Enterohemorrhagic *E. coli* strains are a recently emerged group of food-borne pathogens that are a significant public health threat, due mainly to the severity of clinical outcomes (15, 26). *E. coli* O157:H7 is the most clinically relevant serotype of enterohemorrhagic *E. coli* strains in most industrialized countries, including the United States (1, 15). Cattle are the primary reservoir of *E. coli* O157:H7, and cattle-derived foods, particularly ground beef, have principally been associated with human morbidity (1, 14, 26). *E. coli* O157:H7 impacts beef production security, trade, and consumer confidence. The employment of stringent measures to exclude this pathogen from production security, trade, and consumer confidence. The employment of stringent measures to exclude this pathogen from production support an association between levels of fecal excretion of *E. coli* O157:H7 and RAJ colonization in pens of feedlot cattle and suggest that the presence of supershedders influences group-level excretion parameters. An improved understanding of individual and population transmission dynamics of *E. coli* O157:H7 can be used to develop preslaughter- and slaughter-level interventions that reduce contamination of the food chain.

Conjecture exists as to the relevance and nature of gastrointestinal colonization by *E. coli* O157:H7 (7, 19). Recently, an anatomical area within the terminal rectum of cattle known as the rectoanal junction (RAJ) was proposed to be a site of preferential colonization (21). The research that followed aimed to confirm the phenomenon of RAJ colonization, investigate its relationship with fecal excretion, and explore RAJ swabbing as a method of testing for *E. coli* O157:H7 (8, 17, 25). Further work is needed to explore the potential importance of RAJ colonization for excretion and transmission dynamics in cattle populations. Several studies have noted wide variation in *E. coli* O157:H7 fecal concentrations between animals (2, 4, 9, 17, 22, 24). Some authors have suggested that certain cattle, designated “supershedders,” have greater *E. coli* O157:H7 transmission potential than other cattle, whether through greater incidence or persistence of excretion, excretion of...
greater concentrations of E. coli O157:H7, or a combination of these factors (18, 21). We hypothesize that this concept of supershedders may be related to the phenomenon of RAJ colonization and that, together, these are at least partly responsible for the large intergroup variation in bovine E. coli O157:H7 prevalence. The aims of the current study were to (i) compare E. coli O157:H7 RAJ colonization prevalence, count, and persistence data between individual feedlot cattle and pens of cattle in order to identify potential supershedders, (ii) determine whether the E. coli O157:H7 status of cattle within a feedlot pen is associated with the presence of a supershedder within that pen, and (iii) examine RAJ colonization relative to fecal excretion and compare sensitivities of RAJ swabbing and fecal sampling for E. coli O157:H7 status determination.

MATERIALS AND METHODS

Experimental approach. A cross-sectional survey of natural RAJ colonization with and fecal excretion of E. coli O157:H7 in feedlot cattle was conducted. Cattle were maintained and sampled within experimental research pens located at Lakeside Feeders, Alberta, Canada, a commercial feedlot and slaughter operation. Mixed-breed and mixed-sex cattle were introduced into the feedlot at approximately 350 kg in weight and randomly assigned to 20 pens, with eight head of cattle per pen. Cattle in different pens could not directly contact each other, and there was no sharing of feed or water sources between pens. Apart from the sampling procedures, cattle were fed, managed, and slaughtered in a manner typical to commercial feedlot practice.

Sample collection. Cattle were individually sampled twice per week, commencing 3 days following pen assignment, over a 14-week period from 21 July to 27 October 2003. Over the course of the sampling period, four cattle were withdrawn from the study due to physical or behavioral problems in repeat handling. Two samples were collected from each subject at each sampling date: a 10-g feed sample collected into Whirl-Pak bags (Nasco, Fort Atkinson, WI) from freshly passed manure and recto-anal mucosal swabs (RAMS). The collection of RAMS has previously been described (8, 25) but briefly involves vigorous mucosal surface swabbing of the rectal area 5 to 10 cm cranial to the anus by using sponge-tipped swabs (VWR International, Buffalo Grove, IL). Samples were transported at 4°C to Washington State University laboratories. Personnel sampling cattle and processing samples were blinded to results throughout the study.

