studied [30, 50]. Backgrounding operations and feedlots collect calves from a number of sources with potential for exposure of calves to microorganisms which they may not have experienced previously. It is logical to assume that this mixing of calves and subsequent transmission of pathogens is responsible for the widespread infection with *E. coli* O157:H7 present in feedlot cattle. However, range beef herds had not been evaluated systematically prior to this study. Range cow-calf herds tend to be isolated relative to other stages of beef production. The fact that nearly three quarters of the herds participating in this survey had not introduced new cattle into their herds in the last four or more years supports this

contention. Furthermore, range cow-calf operations tend to be extensive rather than intensive management systems, with cattle grazing over large tracts of land, as opposed to high-density penning. Thus, it is somewhat surprising that essentially all herds in this survey had evidence of widespread exposure to *E. coli* O157:H7. This suggests that not only does this bacteria transmit efficiently within herds but that there must exist a mechanism other than movement of cattle by which *E. coli* O157:H7 is introduced into such herds. The presence of similar PFGE patterns among isolates from geographically distinct herds, observed in this study and others, would also seem to indicate that such mechanisms exist [25, 51, 52]. A source of infection external to the ranching operation, other domestic animals, water, wildlife, feed, humans, etc., may be responsible for introduction of *E. coli* O157:H7 into these herds. Several wildlife species, especially deer, are known to become infected with *E. coli* O157:H7 [4, 52–54]. Through normal migration, as well as sharing of feed and water sources, transmission from wildlife to cattle and vice versa may occur. Contaminated surface water has been associated with outbreaks of *E. coli* O157:H7 infection in humans and could contribute to introduction into cattle herds [55–57]. Similarly, human-to-human transmission via asymptomatic carriers has been documented and we must assume that human to cattle transmission is possible [1, 58]. Which of these mechanisms is responsible for dissemination of *E. coli* O157:H7 is not known and may be unknowable, given that introduction is likely a rare event, there are no clinical signs in cattle to indicate a new introduction, the bacteria is likely to be non-homogenously distributed in sources such as feeds, and infectious doses for calves may be less than 250 c.f.u. (T. E. Besser, unpublished observation).

The results of this study provide an alternative explanation for the higher prevalence of *E. coli* O157:H7 in fall weaned calves which have been on feed for the shortest period of time. It appears likely that a significant proportion of calves arrive at the feedlot already having been infected with *E. coli* O157:H7 and that this proportion declines during the early to mid feeding period. Thus, if one of the goals of an *E. coli* O157:H7 control strategy is to prevent initial infection in beef calves, control measures will have to be implemented in the cow-calf herd, probably quite early in the life of a calf. Further study is necessary to identify the on-farm ecological factors which might influence within and between herd transmission of *E. coli* O157:H7 to cattle and control strategies which can reduce the incidence of infection in range beef calves.

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