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Survival of *Escherichia coli* O157:H7 on Cattle Hides[▽]

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The objective of this study was to determine the time period that *Escherichia coli* O157:H7 survives on the hides of cattle. Extensive research has been conducted and is ongoing to identify and develop novel preharvest intervention strategies to reduce the presence of *E. coli* O157:H7 on live cattle and subsequent transfer to processed carcasses. If a reduction of *E. coli* O157:H7 levels in feces can be achieved through preharvest intervention, it is not known how long it would take for such reductions to be seen on the hide. In the study presented herein, three trials were conducted to follow *E. coli* O157:H7 hide prevalence over time. For each trial, 36 animals were housed in individual stanchions to minimize or prevent hide contamination events. Through prevalence determination and isolate genotyping with pulsed-field gel electrophoresis, survival of *E. coli* O157:H7 on the hides of live cattle was determined to be short lived, with an approximate duration of 9 days or less. The results of this study suggest that any preharvest interventions that are to be administered at the end of the finishing period will achieve maximum effect in reducing *E. coli* O157:H7 levels on cattle hides if given 9 days before the cattle are presented for processing. However, it should be noted that interventions reducing pathogen shedding would also contribute to decreasing hide contamination through lowering the contamination load of the processing plant lairage environment, regardless of the time of application.

Escherichia coli O157:H7 remains the major food-borne pathogen of concern for the beef industry. Extensive research has been conducted and is ongoing to identify and develop novel pre- and postharvest intervention strategies to reduce *E. coli* O157:H7 from live cattle and processed carcasses (24, 30). Recent work has shown that hides are the main source of beef carcass contamination at slaughter, and as such, reductions in the prevalence of *E. coli* O157:H7 on the hide are directly correlated to lower carcass prevalence rates (7, 12, 28). While postharvest interventions have been designed that address hide contamination directly, the aim of preharvest intervention is to reduce hide contamination indirectly through lowering the prevalence and levels of *E. coli* O157:H7 shed in the feces of cattle.

In order to design effective preharvest intervention strategies, it is necessary to determine the duration of survival of *E. coli* O157:H7 following a hide contamination event. If a reduction of *E. coli* O157:H7 levels in feces can be achieved, it is not known how long it would take for such reductions to be seen on the hide. This information is crucial as various preharvest interventions currently undergoing testing are to be applied to the animal at the end of the finishing phase just prior to shipment for processing (15, 17, 26). While short-duration strategies may produce reductions in the fecal load of *E. coli* O157:H7 in as little as 3 days (3, 37), the bacterial pathogen may persist on the hide for longer periods of time, potentially negating the value of the intervention if the animals are processed immediately.

E. coli O157:H7 survival in a variety of environments has been determined. Studies have shown that *E. coli* O157:H7 can persist in manure-amended soil for greater than 100 days (18, 22). Similarly, long durations of survival of up to 109 days have been reported for *E. coli* O157:H7 in water environments (8, 27, 31).

The survival of *E. coli* O157:H7 on the hides of living cattle is not well understood. Some studies have documented fluctuations over time in the *E. coli* O157:H7 population found on cattle hides. It has been shown that the hide prevalence of *E. coli* O157:H7 can decrease from 84% to 0% in 2 weeks' time for cattle housed in a feedlot pen (4). Other studies have reported declines in hide prevalence across multiple feedlot pens of approximately 60% in time spans of 7 and 28 days (6, 35). These studies were not designed to analyze the duration of *E. coli* O157:H7 survival on hides, but the results provide preliminary evidence that survival on cattle hides would likely be of a shorter time frame than that for persistence in soil or water. Information about the length of the survival is expected to help improve the design of new intervention strategies targeted for application at the end of the feeding period to further reduce or eliminate *E. coli* O157:H7 from the meat production system. The objective of this study was to determine the length of time of *E. coli* O157:H7 survival on the hides of cattle.

MATERIALS AND METHODS

Experimental design. The study was performed in three phases to establish the duration of *E. coli* O157:H7 survival on the cattle hides. Phase I was conducted to identify the approximate duration of survival of the pathogen on cattle hides.

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Phases II and III utilized more frequent sampling times to define the time span of survival more precisely.

