Shiga toxin-producing *Escherichia coli* in the feces of Alberta feedlot cattle

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Abstract

Shiga toxin-producing *Escherichia coli* (STEC) are a public health concern. Bacterial culture techniques commonly used to detect *E. coli* O157:H7 will not detect other STEC serotypes. Feces from cattle and other animals are a source of O157:H7 and other pathogenic serotypes of STEC. The objective of this study was to estimate the pen-level prevalence of Shiga toxins and selected STEC serotypes in pre-slaughter feedlot cattle. Composite fecal samples were cultured and a polymerase chain reaction (PCR) was used to detect genes for Shiga toxins (*stx*1 and *stx*2) and genes for O157:H7, O111:H8, and O26:H11 serotypes. Evidence of Shiga toxins was found in 23 pens (92%), O157:H7 in 2 (8%), O111:H8 in 5 (20%), and O26:H11 in 20 (80%) of the 25 pens investigated. Although pen-level prevalence estimates for Shiga toxins and non-O157 serotypes seem high relative to O157:H7, further effort is required to determine the human health significance of non-O157 serotypes of STEC in feedlot cattle.

Résumé

*Les isolats d'*Escherichia coli *producteurs de shiga-toxine (STEC) sont une préoccupation en santé publique. Les techniques de culture bactériologique couramment utilisées pour détecter* E. coli *O157:H7 ne permettront pas de mettre en évidence les autres sérotypes de STEC. Les fèces de bovins et d'autres espèces animales sont une source de O157:H7 de même que des autres sérotypes pathogènes de STEC. Cette étude visait à estimer chez des bovins d'embouche avant abattage la prévalence par enclos de toxine Shiga et de sérotypes choisis de STEC. Des échantillons composites de matières fécales ont été cultivés et une réaction d'amplification en chaîne par la polymérase (PCR) utilisée pour détecter les gènes codant pour la production de toxine Shiga (*stx1 *et* stx2*) et les gènes pour les sérotypes O157:H7, O111:H8 et O26:H11. La présence des gènes codant pour la toxine Shiga a été mise en évidence dans 23 enclos (92 %), ceux pour O157:H7 dans 2 (8 %), O111:H8 dans 5 (20 %) et O26:H11 dans 20 (80 %) des 25 enclos étudiés. Bien que les estimés de la prévalence par enclos pour les gènes de la toxine Shiga et des sérotypes non-O157 semblent élevés par rapport à ceux de O157:H7, des efforts supplémentaires sont requis pour déterminer l'impact sur la santé humaine des sérotypes de STEC autres que O157:H7 rencontrés chez les bovins d'embouche.*

(Traduit par Docteur Serge Messier)

Shiga toxin-producing *Escherichia coli* (STEC) are increasingly recognized as an important cause of human illness. Shiga toxins (also referred to as verotoxins or vero cytotoxins) are a principal virulence factor of STEC and are thought to account for clinical complications in humans, such as hemolytic-uremic syndrome (1). Shiga toxin-producing *Escherichia coli* can cause severe, potentially life-threatening human illness with most infections resulting from the consumption of food or water that has been contaminated with feces (1). More than 200 serotypes of *E. coli* may produce Shiga toxins, but only a subset of STEC are thought to be human pathogens (1). Although *E. coli* O157:H7 is the primary serotype of public health importance in North America, other STEC serotypes, particularly O111 and O26, have emerged as significant causes of human disease (1–3). Bacterial culture techniques that are commonly used to detect *E. coli* O157:H7 in food, water, and human clinical specimens will not detect other pathogenic STEC serotypes (1–3). Therefore, individual cases of non-O157 STEC may go unreported and the national prevalence of human infections is often very difficult or impossible to estimate (1–3).

The feces of clinically normal animals, particularly cattle, are considered a major source of *E. coli* O157:H7 and other STEC (4). Although there is considerable interest in controlling *E. coli* O157:H7 in cattle, information on the epidemiology of other serotypes of pathogenic STEC in cattle populations is sparse. Our objective was to determine the frequency at which evidence for Shiga toxins and select pathogenic STEC serotypes (O157:H7, O26:H11, and O111:H8) could be detected in feedlot pens using composite fecal samples and PCR.

