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Prevalence and Clonal Nature of *Escherichia coli* O157:H7 on Dairy Farms in Wisconsin

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A survey was conducted between March and October of 1994 to determine the prevalence and identify the sources of serotype O157:H7 isolates of *Escherichia coli* in Wisconsin dairy herds. A stratified sample of 400 farms was identified, and 70 farms with weaned calves less than 4 months old were included in the study. During the prevalence study, 5 of the 70 farms (herd prevalence, 7.1 ± 4.5%) and fecal samples from 10 of 560 calves (animal prevalence, 1.8%) tested positive for serotype O157:H7. In a follow-up study, the five O157:H7-positive farms and seven of the O157:H7-negative farms identified in the prevalence study were visited again. An additional 517 fecal samples from cattle of various ages were tested, and a total of 15 animals from four of the five herds that were previously positive and 4 animals from two of seven herds that were previously negative tested positive for *E. coli* O157:H7. Observations made during the follow-up study suggested that horizontal transmission was an important means of *E. coli* O157:H7 dissemination on the farms. A total of 302 environmental samples, were examined, and 2 animal drinking water samples from one previously negative farm and 1 animal drinking water sample from a previously positive farm contained *E. coli* O157:H7. Analyses by the pulsed-field gel electrophoresis technique of contour-clamped homogeneous electric field electrophoresis revealed that isolates from the same farm displayed identical or very similar *Xba*I restriction endonuclease digestion profiles (REDP), whereas isolates from different farms typically displayed different REDP. However, more than one REDP was usually observed for a given herd over the 8-month sampling period. Analyses of multiple isolates from an animal revealed that some animals harbored O157:H7 strains that had different REDP, although the REDP of isolates obtained from the same fecal sample were very similar. Collectively, 160 bovine isolates obtained from 29 different animals and three water isolates displayed 20 distinct *Xba*I REDP. Our data revealed that there are several clonal types of serotype O157:H7 isolates in Wisconsin and indicated that there is probably more than one source of this pathogen on the dairy farms studied. However, animal drinking water was identified as one source of *E. coli* O157:H7 on one farm.

Since *Escherichia coli* O157:H7 was first recognized as a human pathogen (23), bovines have been suspected to be a primary reservoir of this organism because ground beef has been implicated in a majority of outbreaks (10). However, other foods, such as apple cider, turkey roll, and fermented sausage, have also been linked with O157:H7 outbreaks (1, 2, 10). The illnesses resulting from *E. coli* O157:H7 infection range from hemorrhagic colitis to more serious syndromes, including hemolytic uremic syndrome and thrombotic thrombocytopenic purpura (9). To date, the largest outbreak associated with this pathogen involved ground beef distributed through a single fast-food chain in California, Idaho, Nevada, and Washington in late 1992 and early 1993. This western states outbreak resulted in more than 500 confirmed cases and four deaths (7). Between 1982 and mid-1995, *E. coli* O157:H7 was implicated in 75 outbreaks involving 2,562 individuals (8a).

The results of surveys of cattle and traceback studies support epidemiological data that link *E. coli* O157:H7 with a bovine reservoir (8, 11, 25). In a Washington state survey, *E. coli* O157:H7 was isolated from 10 of 3,570 dairy cattle (0.3%), 10 of 1,412 beef cattle (0.7%), and 2 of 600 feedlot beef cattle (0.3%) (11). The herd prevalences of O157:H7 strains in dairy and beef cattle were 8.3% (5 of 60 herds) and 16% (4 of 25 herds), respectively. In another survey conducted in 1991 and 1992, preweaned dairy calves in 28 states throughout the United States were analyzed for *E. coli* O157:H7, and 0.4% (25 of 6,894) of the calves and 1.8% (19 of 1,068) of the herds tested positive (12). In a traceback study of nine farms that may have been sources of meat involved in an outbreak of hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura, five of the farms (55.5%) and 7 of 315 heifers (2.2%) tested positive for *E. coli* O157:H7 (25). In a case-control study of two farms that were the sources of raw milk associated with two cases of hemolytic uremic syndrome (case farms) and 11 control farms, researchers found that 5 of 85 heifers (5.9%) from the case farms and 4 of 158 heifers (2.5%) from three of the control farms, including one control farm adjacent to the case farms, were positive for *E. coli* O157:H7 (25). Although it is difficult to directly compare the results of previous studies because of different sampling and testing procedures, as well as a lack of information on the shedding of *E. coli* O157:H7, previously published results (11, 12) reveal that the prevalence of *E. coli* O157:H7 in cattle ranges from 0.3 to 0.7% and that the prevalence of this pathogen in cattle herds ranges from 1.8 to 16%.