Individual stanchions. To prevent hide contamination events during the study, stanchions were used to house individual animals. The stanchions consisted of tubular iron railings mounted in a concrete floor. The interior dimensions of the stalls were 7 ft in length and 3 ft in width. Each stanchion had a mechanical head restraint and individual food and water stations. In addition, the floor in each stanchion was covered by rubber matting to prevent foot irritation. Animal-to-animal contact was prevented. The space was adequate for the cattle to lie down. Individual grooming could take place, but not at the hide sample sites.

Feedlot cattle (300 to 450 kg) were used in this study. Before test cattle could be transferred to the stanchions, they were held in small holding pens (5 to 8 cattle per pen) for 1 to 2 weeks to allow them to acclimate to human interaction in order to prevent animal or personnel injury by taking cattle straight from the feedlot to the individual stanchions. Based on previous observations of keeping animals in small groups, it was presumed that hide contamination would decline due to the cattle threshold number and animal density of these pens being below that needed to maintain colonization. In order to maximize the number of animals that harbored *E. coli* O157:H7 on their hides following this holding period, cattle were prescreened at the feedlot to identify animals that harbored *E. coli* O157:H7 on their hides.

Phase I. (i) Animals. One hundred twenty-five cattle from 5 feedlot pens were screened for *E. coli* O157:H7 on their hides. *E. coli* O157:H7 was detected on 123 animals. One week after screening, 8 cattle from each pen were transferred to smaller holding pens adjacent to the facility hosting the single-animal stanchions. Another 24 cattle from three unscreened pens were also transferred to this facility. A facility veterinarian and cattle-feeding personnel evaluated the behaviors of individual animals in small holding pens upon closer interactions with humans. Individual animals showing aggressive behaviors or abnormal feeding patterns were released from the small holding pens. After a week of acclimation in the holding pens, 36 animals were placed in thoroughly cleaned individual stanchions. Each animal was restrained by a head restraint at the time of sampling. Floors and railings of individual stanchions were sampled before hosting animals, and no *E. coli* O157:H7 isolates were detected in those samples. Each animal received standard feed and water rations for the duration of the experiment. Animal excretion was cleaned twice a day to minimize recontamination of hides. To aid in minimizing hide recontamination, half of the animals received two doses of neomycin (trade name Biosol) administered by the facility veterinarian on day 0 and day 1 via drinking water.

(ii) Sampling. Hide and fecal samples were collected from individual animals on days 0, 4, 7, 11, 14, 18, 25, and 32 for detection of *E. coli* O157:H7. Hide samples were collected from the accessible side of the cattle in the holding stanchions while the animals were being restrained using a mechanical device equipped on individual stanchions. A sterile WhirlPak sponge (Nasco, Fort Atkinson, WI) premoistened in buffered peptone water (BPW; Difco, Becton Dickinson Microbiology Systems, Sparks, MD) was used to sweep the shoulder and back area in a Z pattern in one streak, followed by turning the sponge and retracing the Z pattern in the opposite direction. The Z pattern covered approximately 1,000 cm^2, and a different angle for the Z pattern was applied for each sampling to avoid resampling the same area. Fecal samples were collected from unperturbed fresh fecal pats in the early morning before cleaning. Floors and railings for each stanchion were sampled using a sterile WhirlPak sponge (Nasco) premoistened in BPW (Difco). Areas of approximately 3,000 cm^2 were sampled.

(iii) Detection and enumeration. For each sponge sample (floors, hides, railings), the sponge was thoroughly massaged in 100 ml tryptic soy broth (TSB; Difco, Becton Dickinson). For each fecal sample, 10 g fecal material was homogenized by use of hand massaging in 100 ml TSB. One milliliter of the sponge or fecal sample was removed for direct detection of *E. coli* O157:H7 by immunomagnetic separation (IMS), while the rest of the sample was enriched as described previously (10, 11). *E. coli* O157:H7 isolates in the preenrichment samples were enumerated by being plated on CT-SMAC (sorbitol MacConkey agar [Becton Dickinson] supplemented with cefixime [0.05 mg/liter] and potassium tellurite [2.5 mg/liter; Invitrogen] and ntCHROMagar [CHROMagar-O157; DRG International, Mountainside, NJ] supplemented with novobiocin [20 mg/liter; Sigma, St. Louis, MO] and potassium tellurite [0.8 mg/liter; Sigma]) plates following IMS using a PickPen device. For those that produced negative results by direct detection of preenrichment samples, 1 ml enriched samples was similarly processed the second day for detection of *E. coli* O157:H7.