The study was conducted in July 2002, in 4 feedlots in southern Alberta. Feedlot capacity ranged from 5000 to 18 000 head. All pens containing cattle within 90 d of slaughter were considered eligible for sampling. A random stratified sampling method was used to choose 25 pens for this study from a total of 77 eligible pens available in the 4 feedlots. Within each feedlot, pens were chosen randomly from available pens within each class of cattle (yearling, fall-placed calves, and winter-placed calves). The number of pens chosen per feedlot was relative to the size of the feedlot and number of eligible pens.

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Received May 13, 2003. Accepted August 28, 2003.

Table I. Prevalence of genes for Shiga toxins and Shiga toxin-producing *Escherichia coli* serotypes in composite fecal samples from pens of feedlot cattle in southern Alberta

	Feedlot pens		Feedlots[a]
Targeted genes/pathogens	Positive/total	Percent positive	Positive/total
Shiga toxins (*stx*1 and/or *stx*2)	23/25	92% (74.0% to 99.0%)[b]	4/4
O157:H7[c]	2/25	8% (1.0% to 26.0%)	2/4
O111:H8[c]	5/25	20% (6.8% to 40.7%)	2/4
O26:H11[c]	20/25	80% (59.3% to 93.2%)	4/4

[a] Feedlots were considered positive when 1 or more pens in the feedlot tested positive

[b] Figures in parenthesis indicate 95% exact confidence intervals

[c] O157:H7, O111:H8, and O26:H11 positives include those positive for serotype-specific gene targets (*eaeA* genes) and Shiga toxin genes (*stx*1, *stx*2, or *both*)

Composite fecal samples (600 g) were collected from each pen by pooling feces from 30 fresh fecal pats. Fecal pats were identified by walking pens using a predetermined pattern that began along the fence aside the gate, continued along the opposite fence, then returned diagonally to the gate. Samples were refrigerated, immediately transported to the laboratory and processed within 24 h of collection.

Bacterial culture and polymerase chain reaction (PCR) were used to determine the presence of genes for Shiga toxins (*stx*1 and *stx*2) and genes for O157:H7, O111:H8, and O26:H11 serotypes. Fecal samples were homogenized after adding 200 mL of physiological saline. Four MacConkey plates per sample were swab inoculated and streaked for isolation. After incubating at 35°C for 18 to 24 h, a sterile loop was used to take a sweep of bacterial growth off each plate (4 plates per composite pen sample). Bacterial growth was suspended in 200 μL of reagent (UltraPrepman reagent; PE Applied Biosystems, Foster City, California, USA) and placed in a boiling water bath for 10 min. Samples were cooled to room temperature and centrifuged at 18 000 × *g* for 3 min. Seventy-five microliters of supernatant were mixed with 75 μL of 12 mM Tris buffer, pH 8.0 before storing samples at 4°C for 1 wk or at –70°C long term.

Previously described PCR primers were optimized and used for detection (5–7). Primers targeting the Shiga toxin (*stx*1 and *stx*2) genes (5) were used in multiplex PCR to screen samples for STEC. Reactions consisted of 1 × PCR buffer (Invitrogen, Burlington, Ontario), 2.5 mM $MgCl^2$, 0.2 mM dNTPs (Invitrogen), 1 μM *stx*1 primers, 0.5 μM *stx*2 primers, 1U of *Taq* polymerase (Platinum Taq polymerase; Invitrogen), and 10 μL of DNA template in a 50 μL reaction volume. Amplification was performed in a thermalcycler (PTC 200 thermalcycler; MJ Research, Incline Village, Nevada, USA) beginning with a hot start at 94°C for 5 min followed by 35 cycles of 94°C for 1 min; 60°C for 1 min; 72°C for 2 min; and a final extension at 72°C for 5 min.

