**Escherichia coli** O157:H7 is a foodborne pathogen that can cause severe bloody diarrhea and hemolytic uremic syndrome in humans,¹ and it has become a major public health issue in the United States and elsewhere. Outbreaks of disease attributable to *E coli* O157:H7 have been associated with beef products, especially ground beef, and other foods, such as leafy vegetables.

**Objective**—To evaluate seasonal patterns and risk factors for *Escherichia coli* O157:H7 in feces in a beef cattle herd and determine strain diversity and transition in *E coli* over time by use of multiple-locus variable-number tandem-repeat analysis (MLVA) and pulsed-field gel electrophoresis (PFGE).

**Sample Population**—456 samples of freshly passed feces collected over a 1-year period from cattle in a range-based cow-calf operation located in the foothills of the Sierra Nevada Mountains in California.

**Procedures**—*E coli* O157:H7 was recovered from feces by use of immunomagnetic separation and 2 selective media. Virulence factors were detected via reverse transcriptase–PCR assay. *Escherichia coli* O157:H7 isolates were subtyped with MLVA and PFGE. Prevalence estimates were calculated and significant risk factors determined. A dendrogram was constructed on the basis of results of MLVA typing.

**Results**—Overall prevalence estimate for *E coli* O157:H7 was 10.5%, with the prevalence lowest during the winter. Mean temperature during the 30 days before collection of samples was significantly associated with prevalence of *E coli* O157:H7 in feces. Nineteen MLVA and 12 PFGE types were identified.

**Conclusions and Clinical Relevance**—A seasonal pattern was detected for prevalence of *E coli* O157:H7 in feces collected from beef cattle in California. Subtyping via MLVA and PFGE revealed a diversity of *E coli* O157:H7 strains in a cow-calf operation and noteworthy turnover of predominant types. Given the importance of accurately determining sources of contamination in investigations of disease outbreaks in humans, MLVA combined with PFGE should be powerful tools for epidemiologists. (Am J Vet Res 2010;71:1339–1347)

---

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>CI</td>
<td>Confidence interval</td>
</tr>
<tr>
<td>CT-SMAC</td>
<td>MacConkey agar containing sorbitol, cefixime, and tellurite</td>
</tr>
<tr>
<td>IMS</td>
<td>Immunomagnetic separation</td>
</tr>
<tr>
<td>MLVA</td>
<td>Multiple-locus variable-number tandem-repeat analysis</td>
</tr>
<tr>
<td>NT-SCCM</td>
<td>Selective, chromogenic culture medium containing novobiocin and tellurite</td>
</tr>
<tr>
<td>OR</td>
<td>Odds ratio</td>
</tr>
<tr>
<td>PFGE</td>
<td>Pulsed-field gel electrophoresis</td>
</tr>
<tr>
<td>RT</td>
<td>Real time</td>
</tr>
<tr>
<td>TBS</td>
<td>Tris-buffered saline</td>
</tr>
</tbody>
</table>

---

Received July 30, 2009.
Accepted October 28, 2009.
From the Departments of Medicine and Epidemiology (Kondo, Hoar, Villanueva) and Population Health and Reproduction (Atwill), School of Veterinary Medicine, University of California-Davis, Davis, CA 95616; and USDA Agricultural Research Service, 800 Buchanan St, Albany, CA 94710 (Mandrell). Dr. Kondo's present address is Ministry of Agriculture, Forestry and Fisheries, 1-2-1 Kasumigaseki, Chiyoda-ku, Tokyo, 100-8950 Japan. Supported by the Center for Food Animal Health, School of Veterinary Medicine, University of California-Davis.
The authors thank Daniel Myers and Justin Tindall for assistance with sample collection and data collection and Dr. Michael Cooley, Diana Carychao, and Samaripa Fontanez for assistance with multiple-locus variable-number tandem-repeat analysis and pulsed-field gel electrophoresis.
Address correspondence to Dr. Hoar (b rhoar@ucdavis.edu).
etables that may have been contaminated with animal feces.1-4 Cattle are considered to be a reservoir for this bacteria,3 and several studies1-4 have been conducted to help clinicians and researchers understand the ecology of E coli O157:H7 in farm environments as well as transmission routes into the human food chain.