Phase II. (i) Animals. One hundred sixty-two crossbred cattle were screened for *E. coli* O157:H7 on the hides and in feces. Animals ($n = 78$) were selected for further study if *E. coli* O157:H7 was detected on their hides. The animals were sorted into two groups, referred to in this report as "control" and "treated." The control group ($n = 39$) remained in a single feedlot pen (100 by 250 ft) where hide contamination could readily occur. The treated animals would be transferred to single-animal stanchions where contamination of the hide would be minimized, if not prevented. Each stanchion had a dedicated feed and water supply. Following sorting, but prior to placing the treated animals in the stanchions, the animals ($n = 39$) were transferred to small holding pens (≈6 cattle/pen). There were 36 individual stanchions for housing the animals during the experiment. Three extra animals were included at this point, in the event that some animals would not acclimate to the new environment and could not be used for the study. After the 2-week acclimation period, the animals of the treated group ($n = 36$) were placed in the individual stanchions. Half ($n = 18$) of the animals in individual stanchions were treated with neomycin for 2 days upon being placed in the stanchions. The day the animals were moved to the individual stanchions was considered day 0. On day 0, hide and fecal samples were collected from both the control and treated animals as they passed through a squeeze chute.

(ii) Environmental samples. Prior to the arrival of the animals in either the small holding pens or the individual stanchions, the pens and stanchions were screened for *E. coli* O157:H7. Sponge samples were collected as described for phase I. Sample processing was carried out as described below for hide samples in phase II.

(iii) Cattle sampling. Hide and fecal samples were collected on Mondays, Wednesdays, and Fridays for treated animals and on Mondays and Fridays for the control animals maintained at the feedlot. Hide samples were collected for all animals using a sterile sponge (Nasco) premoistened with BPW (Difco) and by swabbing an area of approximately 1,000 cm^2 behind the shoulder. For the control animals, fecal samples (10 g) were collected by rectal palpation. Due to personnel safety issues, it was not possible to obtain fecal samples by rectal palpation from animals housed in the individual stanchions. Once the animals were placed in the individual stanchions, fecal samples were collected by swabbing of the recto-anal junction (Spongecicle; Biotrace International Inc., Bothell, WA).

(iv) Enumeration. *E. coli* O157:H7 was enumerated from hide and fecal samples using a spiral plater (Spiral Biotech, Norwood, MA), following the method described by Brichta-Harhay et al. (14). For hide samples, the sponge sample was homogenized by using hand massaging prior to the addition of enrichment medium, and 250 μl of solution was removed to a microcentrifuge tube. Each tube was vortexed and then held static for 3 min to allow the debris to settle. Following the settling period, 50 μl of sample was spiral plated onto ntCHROMagar (CHROMagar-O157 [DRG International] supplemented with novobiocin [5 mg/liter; Sigma] and potassium tellurite [2.5 mg/liter; Sigma]) plates. When being enumerated from fecal grab samples, the enrichment medium (90 ml TSB [Difco, Becton Dickinson] with phosphate buffer [TSB-PO_4; 30 g TSB, 2.31 g KH_2PO_4, and 12.54 g K_2HPO_4 per liter of solution]) was added to the 10-g fecal sample and the mixture was homogenized by hand massaging. For fecal swab samples, 90 ml TSB-PO_4 was added and the sample was homogenized by hand. One milliliter of each fecal sample mixture was removed to a microcentrifuge tube and vortexed. The enumeration was then carried out as described for hide samples.

(v) Sample processing for prevalence. Samples were processed according to previously described methods, with slight modifications (10, 11). Hide sponge samples were enriched with 80 ml TSB after the 250-μl aliquot was removed for enumeration. Fecal samples were enriched in the 90 ml TSB-PO_4 used for enumeration dilution. The sample bags were incubated at 25°C for 2 h and then at 42°C for 6 h prior to being held at 4°C overnight. Following incubation, the samples were processed by immunomagnetic separation, in which 1-ml samples from each enrichment were subjected to an anti-O157 immunomagnetic bead cell concentration (Dynal). Fifty-microliter volumes of the final bead-bacterium complexes were spread-plated onto ntCHROMagar and CT-SMAC (sorbitol MacConkey agar [Difco] supplemented with cefixime [0.05 mg/liter] and potassium tellurite [2.5 mg/liter; Dynal]). All plates were incubated at 37°C for 18 to 20 h, after which up to three suspect colonies were picked and tested by latex agglutination (DrySpot *E. coli* O157; Oxoid). PCR analysis was used to confirm that each isolate harbored genes for the O157 antigen, H7 flagella, and at least one of the Shiga toxins (20). Isolates were maintained as frozen stocks for later use in strain typing by pulsed-field gel electrophoresis (PFGE).