Samples positive for *stx*1, *stx*2, or both were run in PCR assays for the detection of *E. coli* attaching and effacing (*eaeA*) genes specific for serotypes O26:H11 ($eaeA_{O26:H11}$) (6), O111:H8 ($eaeA_{O111:H8}$) (6), and O157:H7 ($eaeA_{O157:H7}$) (7). For $eaeA_{O26:H11}$ and $eaeA_{O111:H8}$ detection, the reaction consisted of 1 × PCR buffer (Invitrogen), 2.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.25 μM of $eaeA_{O26:H11}$ or $eaeA_{O111:H8}$ primers, 1 U of *Taq* polymerase (Platinum Taq polymerase; Invitrogen), and 10 μL of DNA template in a 50 μL reaction volume. Amplification parameters consisted of a single cycle at 94°C for 5 min followed by 35 cycles of 94°C for 1 min; 56°C for 1 min; 72°C for 1 min and final extension at 72°C for 3 min. The *E. coli* O157:H7 multiplex assay (6,7) detects *stx*1 and *stx*2 genes (to confirm screening results), $eaeA_{O157:H7}$ gene, and the H7 flagellar antigen gene (*fliC*). Reagent and amplification parameters were identical to the *stx*1/*stx*2 multiplex reaction except 2.0 mM $MgCl_2$ and 0.2 μM of each primer were used and the annealing temperature was 65°C. All PCR assays included positive (genomic DNA) and negative (water) controls. The PCR products were visualized using a 100 base pair (bp) ladder (Invitrogen) on a 1.2% agarose gel stained with ethidium bromide and documented with a digital camera (DC290; Kodak, Toronto, Ontario).

A pen was considered positive if bacterial growth from 1 or more of the 4 MacConkey plates from the composite pen sample were positive by PCR. All *E. coli* O157:H7, O26:H11, and O111:H8 positives were from the same bacterial composites (colony sweeps) that were first determined positive for *stx*1, *stx*2, or both. The proportion of positive samples for each pen and feedlot were determined and 95% exact confidence intervals calculated (PEPI, version 4.0; Sagebrush Press, Las Vegas, Nevada, USA.).

The 25 pens sampled included 10 pens from 1 feedlot and 5 from each of 3 other feedlots. All of the pens sampled were within 90 d of the projected slaughter dates, with 14 pens within 30 d (56%) and 5 pens within 60 d (20%). Cattle in 10 (40%), 13 (52%), and 2 (8%) pens were placed in the feedlots as yearlings, fall-placed calves, and winter-placed calves, respectively. Twenty pens contained steers (80%) and 5 pens contained heifers (20%). There were an insufficient number of pens to compare prevalence estimates among different classes and types of cattle.

Shiga toxin genes (*stx*1, *stx*2, or both) were detected in samples from 23 of 25 (92%) pens and 4 of 4 feedlots (100%) (Table I). Nineteen of the positive samples (83%) and 43 of 70 positive plates (61%) had both *stx*1 and *stx*2, while 4 samples (17%) and 20 plates (29%) had *stx*2 only, and 0 samples and 7 plates (10%) had *stx*1 only.

Plates from twenty (80%) and 5 (20%) pens contained Shiga toxin genes and the eaeA genes for O26:H11 and O111:H8, respectively. Plates from 2 pens (8%) contained all the *E. coli* O157:H7 markers (*stx(s)*, $eaeA_{O157:H7}$, *fliC*), but there could be no certainty that these genes were all contributed by an *E. coli* O157:H7 strain(s). Six pens (24%) and 2 feedlots (50%) had evidence of both O26:H11 and O111:H8. We detected O26:H11 markers in both samples where *E. coli* O157:H7 markers were detected.

The frequency distribution of *stx*1 and *stx*2 found here is similar to previous reports. The presence of both genes in the majority of the Shiga toxin positives, only *stx*2 in a smaller percentage and only *stx*1 in very few, was similar to the frequency distribution described in other studies of cattle isolates (8). Strains of O157:H7 containing only Shiga toxin 2 are generally thought to be more virulent in people, but STEC strains with any combination Shiga toxins are potential pathogens (1). The concurrent detection of multiple STEC serotypes (9) and multiple molecular subtypes of the same serotype (4,8) in cattle herds has been previously reported. Although a particular STEC strain may be found in a herd more frequently, these predominate strains do not appear to lead to the exclusion of other STEC (4,8).

A previous study of Alberta cattle found Shiga toxins (verotoxins) in 80% of lots and 38% of individual samples of yearling feedlot cattle at slaughter (9). Considering that 30 individual samples were pooled and the individual prevalence of Shiga toxins in cattle feces can be more than 30% (4,9), it is not surprising that we detected evidence of Shiga toxins in nearly all pens. Furthermore, the use of bacterial colony sweep techniques allowed us to screen multiple isolates per culture plate, thereby increasing our ability to detect the gene markers if present. Although PCR detection of these genes suggests the presence of STEC, we did not subsequently culture and characterize individual STEC isolates. It is important to consider that genes for Shiga toxin production have been detected in other bacterial species and apparently nonvirulent STEC (1).