Prevalence of E coli O157:H7 in beef cattle is highly variable, depending on several factors such as type of cattle operation, geographic area, season of sample collection, method of sample collection, and method for bacterial detection. In a review article,4 it was reported that the prevalence of E coli O157:H7 ranged from 0.3% to 19.7% in beef cattle in feedlots and from 0.7% to 27.3% in beef cattle on pasture. Factors that affect the presence of E coli O157:H7 in pasture-based cow-calf operations may differ from those that affect the presence in cattle in feedlots because of differences in management systems, feeds, and environment.3 A seasonal pattern for the prevalence of E coli O157:H7 in feces of range-based cattle has been reported in several studies.5-8 It may be explained by factors such as temperature, precipitation, herd management, pasture condition and management, feed and water sources, wildlife activity, and persistence of bacteria in feces. Understanding the complex ecology of E coli O157:H7 in pasture-based operations and identifying risk factors that are important in transmission and maintenance of disease may be critical for use in planning preharvest control measures9 and in limiting possible environmental contamination.

Genetic subtyping is a powerful tool for use in investigating outbreaks of disease attributable to E coli O157:H7 by allowing identification of possible sources of infection and routes of transmission of the organism. Increasingly, PFGE has been used in investigations of disease outbreaks in the United States. PulseNet, the molecular subtyping–based surveillance system for foodborne bacterial diseases initiated by the CDC, currently uses PFGE for investigating outbreaks of disease in humans associated with E coli O157:H7.10-11 Although PFGE has been an efficient tool for use in detection and investigation in foodborne disease outbreaks, it is a time-consuming and labor-intensive method that requires rigorous standardization of protocols and trained personnel to provide comparable results among multiple laboratories. In addition, PFGE subtyping data do not always provide information of value to epidemiologists, even when multiple enzymes are used in the analysis.12 In contrast, MLVA can be useful for genetic subtyping of E coli O157:H7 because it is a less labor-intensive method than PFGE, and it can yield results rapidly and has high discriminatory power.13-16 In MLVA, the difference in the number of tandem repeats in multiple loci is determined by the use of PCR amplification. The size and number of tandem repeats are easily defined, which results in more rapid and accurate comparison of the genotyping data among laboratories. Although additional information is needed to develop standard protocols and interpretation guidelines for MLVA, this technique should be considered as an alternative method to PFGE for subtyping E coli O157:H7 isolates.15,17

To use molecular subtyping methods such as MLVA and PFGE in investigations of disease outbreaks, it is critical to have knowledge of the distribution and transition of E coli O157:H7 subtypes in a farm environment. Investigators in 1 study12 conducted a 5-month evaluation at a feedlot operation and found the strain diversity within pens, individual animals, and their environment. In another study,20 multiple MLVA types were identified from fecal samples and water samples collected from a ruminant pasture or stream environment during a 19-week period. To the authors’ knowledge, no longitudinal study conducted throughout a year on E coli characterized by use of MLVA in pasture-based beef cattle in a cow-calf operation has been reported. Therefore, the objectives of the study reported here were to evaluate seasonal patterns and risk factors for prevalence of E coli O157:H7 in feces in a pasture-based cow-calf operation and to determine the diversity and transition of E coli O157:H7 strains by use of both MLVA and PFGE.

Materials and Methods

Sample population—A herd of beef cattle located at the University of California Sierra Foothill Research and Extension Center in Yuba County, Calif, was used for the study. The herd consisted of approximately 400 adult beef cows that were divided into two groups; number of cattle in each group varied, depending on pasture size, grass availability, and calving season. Pastures were native range or irrigated pasture consisting of a legume-grass mixture. Protein supplement products1 were provided to groups, depending on their reproductive stage and season. Alfalfa hay was fed to groups grazing native pastures in winter (December through February) to supplement forage from pastures. Calves were born between October and November and remained with their dams until weaning at 7 months of age. No new cattle were introduced into the herd during the study period.

Sample collection—Fecal samples were collected once each month from March 2007 through February 2008. Approximately 40 samples were collected from multiple groups of cows in different pastures each month. Freshly passed fecal material was collected with sterile disposable spatulas and clean examination gloves. Fecal samples were placed into sterile plastic bags, stored in a cooler with ice, and immediately transported to our laboratory. Samples were processed within 4 hours after collection. Air temperature and precipitation data were retrieved from the California Irrigation Management Information System.