Phase III. (i) Cattle. Hide and fecal samples were collected from feedlot cattle ($n = 147$) for *E. coli* O157:H7 screening. Cattle that were positive for *E. coli* O157:H7 on their hides were selected and assigned to a treated or control group as described for phase II. In phase III, the control group consisted of 36 cattle placed in a 50-ft by 250-ft feedlot pen.

(ii) Sampling. Hide samples were collected as described for phase II. Hide samples were collected on days 0, 2, 4, 7, 9, and 11 for the treated group and days 0, 4, 7, 9, and 11 for the control group. Fecal samples for both the treated and

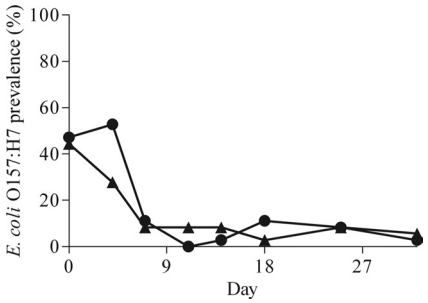

FIG. 1. Hide and fecal prevalence of *E. coli* O157:H7 in phase I sampling. Hide (circle) and fecal (triangle) samples were collected from 36 cattle in individual stanchions over a 32-day period. Samples were collected on days 0, 4, 7, 11, 14, 18, 25, and 32. The data points represent the percentage of total samples in which *E. coli* O157:H7 was detected.

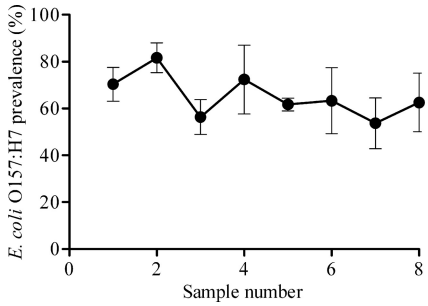

FIG. 2. Effects of repeated sampling on hide prevalence of *E. coli* O157:H7. Multiple hide samples were collected from individual animals. The average *E. coli* O157:H7 prevalence (%) is presented from three trials with at least 10 animals per trial. Error bars represent the standard errors of the means.

control animals were collected by swabbing the recto-anal junction (swab catalog no. 10812-022; VWR International, Buffalo Grove, IL) as described by Rice et al. (29). Immediately following sample collection, the swabs were placed into 4 ml TSB-PO_4 and held on ice during transportation to the lab. Fecal swabs were collected from the control and treated groups on days 0, 4, 7, 9, and 11.

(iii) Laboratory procedures. Enumeration and prevalence determination of *E. coli* O157:H7 was conducted for all samples as described for phase II.

(iv) Repeated hide sampling. Multiple hide samples were collected from individual animals to determine if repeated sampling of the same sample site would affect the *E. coli* O157:H7 population. In the first trial, 10 animals were sampled seven times each. In trials two and three, 12 animals were sampled eight times each. The prevalence rates for each replication were averaged over three trials, and the standard error was determined using Prism 5.0 GraphPad software. Hide samples were collected and processed for enumeration and prevalence as described for phase II.

(v) PFGE. *E. coli* O157 isolate fingerprints generated and analyzed in this study were based on PFGE separation of XbaI-digested genomic DNA as described by PulseNet (http://www.cdc.gov/pulsenet/protocols.htm). Agarose certified for pulsed-field gels (SeaKem gold agarose) was obtained from Cambrex Bio Science Rockland Inc. (Rockland, ME); Tris-borate-EDTA running buffer and proteinase K were purchased from Sigma (St. Louis, MO). XbaI was purchased from New England BioLabs (Beverly, MA); *Salmonella enterica* serotype Braenderup strain H9812 was used as a control and for standardization of gels (21). Banding patterns were analyzed and comparisons were made using BioNumerics software (Applied Maths, Sint-Martens-Latem, Belgium), employing the Dice similarity coefficient in conjunction with the unweighted pair group method using arithmetic averages for clustering.