E. coli O157:H7 and E. coli enumeration. Fecal samples were decimally diluted in tryptone soy broth (Difco, BD, Franklin Lakes, NJ) and 100-μl aliquots plated onto sorbitol MacConkey agar plates (Remel, Lenexa, KS) containing ceftaxime (50 ng/ml) and potassium tellurite (2.5 g/ml) (SMAC-CT; Sigma Chemical Co.). After incubation at 37°C for 18 to 24 h, sorbitol-negative colonies were enumerated. Final E. coli O157:H7 counts were calculated based on the sorbitol-negative colony count multiplied by the proportion of sorbitol-negative colonies confirmed as E. coli O157:H7 multiplied by the dilution factor. RAMS were placed in 100 ml tryptone soy broth and vortexed for 60 s before being diluted, plated, and counted as per fecal samples. E. coli counts were performed by direct plating of serially diluted samples onto violet-red bile agar containing 4-methylumbelliferyl-β-D-glucuronide (VRB-MUG; VWR International). All E. coli O157:H7 isolates were banked at −80°C in 30% phosphate-buffered glycerol. Limits of detection for direct plating of RAMS and fecal samples were approximately 10^3 CFU/g.

E. coli O157:H7 confirmation. Subsets of sorbitol-negative colonies (five per plate) were confirmed as E. coli O157:H7 through typical colonial morphology on VRB-MUG (VWR International), O- and H-antigen latex agglutination (Oxoid, Ogdenberg, NY), and PCR. The PCR used multiplexed primers directed against markers stx1, (23), stx2, eae, and fliC (10). Reaction mixtures (50 μl) comprised 0.02 nmol/μl primer solutions, 0.02 mM deoxynucleoside triphosphates, 2 mM MgCl2, 2.5 U Taq polymerase (Invitrogen, Carlsbad, CA), and 2 μl of boiled cell lysate template. The cycling conditions were denaturation at 95°C for 3 min, amplification for 35 cycles at 94°C for 1 min, 58°C for 1.5 min, and 72°C for 2.5 min, and elongation at 72°C for 10 min. PCR was performed using an iCycler thermal cycler (Bio-Rad, Hercules, CA) and products visualized in ethidium bromide-stained 1% agarose electrophoresis gels.

PFGE. Isolates were chosen for pulsed-field gel electrophoresis (PFGE) analysis on the following bases: from supershedders; other cattle within the same three pens as supershedders; cattle within three other pens, chosen randomly; multiple isolates from each animal isolated over the feeding period; and RAJ and fecal isolates from the same animal on the same sampling date. PFGE was based on XbaI restriction using PulseNet (CDC) standard marker strains and restriction parameters (11). Bands were resolved using a CHEF-DR II system (Bio-Rad) and gel images digitized (Syngene Gene Genius; Synoptics, Cambridge, United Kingdom). PFGE profiles were analyzed using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium), with both position tolerance and optimization parameters set at 1%. Cluster analyses were performed and dendrograms created with an unweighted-pair group method using arithmetic means with the Dice similarity coefficient.

Data analysis. Data were analyzed using SAS 8.02 (SAS Institute, Cary, NC). Comparative statistics used pens as the unit of analysis rather than individual cattle, as a substantial pen effect was anticipated. Cattle were defined as supershedders on the basis of both high mean RAJ concentration (≥10^4 CFU/RAMS) and persistent RAJ colonization (≥4 consecutive positive RAMS samples). Analysis of pen E. coli O157:H7 parameters excluded supershedder data, i.e., for pens that housed a supershedder, analysis included data only for supershedders’ pen mates. For comparisons of RAMS to fecal counts, CFU/RAMS counts were converted to CFU/g by multiplying by 0.242 g, the mean weight of material absorbed by RAMS. Mean and cumulative count data excluded E. coli O157:H7-negative results. Statistical differences were considered significant when P was <0.05.

RESULTS

RAJ and fecal E. coli O157:H7 prevalence. The mean prevalences for each date over the sampling period (Fig. 1) and the mean prevalences for each feedlot pen (Fig. 2) demonstrated similar general trends for both RAMS and fecal samples. Pen-to-pen variations in both RAMS and fecal prevalences were significant (P < 0.001) (general linear model procedure). Mean pen RAMS and fecal prevalences were highly correlated (Pearson’s correlation coefficient = 0.9708; P < 0.001). The mean RAMS prevalence for E. coli O157:H7 was 11.0%, with a 95% confidence interval (CI) of 9.9% to 12.1%, which was significantly higher than overall fecal prevalence (6.6%; CI, 5.6% to 7.6%) (P < 0.001 by the Wilcoxon signed-rank test).