Recovery of E coli O157:H7—Fecal samples were submitted for bacterial culture, and E coli O157:H7 were recovered by use of a method described elsewhere.18 Briefly, 10 g of feces was placed into 90 mL of tryptic soy broth and incubated for 2 hours at 25°C, then for 8 hours at 42°C with shaking, and then at 4°C without shaking until further processing. Anti-O157 magnetic beads1 were added to the enriched broth. Beads were incubated and washed with PBS solution (pH, 7.4) with 0.05% Tween 20 in accordance with the entero-
pathogenic *E. coli*—verotoxin-producing *E. coli* protocol. Fifty microliters of the resuspended beads was spread on CT-SMAC (ie, sorbitol MacConkey agar) containing cefixime (0.05 μg/mL) and tellurite (2.5 μg/mL) and on NT-SCCM (ie, a selective, chromogenic culture medium) containing novobiocin (20 μg/mL) and tellurite (0.8 μg/mL). Plates were incubated at 37°C overnight. Suspect colonies and a positive control sample of *E. coli* O157:H7 were swabbed onto Luria Bertani broth agar and incubated at 37°C overnight. When there were many potential colonies, we selected 10 from CT-SMAC and 10 from NT-SCCM. Then, immunoblotting by use of nitrocellulose membranes was performed on the set of swabbed colonies that grew on Luria Bertani broth agar. Each membrane was washed and blocked by incubation with 10 mL of casein block at 21°C for 30 minutes with agitation. The membrane was incubated in mouse anti-O157 IgG monoclonal antibody (diluted 1:2,000 in 10 mL of diluting buffer, which consisted of 1X TBS, 1% bovine serum albumin, 0.02% KCl, 0.1% sodium azide, and 0.1% Tween 20). After 30 minutes of agitation, the membrane was washed and then incubated in goat anti-mouse IgG conjugated with alkaline phosphatase (diluted 1:2,000 in diluting buffer as above) at 21°C for 30 minutes with agitation. The membrane was washed and developed by adding 10 mL of alkaline phosphatase substrate.

**Detection of virulence factors by use of PCR assays**—Putative *E. coli* O157:H7 colonies identified via immunoblotting were analyzed further with RT-PCR assays by use of methods reported elsewhere to detect virulence genes. For the detection of the *rfb* gene, 20 μL of a universal PCR master mix, 0.3 μM *rbe* primer, and 0.1 μM FAM probe were used. The DNA of isolates that had positive results for immunoblotting with anti-O157 monoclonal antibody was extracted. Cells were boiled for 20 minutes, then 1 μL of lysate was transferred directly into a PCR plate. The amplification cycles of the RT-PCR machine were 95°C for 5 minutes, then 60 cycles of 95°C for 15 seconds and 60°C for 45 seconds. Colonies producing *rbe* were confirmed as *E. coli* O157 and analyzed further for *fliC* (the structural gene of the H7 serogroup) and other virulence genes (ie, *stx1*, *stx2*, *eae*, and *hly*) in multiplex reactions. The DNA template was prepared by resuspending cells by boiling in 100 μL of distilled water for 20 minutes. Debris from boiled cells was removed by centrifugation at 21,130 × g for 10 minutes. Each 15-μL reaction contained a multiplex PCR master mix, 0.25 μM of each primer, and 1 μL of template. Settings for the thermal cycles were 95°C for 1 minute, 65°C for 2 minutes, and 72°C for 1.5 minutes. Annealing temperature decreased from 65°C to 60°C between cycles 10 and 15, and elongation time increased from 1.5 to 2.5 minutes between cycles 25 and 35. The product was assayed on 2% tris-acetate-EDTA agarose gels, which included a 100-bp ladder.