RESULTS AND DISCUSSION

The objective of this work was to determine the length of time that *E. coli* O157:H7 can persist on the hides of live cattle. In the first phase of the experiment, 36 cattle were selected for transfer from multiple feedlot pens to individual stanchions in which hide contamination events would be minimized if not prevented. All stanchions were sampled prior to entry of cattle. *E. coli* O157:H7 was not detected on the floors or railings of any stanchion prior to animal entry. Upon entry into the stanchions (day 0), each animal was sampled. Hide prevalence of *E. coli* O157:H7 started at 47%, was 53% on day 4, and then decreased to 0% by day 11 (Fig. 1). From day 11 to the last sample day (day 32), hide prevalence rose to a peak of 11% on day 18 and then declined again. Fecal prevalence closely followed hide prevalence (Fig. 1).

The rapid decline and absence of *E. coli* O157:H7 on cattle hides by day 11 indicated that the pathogen does not persist on cattle hides for long periods of time. The mechanism of the reoccurrence of *E. coli* O157:H7 after day 11 was not known. Possible scenarios are as follows: (i) recontamination by the individual animal, (ii) heterogeneous distribution of hide contamination that was missed on previous samplings, or (iii) recontamination from another source occurring at later time periods. *E. coli* O157:H7 isolates were not kept for PFGE analysis; hence, it was not possible to determine the source of hide contamination.

In order to reduce the likelihood of missing sites of contamination due to heterogeneous distribution, the sampling scheme was changed for phases II and III. Instead of sampling in alternating Z patterns, samples were collected from a 1,000-cm^2 rectangular area behind the shoulder. To ensure that any reductions in *E. coli* O157:H7 prevalence seen in phases II and III were not due to multiple samplings of the same area of the hide, three replicates of repeated sampling were performed. On three separate occasions, 10 to 12 cattle not included in the survival experiments were randomly chosen from a feedlot. As the cattle were restrained in a squeeze chute, multiple samples were collected from the same sample site. The results of this sampling show that repeated sampling of the same sample site does not lead to a decrease in *E. coli* O157:H7 prevalence (Fig. 2).

In phase II, a control group of cattle was added and comprised 39 animals that were kept in a single feedlot pen. The control group was added in order to show changes in hide prevalence when contamination events were not minimized. Also in phase II, all animals were prescreened for *E. coli* O157:H7 hide contamination and all *E. coli* O157:H7 isolates were kept for PFGE analysis. Prescreening was conducted in an attempt to increase the initial O157:H7 prevalence at day 0. Based on the enumeration data from the screening period and the sorting assignments, 17 animals in the treated group and 9 animals in the control group were shedding *E. coli* O157:H7 at high levels ($\geq$200 CFU/g feces). As the cattle were sorted, 1 week after prescreening, they were again tested for O157:H7 on their hides and in their feces. Animals destined for the individual stanchions (treated) had hide and fecal prevalences of 100% and 67%, respectively, with 6 animals shedding high levels of *E. coli* O157:H7, while those animals that were to stay in the feedlot had hide and fecal prevalences of 87% and 85%, respectively, with 11 animals shedding high levels of *E. coli* O157:H7 (data not shown).

The treated animals were moved to small holding pens (5 to

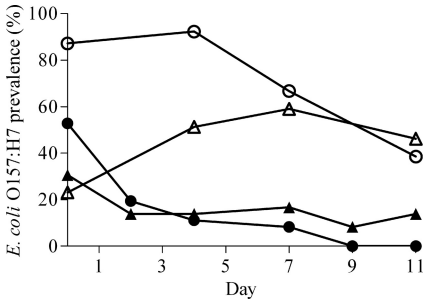

FIG. 3. Hide and fecal prevalence of *E. coli* O157:H7 in phase II sampling. Hide (circle) and fecal (triangle) samples were collected from 36 cattle in individual stanchions (solid symbols) and 39 cattle in a feedlot pen (open symbols) over an 11-day period. Samples were collected on days 0, 2, 4, 7, 9, and 11. The data points represent the percentage of total samples in which *E. coli* O157:H7 was detected.