The prevalence of Shiga toxins, STEC, or both in cattle feces is often considerably higher than the prevalence of individual serotypes such as *E. coli* O157:H7 (4). Pen- and feedlot-prevalence of *E. coli* O157:H7 markers in this report are similar to several other reports of *E. coli* O157:H7 prevalence (4). Other recently reviewed reports of pen- and feedlot-prevalence have been much higher, however it is well recognized that one-time sampling may underestimate the pen-prevalence of these bacteria, since they are transiently shed by cattle (4). Our study was conducted during the summer, which is considered the peak season for detecting *E. coli* O157:H7 and other STEC in cattle feces. Including immunomagnetic separation significantly improves the sensitivity of *E. coli* O157:H7 culture (1,4), however selective enrichment would lead to an over-estimation of this serotype relative to others. The diagnostic sensitivity and specificity of our composite fecal and PCR protocol has not been quantified so we are unable to estimate true prevalence based on apparent prevalence.

Escherichia O111 and O26 serotypes have been detected in Ontario dairy cattle (10) and estimates exist for the overall STEC prevalence in cattle (4,9,10). A study of 1,000 slaughtered beef cattle from Prince Edward Island found one O26 and no O111 isolates from 40 positive samples (11). Pen- and feedlot-prevalence estimates of O111 and O26 serotypes have not been reported for feedlots in western Canada. Although we expected our pooled samples to be positive more frequently than would individual samples, our relatively high pen-level prevalence of O26:H11 was not initially anticipated. However, a recent study of German cattle found O26:H11 on 3 of the 4 farms investigated and in more samples than any other STEC serotype (24%) (12). Of STEC isolates recovered from Australian dairy cattle, 10.2% were serotype O26:H11 and 11.2% serotype *E. coli* O157:H7, with the individual animal prevalence estimates of 1.7 and 1.9%, respectively (13). There were differences in sampling and/or laboratory methods among these studies, which makes it extremely difficult to compare prevalence estimates, as others have discussed in detail (4).

In North America, *E. coli* O157:H7 is considered the most significant human pathogen of the STEC serotypes, but in other parts of the world non-O157, including O111 and O26, are responsible for a significant portion of STEC-related disease (1,14,15). A recent U.S. report on the incidence of foodborne illness indicates that the most common non-O157 STEC serotypes isolated from human clinical cases were O26 and O111 (16). Clinical case data collected through 1996 in Canada indicate that 93% of human-disease-associated strains were O157:H7 followed by O55 (1.1%); O125 (0.8); O26 (0.6); and, less frequently, O111 (14). More recent data reported by Health Canada also shows that the majority (95%) of pathogenic *E. coli* isolates from human case reports were O157 (17). However, most laboratory techniques commonly used to detect *E. coli* O157:H7 will not detect other STEC serotypes (1–3). The potential under-reporting of non-O157 STEC is indicated by an Alberta study which showed that testing human clinical specimens for all STEC increases the diagnosis of STEC infection 3-fold, as compared to culturing specimens only for *E. coli* O157:H7 (18). The need for complex methods for detection of non-O157 STEC and the resulting under-reporting of infections hinders surveillance activities and prevents accurate estimates of disease burden (2,3,15).

Recent evidence indicates that human disease-associated and bovine-associated STEC differ in their ability to produce Shiga toxins (19). The relatively high prevalence of STEC in cattle feces and ground beef in the absence of a high number of human cases suggests that not all STEC are pathogenic (1–3,20) and there is a need to distinguish between virulent and avirulent STEC (20). Therefore, the relatively high number of Shiga toxin positive pens in this study does not necessarily represent a high human health risk. In addition, we did not recover and characterize individual isolates for virulence markers, although *E. coli* serotypes O157:H7, O111:H8, and O26:H11 are known pathogens (1).

Standard *E. coli* O157:H7-specific culture methods will not identify other pathogenic serotypes of STEC and our data suggests that some of these non-O157 serotypes are not rare in pre-slaughter pens of feedlot cattle. Further effort to determine the human health significance of non-O157 serotypes of STEC in cattle feces is warranted.

Acknowledgments

The authors acknowledge Dr. Craig Dorin for his assistance in enrolling feedlots for the study. We also acknowledge the vital technical support provided by Marg McFall and Wayne Lazaroff in performing laboratory analyses. Alberta Agriculture, Food and Rural Development, and the Western College of Veterinary Medicine, University of Saskatchewan provided funding for this project.

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