**Strain typing by use of MLVA and PFGE**—The MLVA was performed by use of capillary electrophoresis techniques based on methods described elsewhere. Eleven loci were amplified in 3 multiplex PCR reactions with fluorescent primers, as described elsewhere. Reaction 1 contained primers for Vhec1-VIC, Vhec3-NED, Vhec4-FAM, and Vhec5-FAM; reaction 2 contained primers for Vhec1-VIC, Vhec2-NED, Vhec6-VIC, and Vhec7-FAM; and reaction 3 contained primers for O157-17-FAM, O157-19-NED, O157-25-NED, and O157-37-VIC. Each 10 μL of PCR reaction contained multiplex PCR master mix; 0.2 μM of each primer, and 1 μL of template. Thermal cycling settings for reactions 1 and 2 were 95°C for 15 minutes; then 25 cycles at 94°C for 30 seconds, 63°C for 92 seconds, and 72°C for 90 seconds; and then a final extension at 72°C for 10 minutes. Thermal cycling settings for reaction 3 were 95°C for 15 minutes; then 35 cycles at 94°C for 20 seconds, 65°C for 20 seconds, and 72°C for 20 seconds; and then a final extension at 72°C for 10 minutes. Products for the PCR reactions were pooled and diluted 1:50 with distilled water. One microliter of the diluted product was added to a mixture of 12 μL of formamide and 0.05 μL of a 100-bp ladder. The mixture was heat-denatured for 5 minutes at 95°C, cooled on ice for 2 minutes, and then loaded onto a sequencer. The size of fragments was determined by use of commercial software. A dendrogram was constructed with categorical coefficients and the Ward algorithm by use of commercially available software. The MLVA types were assigned the next available number on the basis of a unique profile (at least 1 tandem-repeat difference), compared with results in an internal database.

**Statistical analysis**—Prevalence estimates were calculated by dividing the number of samples with positive results for *E. coli* O157:H7 by the total number of samples. Seasonal differences in prevalence were determined by use of χ² tests; season was classified as winter (December through February), spring (March through May), summer (June through August), and fall (September through November). Associations between *E. coli* O157:H7 in feces and management factors (animal density in pasture, presence of calves, and pasture type) and climate factors (7-day cumulative precipitation and 30-day mean temperature before sample collection) were determined by use of univariable and multivariable logistic regression. Animal density was classified into 3 categories (< 1 animal/ha, ≥ 1 to < 5 cattle/ha, and ≥ 5 cattle/ha). Similarly, 30-day mean temperature before sample collection was classified into 3 categories (< 13°C, ≥ 13°C to < 20°C, and ≥ 20°C). The 7-day cumulative precipitation was classified into 2 categories (< 10 mm or ≥ 10 mm). Variables that remained in the final multivariable logistic regression model were determined by use of a backward elimination approach, with P ≤ 0.05 as the value needed for inclusion. Commercially available software was used for all statistical analyses.

**Results**

A total of 456 fecal samples were collected during the 12-month sample collection period. Overall preva-
lence estimate of fecal samples with positive results for \( E. coli \) O157:H7 was 10.5% (48/456). The prevalence differed significantly \( (P = 0.001) \) among seasons, with the lowest prevalence in winter \( (\text{Figure 1}) \). Fall had a higher prevalence than did spring or summer.

Results for environmental and management factors of the location for collection of fecal samples were determined \( (\text{Table 1}) \). The 30-day mean temperature before sample collection and 7-day cumulative precipitation before sample collection were significantly associated with \( E. coli \) O157:H7 prevalence in feces for the univariate analysis \( (\text{Table 2}) \). In the multivariable logistic regression analysis, 30-day mean temperature before sample collection was positively associated with detection of \( E. coli \) O157:H7 in feces. The association between the 30-day mean temperature and \( E. coli \) O157:H7 prevalence was not linear because the OR was higher for moderate temperatures \( (\text{OR} = 20.6; \text{95\% CI, 2.8 to 154.2}; P = 0.003) \), compared with the OR for high temperatures \( (\text{OR} = 18.0; \text{95\% CI, 2.4 to 135.7}; P = 0.005) \). In the logistic regression analysis, animal density, presence of calves, 7-day cumulative precipitation before sample collection, and irrigation of pasture were not significantly associated with detection of \( E. coli \) O157:H7 in fecal samples.