RAJ and fecal E. coli O157:H7 counts. The mean RAMS E. coli O157:H7 count (4.06 log_{10} CFU/g; CI, 3.94 to 4.17) was significantly higher than the mean fecal count (3.21 log_{10} CFU/g; CI, 3.08 to 3.33) over the feeding period (P < 0.001 by the Wilcoxon signed-rank test). Although these parameters fluctuated over the sampling period (data not shown), the RAMS count was always higher than the fecal count. Mean E. coli O157:H7 counts varied between pens, but this variation was not significant when considered for E. coli O157:H7-positive animals alone. The frequency distributions of E. coli O157:H7 counts for each animal on each sampling date (cattle day) also differed for RAMS and fecal samples (Fig. 3). The RAJ colonization count followed a log-normal distribution, and the fecal count followed a declining log-linear distribution. RAMS and fecal sample mean counts were not correlated.

Ratios of E. coli O157:H7 to E. coli. Pen day RAMS and fecal ratios (mean pen E. coli O157:H7/E. coli ratio for each pen on each sampling date) were correlated (Spearman’s p, 0.479; P < 0.001). Median pen day ratios were significantly higher for RAMS (−2.22 log_{10}) than for fecal samples (−3.14 log_{10}) (P < 0.001 by the Wilcoxon signed-rank test). Based on median ratios, for every E. coli O157:H7 cell detected in RAMS, there were 168 E. coli cells. The median proportion of E. coli cells that were E. coli O157:H7 for fecal samples was >8 times less (for each E. coli O157:H7 cell, there were 1,373 E. coli cells). Temporal trends for RAMS and fecal sample ratios over the sampling period are demonstrated in Fig. 4. Median pen date E. coli O157:H7/E. coli ratios for RAMS were significantly lower than those for fecal ratios.
Pen-level E. coli O157:H7 associations with supershedders.

Based on the defining criteria, five supershedders from three pens were identified. E. coli O157:H7 RAJ colonization and fecal excretion parameters with respect to contact (i.e., sharing a pen) with a supershedder are summarized in Table 1. Cattle that were housed in pens with no supershedder present had significantly lower RAMS and fecal prevalences than those that were copenned with supershedders. The mean RAMS and fecal counts for supershedder-present pens were higher than those in which no supershedders were identified, though this difference was not significant. When cumulative fecal counts were calculated (by multiplying mean pen prevalence with mean pen count), however, the presence of a supershedder in pens was significantly associated with higher fecal outputs of E. coli O157:H7 over the sampling period. Odds ratio calculations indicate that cattle negative for E. coli O157:H7 fecal excretion throughout the sampling period were five times more likely to have been housed in a pen without a supershedder than one with a supershedder.

PFGE results. A total of 112 isolates were subjected to PFGE analysis. Based on isolate matching at the ≤1 band (i.e., ≥97% similarity) level, 20 individual PFGE patterns were identified. Pen clustering was noted (i.e., isolates from within the same pen were more similar to each other than to those from other pens), although some pattern types were noted across many pens. RAMS and fecal isolates were generally highly related; of 30 comparisons for animal- and date-matched RAMS and fecal isolates, 26 (87%) were ≤1 band different. RAMS isolate pattern type tended to be stable over

FIG. 1. Mean pen RAMS and fecal E. coli O157:H7 prevalences for feedlot cattle over the sampling period. Error bars represent 95% confidence intervals.

FIG. 2. Mean RAMS and fecal E. coli O157:H7 prevalences for feedlot pens. Error bars represent 95% confidence intervals.
time; of 20 longitudinal comparisons of isolates from the same animal, 16 (80%) were within one band similarity. Isolates from supershedders were compared to those from other cattle in their pens and in other pens, as summarized in Table 2. The proportion of matching comparisons between supershedders and copenned cattle was significantly greater than for supershedders and cattle from different pens.