6 animals per pen) for a 2-week acclimation period. During this time, neither the animals in the holding pens nor the animals in the feedlot pen were sampled. Following the 2-week acclimation, 36 treated animals were moved into individual stanchions. At that time, each animal was sampled, as were the control animals in the feedlot pen. The time of this sampling was considered to be day 0. It was found that following the 2-week period, the hide prevalence for animals in the feedlot was the same as that for the prior sampling, but the hide prevalence for animals in the small holding pens had dropped to 53%. As the animals were maintained in the individual stanchions, the hide prevalence steadily dropped from 53% at day 0 to undetectable levels by day 9 (Fig. 3). The *E. coli* O157:H7 hide prevalence for the feedlot animals maintained a level above 66% through day 7 but dropped to 39% by day 11.

The results from phase II supported the phase I results, suggesting that in the absence of recontamination, *E. coli* O157:H7 is not maintained on the hides of cattle for a lengthy period of time. In this phase of the experiment, when recontamination was prevented, *E. coli* O157 levels dropped below detectable limits after 9 days. In two of the three cases where animals had detectable levels of *E. coli* O157:H7 at the day 7 sample period, hide sample results from the previous two sampling periods (days 2 and 4) were negative for *E. coli* O157:H7. Either the area of hide contamination was localized and missed in the previous samples or the hide was recontaminated. Because neither of these animals was shedding *E. coli* O157:H7 at the day 7 time point, recontamination from the individual animals was not likely. However, recontamination could have occurred from other sources. At the time of sampling, it was noted that there were a large number of flies congregating on all of the cattle in the barn. Several varieties of flies have been shown to harbor *E. coli* O157:H7 and even transfer the bacteria to new locations, including transmission to cattle (1, 23, 33). PFGE analysis supported the hypothesis of recontamination from another source. The PFGE patterns for *E. coli* O157:H7 isolates recovered from the day 7 hide samples from these two animals were similar to each other, but they did not match any PFGE patterns that had been seen on these animals previously (Fig. 4).

For the third animal that harbored *E. coli* O157:H7 on its hide at day 7, the hide samples for that animal contained *E. coli*

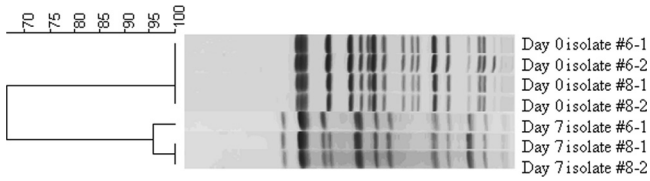

FIG. 4. PFGE profiles of *E. coli* O157:H7 strains collected in phase II. *E. coli* O157:H7 isolates collected from animals 6 and 8 that were housed in individual stanchions. Two isolates from each animal's day 0 hide samples were analyzed. On day 7, isolates were analyzed for animal 8, while only one isolate was recovered from animal 6's day 7 hide sample.

O157:H7 on all sampling occasions up to and including day 7. This animal was found to be shedding O157:H7 at every sampling period of this experiment. On several of the sampling days (days 0, 4, 7, and 9), this animal was shedding *E. coli* O157:H7 at high levels (range, 4.4×10^2 to 8.0×10^4 CFU/g feces). PFGE analysis showed that the isolates recovered from the hide were of the same genotype as those being shed in the feces of that animal (data not shown). It is likely in this case that recontamination of the hide occurred at some point. These results imply that the duration of survival of *E. coli* O157:H7 on hides is less than 9 days, although it may actually be 7 days or less, but an additional trial was deemed necessary for confirmation.

Phase III was conducted in a manner similar to that of phase II, with the exception that no animals were treated with neomycin. Following the trend observed in phase II, hide prevalence for animals housed in the individual stanchions steadily declined from day 0 (82%) to day 7 (8%) (Fig. 5). On day 9, the prevalence increased to 14% and then dropped to 3% on day 11. The increase to five *E. coli* O157:H7-positive animals seen on day 9 included three animals that had not yielded an O157:H7-positive hide sample for at least the three previous sample times. The other two animals, while positive for O157:H7 on sample time points previous to day 9, had isolates with novel PFGE patterns at day 9 (Fig. 6A and B). The one animal with a positive result on day 11 had not had a positive hide result since day 0, and the PFGE patterns for isolates collected on days 0 and 11 were divergent (Fig. 6C).