A total of 113 isolates from 48 fecal samples collected during a 12-month period were confirmed as \( E. coli \) O157:H7. An isolate was defined as a bacterial colony cultured on CT-SMAC or NT-SCCM that had a unique morphological appearance confirmed to be consistent with that of \( E. coli \) O157:H7. Ten fecal samples yielded 1 isolate, whereas 20 samples yielded 2 isolates, 11 samples yielded 3 isolates, 6 samples yielded 4 isolates, and 1 sample yielded 6 isolates. All \( E. coli \) O157:H7 isolates had positive results for \( fliC, eae, \) and \( hly \) when evaluated by use of RT-PCR assay. Forty fecal samples yielded isolates that had positive results for \( stx1 \) and \( stx2 \), whereas 2 samples yielded isolates that had positive results for \( stx1 \) and negative results for \( stx2 \). Positive results for \( stx2 \) were not detected again during the study period.

Of the 113 \( E. coli \) O157:H7 isolates, 103 were submitted for MLVA. Ten isolates from 4 fecal samples collected in November were not submitted for MLVA because they were misplaced after testing to detect virulence genes. Nineteen MLVA types were identified from the 103 isolates obtained from the 44 fecal samples \( (\text{Figure 2}) \). Eight fecal samples had multiple isolates with different MLVA types \( (2 \text{ samples each had } 3 \text{ MLVA types, and 6 samples each had } 2 \text{ MLVA types}) \). The remaining 36 fecal samples each had only 1 MLVA type. Of 11 loci used for MLVA, Vhec1 had the most variation in the number of repeats, which varied from 18 to 31. In contrast, Vhec6 and O157-37 did not have any variation in the number of repeats. Samples collected from the same group of cattle during the same month typically had identical or extremely similar MLVA profiles.

In creating the dendrogram based on results of MLVA typing, isolates with indistinguishable MLVA profiles obtained from the same sample were considered as 1 strain \( (\text{Figure 2}) \). As a result, 54 isolates contributed to the dendrogram. The isolates were divided into 2 clusters, with some exceptions. Cluster A included samples collected from March through August, whereas cluster B included samples collected from June through January. Three isolates were not included in these clusters. Notably, MLVA type 476 was substantially different from any other isolates.

We observed that some of the MLVA types were detected repeatedly and continuously throughout the sample collection period. For example, MLVA type 346 was continuously identified in samples collected in March, April, and May; MLVA type 347, which was different from type 346 by only 1 tandem-repeat difference on the Vhec1 locus, was identified in samples collected in April and in June through August. These 2 MLVA types were not detected again during the study period.

Table 1—Effects of environmental and management factors on results for \( E. coli \) O157:H7 in fecal samples collected during a 12-month period in 2007 and 2008 from a range-based herd of beef cattle located at the University of California Sierra Foothill Research and Extension Center.

<table>
<thead>
<tr>
<th>Variable (n = 408 fecal samples)</th>
<th>Negative results (n = 408 fecal samples)</th>
<th>Positive results (n = 408 fecal samples)</th>
<th>( P ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Density (cattle/ha)*</td>
<td>4.69 ± 0.23</td>
<td>5.92 ± 1.02</td>
<td>0.72</td>
</tr>
<tr>
<td>30-day mean temperature before sample collection (°C)*</td>
<td>16.86 ± 0.30</td>
<td>18.96 ± 0.63</td>
<td>0.004</td>
</tr>
<tr>
<td>7-day cumulative precipitation before sample collection (mm)*</td>
<td>15.75 ± 0.87</td>
<td>8.18 ± 1.75</td>
<td>0.006</td>
</tr>
<tr>
<td>Presence of calves§</td>
<td>291 (71.3)</td>
<td>35 (72.9)</td>
<td>0.82</td>
</tr>
<tr>
<td>Pasture irrigation§</td>
<td>101 (24.8)</td>
<td>14 (29.2)</td>
<td>0.51</td>
</tr>
</tbody>
</table>

*Values were considered significant at \( P < 0.05 \). Value reported is mean ± SE. §Value reported is number (%) of fecal samples collected from pastures that contained calves. 

Figure 1—Prevalence of \( E. coli \) O157:H7 in fecal samples of adult beef cattle \( (\text{triangles}) \), 30-day mean temperature before collection of fecal samples \( (\text{diamonds}) \), and 7-day cumulative precipitation before collection of fecal samples \( (\text{squares}) \). Approximately 40 fecal samples were collected each month for a 1-year period from a range-based herd of beef cattle located at the University of California Sierra Foothill Research and Extension Center.