DISCUSSION

Since the emergence of *E. coli* O157:H7, many surveys have examined its epidemiology within cattle populations. Although fecal excretion prevalence data have traditionally been used as the principal basis for such studies, it is important to also recognize the relevance of concentrations of *E. coli* O157:H7 excreted. An individual animal excreting large numbers of *E. coli* O157:H7 will arguably pose a greater risk than the combined output of many animals excreting at low levels, with respect to both potential food contamination and transmission to other cattle (3). This is substantiated by data generated within the relatively small number of studies that have quantified *E. coli* O157:H7 shedding. Omisakin et al. (24) determined that >96% of all *E. coli* O157:H7 cells shed by slaughter cattle were done so by a minority of individuals demonstrating high fecal concentrations. Other authors have similarly noted large variations in fecal *E. coli* O157:H7 concentrations, for both natural (9, 17, 22, 24) and experimental (2, 4) excretions, with a minority of individuals demonstrating uncommonly high levels of excretion. Matthews et al. (18) modeled *E. coli* O157:H7 transmission dynamics and concluded that a model that incorporated *E. coli* O157:H7 “supershedders” best represented cross-sectional epidemiological data. The current study aimed to examine the potential for some cattle to be *E. coli* O157:H7 supershedders, how these supershedders may be associated with *E. coli* O157:H7 feedlot cattle epidemiology, and whether they are related to the phenomenon of RAJ colonization (21).

The presence of supershedders in feedlot pens was associated with higher prevalences of *E. coli* O157:H7 colonization and excretion among copenned cattle. Though a similar association was not found for mean pen RAMS or fecal counts, there was a significant difference with respect to cumulative counts. Cumulative counts factored both mean pen prevalence and count parameters and represented the overall *E. coli* O157:H7 “load” that was being excreted within respective pens over the feeding period. Cattle that were exposed to supershedders excreted median *E. coli* O157:H7 counts 6 orders of magnitude greater than those excreted by non-supershedder-exposed cattle, with maximal shedding rate differences exceeding 25 orders of magnitude. The data excluded the *E. coli* O157:H7 prevalences and counts for the supershedders themselves, which would have again added to the cumulative output for these “superpens” of cattle. Such high-excreting cattle rep-

![FIG. 3. Distribution of RAMS and fecal *E. coli* O157:H7 counts among feedlot cattle. Bars represent the frequency of cattle days (number of cattle on each sampling date) within each count interval. The data included only cattle days for which both RAMS and fecal samples were simultaneously positive for *E. coli* O157:H7.](image1)

![FIG. 4. Mean pen ratios of *E. coli* O157:H7 count to *E. coli* count over the sampling period. The data included only pen days for which RAMS and fecal *E. coli* O157:H7 and *E. coli* counts were all available.](image2)
resent the greatest risks with respect to contamination of the food chain and the maintenance of *E. coli* O157:H7 within bovine populations (18, 24). Although no direction for the association could be demonstrated in this study, it could be assumed that the supershedders were responsible for the higher pen-level *E. coli* O157:H7 parameters through greater degrees of transmission of their *E. coli* O157:H7 cells to pen mates. This hypothesis is supported by the high degree of PFGE pattern identity between supershedder and pen mate isolates, although this finding is biased by pen clustering of *E. coli* O157:H7 PFGE types (data not shown).

In concurrence with other studies (8, 12, 25), RAJ swabbing is a more sensitive means of detecting *E. coli* O157:H7 in cattle than fecal sampling. Sampling individual cattle requires direct livestock handling and is more technically and practically challenging than fecal pat sampling. However, it allows the nomination of *E. coli* O157:H7 status to individual animals and overcomes many of the sampling biases and uncertainties inherent to using fecal pat sampling to estimate animal- or lot-level prevalence (6). The use of *E. coli* O157:H7 concentration data, generated in the current study, has the added utility of incorporation within quantitative risk assessments. As well as demonstrating higher cattle prevalences and concentrations for *E. coli* O157:H7, pen prevalence (i.e., the proportion of pens with at least one *E. coli* O157:H7-positive animal) was higher for RAMS samples than for fecal samples (data not shown). RAMS sampling of a proportion of the pen or herd would therefore potentially represent a more sensitive means of determining group-level *E. coli* O157:H7 status.