The data presented here demonstrate that survival of *E. coli*

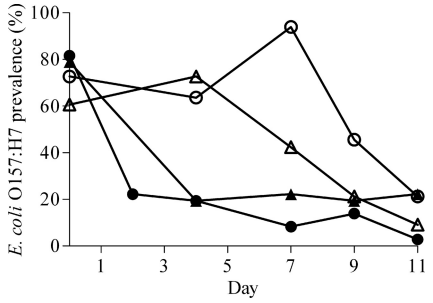

FIG. 5. Hide and fecal prevalence of *E. coli* O157:H7 in phase III sampling. Hide (circle) and fecal (triangle) samples were collected from 36 cattle in individual stanchions (solid symbols) and 36 cattle in a feedlot pen (open symbols) over an 11-day period. Samples were collected on days 0, 2, 4, 7, 9, and 11. The data points represent percentage of total samples in which *E. coli* O157:H7 was detected.

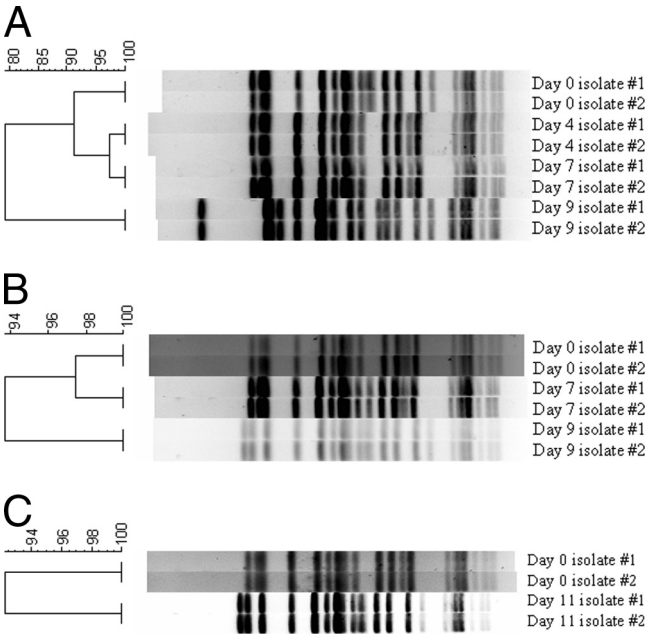

FIG. 6. PFGE profiles of *E. coli* O157:H7 strains collected in phase III. Two animals (2 and 11) which were positive for O157:H7 on sample time points previous to day 9 had isolates with novel PFGE patterns at day 9. (A) Two isolates each from animal 2's hide samples collected on days 0, 4, 7, and 9 were analyzed by PFGE. (B) *E. coli* O157:H7 isolates collected from animal 11 on days 0, 7, and 9 were analyzed by PFGE. (C) One animal (14) had a positive hide sample result on day 11. *E. coli* O157:H7 isolates collected on day 11 were divergent from those collected on the only other positive sampling occasion, day 0.

O157:H7 on the hides of cattle is short lived, with an approximate duration of less than 9 days. Similar results were produced using *in vitro* testing of cattle hide fragments. Small et al. inoculated pieces of cattle hide and monitored the *E. coli* O157:H7 population reduction over time (32). The authors reported that it would take between 5 and 8 days for a reduction in 90% of the pathogen's population on the surfaces of cattle hides (32). It can be seen from the data presented herein and previously (32) that the decrease in *E. coli* O157:H7 on cattle hides occurs rapidly. It is possible that *E. coli* O157:H7 population reductions occurring in less than 9 days may be sufficient to minimize or eliminate finished product contamination simply by lowering hide contamination to levels that are managed by the in-plant interventions. However, in these studies, recontamination was much easier to prevent than would be the case in an actual production setting, where even if fecal shedding were stopped instantaneously, it is likely that there would still be some hide recontamination from the production environment. Therefore, our recommendation of a 9-day time period between application of a short-course intervention and harvest of the animals is based on achieving maximum reductions in the *E. coli* O157:H7 populations on cattle hides.

Survival time of *E. coli* O157:H7 in other matrices has been shown to be considerably longer. *E. coli* O157:H7 has been shown to persist in multiple environments (soil, water, and bovine feces) for over 90 days (19, 31). At this point, the mechanism of bacterial reduction on the hides of cattle is not known. The simplest scenario would involve cell desiccation, because the hide has no moisture retention capabilities. Williams et al. determined that survival of *E. coli* O157:H7 in inoculated feces that were applied to metal and wood surfaces was dependent on moisture content and temperature (36). They showed that survival at 20°C under desiccating conditions was less than or equal to 7 days. The conditions leading to the 7-day survival in the study by Williams et al. would be similar to those found on the hides of cattle (36). Desiccation does not always lead to reduced cell survival, as shown previously by Varma et al. (34), where an outbreak-related *E. coli* O157 strain was isolated from several samples of dust located in the rafters of a barn that had hosted an agricultural fair 42 weeks earlier (34). Therefore, further research is required to understand the mechanism by which *E. coli* O157:H7 bacteria are reduced on the hides of cattle in less than 9 days.