Table 2—Multivariable logistic regression analysis of factors associated with detection of \( E. coli \) O157:H7 in feces. The association between the 30-day mean temperature and \( E. coli \) O157:H7 prevalence was not linear because the OR was higher for moderate temperatures \( (\text{OR} = 20.6; \text{95\% CI, 2.8 to 154.2}; P = 0.003) \), compared with the OR for high temperatures \( (\text{OR} = 18.0; \text{95\% CI, 2.4 to 135.7}; P = 0.005) \).
We detected MLVA type 416 repeatedly in samples collected in June and July and in September and October. This MLVA type was the most frequently detected strain in the study. A total of 20 isolates (6 in June, 1 in July, 7 in September, and 6 in October) were classified as MLVA type 416, although most of them were in fecal samples collected from the same group of cattle (Figure 2).

Eighty-seven isolates were submitted for PFGE. Sixteen isolates from fecal samples collected in March and April were not submitted for PFGE; for the first 2 months of the study, isolates with an identical MLVA type from the same fecal sample were discarded because we assumed that they represented the same strain. Subsequently, all isolates were submitted for PFGE, regardless of MLVA profile. One isolate failed to yield a PFGE result because of the poor state of preservation. Fourteen PFGE XbaI types were identified from 86 isolates, and a dendrogram that represented genetic similarity was created (Figure 3). Obvious clustering of isolates was observed, although XbaI-13 and XbaI-14 were distant from the other 12 PFGE types. Isolates from 6 fecal samples each had 2 PFGE XbaI types, whereas isolates from all other fecal samples each had only 1 PFGE type. As observed for MLVA, certain PFGE types were continuously detected during the study period. For example, XbaI-7 was repeatedly isolated from samples collected in March through August, whereas XbaI-4 was repeatedly isolated in samples collected from June through October and in January.

The relationship between MLVA and PFGE profiles was evaluated (Figure 2). The 2 most frequently identified PFGE types corresponded to multiple MLVA types (eg, isolates with the XbaI-4 type corresponded to 4 MLVA types, and isolates with the XbaI-7 type corresponded to 6 MLVA types). Similarly, some MLVA types were associated with multiple PFGE types. Cluster A was dominated by isolates with the PFGE XbaI-7 type, whereas most of the isolates in cluster B had the PFGE XbaI-4 type.

### Discussion

The overall prevalence (10.5%) of *E. coli* O157:H7 in fecal samples collected from a range-based herd of beef cattle monthly during a 1-year period.
beef cattle in California in the study reported here was higher than that in another longitudinal study conducted in range-based cattle operations in the United States. Prevalence values of 0.7% in Washington, 0.5% in Kansas and Nebraska, 1.3% in Kansas, and 3.6% in 5 states (Alabama, California, North Carolina, Tennessee, and Washington) have been reported. Because several factors, such as management practices, diet, animal factors, environmental factors, and method for Shiga toxin–E coli detection, can influence prevalence, comparisons among studies need to be evaluated carefully.

At least 2 explanations exist for the higher prevalence found in the present study. An obvious possibility is that this herd may truly have had more cattle that shed this organism. Given the wide variation in prevalence estimates that exist in the literature, it is apparent that some groups of cattle have a higher probability of a positive test result for this organism. Data specific to range-based beef cattle in California are limited, but investigators in a recent cross-sectional study found E coli O157:H7 in 26 of 77 (33.8%) cattle fecal samples, which indicates the potential for a higher prevalence in this type of production system.

Another possible explanation for the higher prevalence in the present study is that we used a highly sensitive culture method that included IMS and 2 selective media (NT-SCCM and CT-SMAC). The recovery of E coli O157:H7 from bovine feces can be enhanced by the use of IMS, and use of media with different selective mechanisms enhances the identification of samples with positive results. In addition, we used 10 g of feces for isolation of E coli O157:H7, instead of 1 g of feces or rectal swab specimens that have been used by others. Testing a single 1-g sample/fecal pat by use of IMS may result in a sensitivity of detection as low as 20% because the distribution of E coli O157:H7 in a fecal pat is uneven and typically low. Therefore, use of 10 g of feces probably improved the detection of E coli O157:H7 from fecal pats that contained this organism.