*E. coli* O157:H7/E. coli ratios are an indicator of the specificity of the RAJ for colonization by the O157:H7 serotype. Higher RAMS *E. coli* O157:H7/E. coli ratios for supershedders suggest that they are proportionally colonized with *E. coli* O157:H7 to a greater degree than most cattle. This has been demonstrated elsewhere (21) and may be part of the mechanism responsible for the greater *E. coli* O157:H7 count and persistence that confer supershedder status. Ratio differences between supershedders and other cattle were not reflected in fecal samples, however, suggesting that while supershedders are excreting larger amounts of *E. coli* O157:H7 overall, they are concomitantly excreting large amounts of generic *E. coli*. Notable changes in RAMS and fecal ratios in the early feeding period suggest complexity in *E. coli* O157:H7 colonization and transmission dynamics during this production phase, which may relate to climatic changes, dietary modifications, or mixing of cattle and the effects of stress.

The results for the current study were influenced by how supershedders were defined. Other groups that have quantified *E. coli* O157:H7 excretion or described “supershedders” or “high-level shedders” have proposed their own definitions. Low et al. (17) defined supershedders on the basis of either RAMS or fecal *E. coli* O157:H7 counts of ≥3 log_{10} CFU/g. Another group from the United Kingdom (24) used ≥4 log_{10} CFU/g for fecal samples. Other researchers have more loosely nominated cattle as supershedders by using prevalence parameters or the simple identification of outlying counts (2, 9, 18). For the purposes of the current study, supershedders were identified on the basis of RAJ rather than fecal parameters, as colonization rather than excretion was the focus. *E. coli* O157:H7 concentration and persistence data derived from longitudinal samples were used rather than point prevalence or concentration data, as these data are likely to be a better indicator of true colonization, through the reduction of time-dependent influences such as transient colonization and passive enteric passage (16, 18). During data analysis, a variety of definitions combining prevalence, count, and persistence data were trialed. Although some differences in results were evident, overall outcomes (as per Table 1) remained essentially the same. The current study differed from most others in that direct plating was used for *E. coli* O157:H7 enumeration and detection, without more-sensitive detection methods such as enrichment and immunomagnetic separation. The results, therefore, may have been biased by a failure to identify low-level colonized/excreting cattle. However, the significance of such low-level shedders to public health risk or cattle transmission dynamics is likely to be minimal (19, 24). Further research into the phenomena of RAJ colonization and supershedders requires the standardization of methods and supershedder definition.

The current study supports the hypothesis that the RAJ
represents an important colonization site for \( E. \text{coli} \) O157:H7 and suggests that supershedders represent cattle that have persistent colonization of the RAJ with high concentrations of \( E. \text{coli} \) O157:H7. The data presented also provide evidence that the presence of supershedders within a pen of feedlot cattle is associated with higher shedding of \( E. \text{coli} \) O157:H7 by pen-cohorted cattle. Ongoing research addresses whether supershedders are the cause of this group-level excretion pattern or whether the supershedders’ output is influenced by group-level \( E. \text{coli} \) O157:H7 status and transmission dynamics. Other studies on the epidemiological implications of RAJ colonization and supershedders, e.g., its relevance to other forms of livestock production, other factors mediating pen- pen or herd- herd variation in \( E. \text{coli} \) O157:H7 prevalence, and implications for practical risk management strategies for market-ready cattle, are needed. Prenatal interventions based on the presence of supershedders are envisaged. These might include targeting of supershedders for treatment to eliminate colonizing \( E. \text{coli} \) O157:H7 immediately prior to slaughter, the use of vaccines or competitive exclusion products specifically on supershedders, and logistic slaughter for supershedders or cattle within superpens. These rely on devising practical and cost-effective ways of identifying supershedders and may need to occur earlier in the production process (i.e., on the farm and at sale) rather than in lairage.

ACKNOWLEDGMENTS

This work was funded by the Beef Checkoff, with support from National Institutes of Health Public Health Service grants U54-AI-57141, P20-RR16454, and P20-RR15587.

We give special thanks to Russell McClanahan, Robert Adair, Jennifer Carstens, and the Lakeside research team for laboratory technical assistance. We are also grateful to Margaret Davis for assistance with PFGE analysis.

The use of animals in this research was conducted under Institutional Animal Care and Use Committee Animal Subjects Approval Form no. 1978.

REFERENCES