It should be noted that the animals kept in individual stanchions were in a covered building. While the temperature and humidity were not controlled and the building remained open to the outside atmosphere, the animals were not subjected other environmental factors, such as direct sunlight or precipitation. This may have influenced the survival times for the bacterial pathogen on the cattle hides.

The presence of *E. coli* O157:H7 on the hides of cattle when presented for processing has been shown to be a primary determinant of carcass contamination (7, 12, 13, 28). Postharvest antimicrobial interventions directly targeting the hide have proven effective in reducing the transfer of pathogenic bacteria from the hide to the carcass (12, 25, 28). However, despite effective multihurdle intervention schemes utilized at processing, end product contamination still occurs, indicating the need for additional, effective interventions. The goal of preharvest intervention design is to reduce the pathogen prevalence and load in the feces of the animal. In doing so, the prevalence and load of the target bacteria will be reduced indirectly on the hides of animals through less-frequent contact with contaminated feces.

Some preharvest intervention methods (i.e., vaccination and probiotics) are designed to be applied over a long-duration time span to maintain fecal pathogen levels in a reduced state (16, 26, 30). Other preharvest intervention types (i.e., antibiotics and sodium chlorate) are implemented at the end of the finishing period immediately before cattle are sent to processing (3, 15, 17). It is in the latter scenario that existing hide contamination may render short-duration preharvest interventions ineffective for prevention of carcass contamination. *In vitro* studies utilizing inoculated rumen fluid showed that *E. coli* O157:H7 was reduced from 10^6 CFU/ml to less than detectable levels in 24 h by the addition of sodium chlorate (3). When administered to live cattle as a feed additive within 20 h of harvest, reductions in fecal prevalence of *E. coli* O157:H7 were observed, while no effects were seen for recovery of *E. coli* O157:H7 from hide or carcass samples (2). Based on the data presented herein, a 20-h time period between intervention and slaughter would not provide enough time for reductions in fecal prevalence to be translated into lower levels of hide prevalence.

In addition, the role of the processing plant lairage environment as a major source of *E. coli* O157:H7 contamination can be a confounding factor in the interpretation of preharvest

intervention effects on hide and carcass prevalence during processing. Arthur et al. have shown that over 80% of the *E. coli* O157:H7 isolates recovered from beef carcasses during processing did not come from the feedlot of origin for the associated cattle (5). It was determined that cattle acquire a large amount of hide contamination following arrival at the processing plant as animals move through multiple common spaces of the lairage environment. In this scenario, effects of preharvest interventions in lowering the hide prevalence are likely negated by the time the animals begin processing. Hence, data from hide and carcass samples collected during processing should be interpreted with caution when evaluating the efficacy of preharvest interventions. In order to avoid these confounding effects, processing scenarios which prevent cohabitation of the lairage environment by treated and untreated animals must be employed.

When considering the role of preharvest intervention in the context of lairage environment contributions to hide contamination, it should be noted that any intervention that reduces fecal shedding of *E. coli* O157:H7 would be beneficial in reducing hide contamination at harvest by reducing the contamination potential in the lairage environment. The incoming fecal load of cattle presented for processing is a main determinant of the lairage environment's potential to serve as a contamination source for cattle hides. Therefore, an effective preharvest intervention would serve to reduce *E. coli* O157:H7 hide contamination in both the production and lairage environments. Only in the production environment would the duration of *E. coli* O157:H7 survival on cattle hides be relevant. In the lairage environment, the critical issue is to minimize or prevent hide contamination, not to allow time for die-off the pathogen.

In summary, *E. coli* O157:H7 persistence on cattle hides is short lived. This fact should be taken into account when designing preharvest antimicrobial intervention schemes to maximize the effectiveness of the intervention in preventing carcass contamination. Any preharvest interventions that are to be administered at the end of the feeding period will achieve maximum effect in reducing *E. coli* O157:H7 levels on cattle hides if given 9 days before the cattle are presented for processing.

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