We found that the prevalence of E coli O157:H7 was lowest in the winter and that the prevalence increased when air temperature was moderate to high. This result is consistent with results of some studies in which the largest number of samples that yielded positive results were collected during warmer seasons, whereas fewer isolates were found in the winter. In contrast, other investigators have reported no apparent seasonal difference in fecal shedding of E coli O157:H7. Temperature may affect pasture conditions, such as grass quality, drinking and irrigation water management, wildlife activity, and behavior and distribution of cows in a pasture, thereby influencing apparent prevalence. Further investigation is required to determine the factors that are the most important in this region of California.

The MLVA is a novel, highly discriminatory molecular typing method for E coli O157:H7 that can provide results quickly, is robust, and offers many advantages, compared with other methods such as PFGE. We identified 19 MLVA types from 103 E coli O157:H7 isolates of 48 fecal samples, which indicated the diversity of E coli O157:H7 in this range-based cattle herd and the high discriminatory ability of MLVA. We identified > 1 MLVA subtype in several fecal samples, which may suggest the coexistence of different E coli O157:H7 populations in an animal or a mutational event that causes subtle genetic change in E coli strains. This finding was supported by the identification of 14 PFGE XbaI types from 87 isolates and of multiple PFGE types from a single fecal sample. Isolates from the same sample had identical stx profiles and had similar MLVA profiles with a single- or double-locus variation.

Detection of multiple E coli O157:H7 strains in a fecal sample was consistent with results of other studies. Investigators in 1 study reported that > 1 PFGE XbaI type was detected from a sample when they selected all separate distinct non–sorbitol-fermenting colonies from CT-SMAC plates. Investigators in another study reported a mean of 1.77 XbaI PFGE subtypes/bovine fecal sample when they analyzed up to 3 isolates/sample. They suggested that the number of isolates analyzed per sample may affect the precision of subtype comparisons and that it may be necessary to analyze > 1 isolate/sample to minimize potential misclassification when subtypes from different sources are compared. We selected suspected colonies (on the basis of morphological characteristics) grown on 2 selective media. Although some of the morphologically distinct colonies were identified as indistinguishable MLVA types, others were classified as different MLVA types. Furthermore, some of the colonies grown on each media were classified into different MLVA types. This result indicates that there will be a better chance of identifying multiple E coli O157:H7 strains in a fecal sample by use of multiple selective media and selection of several colonies from 1 culture plate.

We detected multiple MLVA types among and within pastures in 1 sample collection visit. For example, 4 MLVA types were isolated from fecal samples collected in 1 pasture in March and another MLVA type was isolated from fecal samples collected in another pasture during that same month. In other studies, it was...
reported that multiple *E. coli* O157:H7 subtypes were identified from multiple sites, animals, or sources at a single sample collection visit in a pasture-based operation. In the study reported here, no new cattle were introduced into the herd during the study period, thus, there may have been less likelihood of introducing new strains of *E. coli* O157:H7 through introduction of new cattle, compared with the situation at feedlots in which animals from multiple farms are introduced and commingled. Therefore, the diversity of strains in this study may indicate various sources (such as wildlife, soil, and surface water in the farm environment) for the maintenance and transmission of *E. coli* O157:H7.

The diversity of MLVA types may also be explained by the high discriminatory power of this method, therefore, results need to be interpreted with caution. Results indicated that the method is sensitive enough to correctly differentiate epidemiologically related and unrelated isolates during investigations of disease outbreaks. It has been reported in several studies that isolates from the same outbreak had a variation in at least 1 of 7 loci within 5 days after inoculation. The authors of that study suggested that single-locus variants that differed by either a single or double repeat might have arisen from a point source and should be investigated accordingly in outbreak investigations. It has also been reported that MLVA can be used to correctly differentiate isolates in outbreaks when either a single- or double-locus variation in 1 locus is allowed. We agree with other researchers that isolates with a variation of 1 or 2 repeats at a single locus should be considered as closely related strains. However, it is noteworthy that none of the MLVA types identified in the present study matched any strains in a database of > 750 MLVA types.

Results of PFGE were used to differentiate closely related strains in our study; however, it is difficult to evaluate the method (MLVA or PFGE) that has a higher discriminatory power. We used only 1 restriction enzyme for PFGE; the discriminatory power could have been increased if additional enzymes (such as BlnI) were used. In general, restriction digestion with a second, and in some cases a third, enzyme often increases the overall discriminatory power of PFGE. Strains differentiated by use of MLVA were not differentiated by use of PFGE XbaI. At the same time, PFGE XbaI was used to differentiate slight differences among isolates that were classified as identical MLVA types (Figure 2).

Profiles for MLVA and PFGE XbaI were highly correlated. The MLVA cluster A was primarily associated with PFGE XbaI-7, whereas MLVA cluster B was primarily associated with PFGE XbaI-4 (Figure 2). Isolates from 3 fecal samples (2 collected in March and 1 collected in September) were considered as outliers in the MLVA dendrogram, which was consistent with the PFGE result because PFGE XbaI types assigned to those isolates were genetically distant from almost all isolates from other samples.

Although diverse strains were identified in this 1-year study, 3 MLVA types were repeatedly and continuously identified. This finding was confirmed by use of PFGE, which identified 2 dominant PFGE XbaI types. These predominant strains turned over throughout the study period, which was consistent with results of other studies in which investigators used either MLVA or PFGE. In 1 study, investigators identified 79 unique XbaI PFGE subtype patterns from 235 isolates of 92 samples, and although most of the XbaI PFGE subtypes were recovered in samples collected during only 1 collection, the 2 most frequently isolated XbaI PFGE subtypes were recovered repeatedly in samples collected during several collections. The presence of a predominant strain on a farm may suggest that a common source of infection or specific carrier animals contributed to the dissemination or persistence of predominant strains and that selective pressures exist to bring about a shift of *E. coli* O157:H7 type within a herd. Other subtypes isolated only once or twice during the study period could be explained by minor alterations in the genetic material of *E. coli* O157 strains, which may not be maintained in a population, or by rare exposures or introductions of new subtypes from the environment (such as from wildlife or water) that failed to persist. The presence of a predominant strain may play an important role in investigations of outbreaks of disease associated with *E. coli* O157:H7 in humans. If the unique strain that is related to the *E. coli* O157:H7 outbreak in humans were widely spread and persistent in a specific cattle population, the probability of correctly tracing it back to the source of infection would be high. However, our study revealed frequent turnover of predominant types of *E. coli* O157:H7 in feces, which indicates that it may not be possible to trace the infection back to the source if samples are collected a few months after the occurrence of an outbreak.

In the study reported here, we detected a seasonal pattern for prevalence of *E. coli* O157:H7 in cattle feces in a range-based cattle operation in California. The incidence of this pathogen in range-based cattle in California is relevant because of the association of *E. coli* O157:H7 with numerous outbreaks associated with leafy vegetables grown in California, the prevalence of *E. coli* O157:H7 in feral swine, and the fact that there are watersheds near leafy vegetable production.

Results for MLVA and PFGE correlated well and appeared to have equivalent discriminatory power for the strains isolated in our study. Both subtyping methods could be used to identify the diversity of *E. coli* O157:H7 strains on this farm and the turnover of predominant types over time. The MLVA can be a powerful tool for use in investigating disease outbreaks attributable to *E. coli* O157:H7 and could be used as a supplemental method to confirm or complement results obtained with PFGE. An advantage of MLVA is that it is a less labor-intensive method than PFGE, thus facilitating genotyping of multiple colonies from individual samples and enhancing the probability of detection of multiple strains from single samples or strains during outbreak investigations. For example, use of the same MLVA method that was used in the study reported here facilitated identification of outbreak-related strains (subsequently confirmed by use of PFGE) during the 2006 investi-
gation into the disease outbreak attributable to contaminated baby spinach.\textsuperscript{9,23} In numerous situations, MLVA has been used to determine that strains indistinguishable on the basis of results for XbaI and BlnI PFGE were not associated with an outbreak (data not shown). Therefore, use of MLVA can provide a more accurate measure of strain-relatedness than can use of PFGE, especially with insertion-deletion events that alter PFGE fragment sizes\textsuperscript{44} but that do not affect MLVA tandem repeats. Similar to results for other molecular subtyping methods, epidemiological data must be used in conjunction with MLVA and PFGE results.

References