Despite evidence that bovines are a reservoir for *E. coli* O157:H7, relatively little is known about the farm ecology of this pathogen. This study was undertaken to determine the prevalence of *E. coli* O157:H7 on Wisconsin dairy farms, the

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number of genomic types of O157:H7 per farm and per animal, the distribution of the genomic types throughout Wisconsin, and the farming and/or environmental factors that may contribute to the maintenance and/or dissemination of this pathogen.

(Some of the results were presented at the 95th General Meeting of the American Society for Microbiology [7a].)

MATERIALS AND METHODS

Prevalence study design and statistical methods. A stratified sample of 400 dairy farms in Wisconsin was identified. The farmers were contacted by letter and phone, and 70 agreed to participate in the study. On the basis of the number of participating farms, the results were significant at a confidence level of 90% (18). In drawing the sample, the following assumptions were made: (i) approximately 5% of weaned calves shed *E. coli* O157:H7 (8), and (ii) calves less than 4 months old account for 8% of the animals on dairy farms in the United States (26a). The sample of farms was divided into four strata on the basis of the number of milking cows. According to surveys of Wisconsin dairy farms conducted by the Wisconsin Agricultural Statistics Service, 13% of the farms have 1 to 29 milking cows (stratum 1), 40% have 30 to 49 milking cows (stratum 2), 46% have 50 to 199 milking cows (stratum 3), and 1% have 200 or more milking cows (stratum 4). The actual numbers (and corresponding percentages) of participating farms in the four strata were as follows: stratum 1, 5 (7.1%); stratum 2, 22 (31.4%); stratum 3, 41 (58.7%); and stratum 4, 2 (2.8%).

Follow-up study. Positive herds were identified in the prevalence study, and each of these herds had one or more weaned calves that tested positive for *E. coli* O157:H7. Herds that were negative for *E. coli* O157:H7 were identified in the prevalence study and were matched by herd size (i.e., stratum) and geographic location to positive herds.

Sample collection and storage. Samples were collected from March 1994 through October 1994. In the prevalence study, only weaned calves less than 4 months old were tested. All bovine fecal samples (ca. 30 g) were obtained by digital rectal retrieval. Fecal samples from cats and dogs were collected with a rectal loop, and pig and rabbit feces were collected from cages or pens housing individual animals. Raccoon feces were obtained from a barn floor. All fecal samples were transferred to sterile, screw-cap tubes containing 7.5 ml of Bacto Transport Medium Amies without charcoal and agar (Difco Laboratories, Detroit, Mich.) and shaken. Environmental, feed, water, and non-fecal animal samples were collected aseptically and transferred to sterile containers (Whirlpack bags or specimen cups). All samples were then placed in coolers containing cold packs and shipped overnight to the Food Research Institute in Madison, Wis., for testing. If necessary, the samples were refrigerated prior to express delivery, but all samples were tested within 48 h of collection.

After portions of fecal samples were removed for microbiological testing, the remainder of each sample was mixed 1:1 with 2× freezing medium (nutrient broth, 16 g; yeast extract, 10 g; glycerol, 200 ml; distilled H_2O, 800 ml) and stored at −20°C.

Microbiological analyses. A 10-g portion of a sample was added to a flask containing 90 ml of modified EC broth (20) supplemented with novobiocin (final concentration, 20 μg/ml; Sigma Chemical Co., St. Louis, Mo.), and the preparation was incubated at 37°C with shaking (1500 rpm) for 18 to 24 h. When water samples were tested, 100-ml volumes were added to 100-ml portions of 2× modified EC broth, and the preparations were incubated as described above for the other samples. Next, samples were serially diluted in 0.1% Bacto Peptone (Difco), and 0.1-ml portions of the 10^{-5} and 10^{-6} dilutions were spread plated onto duplicate plates of MacConkey sorbitol agar (Difco). In the follow-up study, MacConkey sorbitol agar supplemented with cefixime (50 μg/liter; Lederle Laboratories, Pearl River, N.Y.) and potassium tellurite (25 mg/liter; Sigma) (27) was used in addition to MacConkey sorbitol agar to enhance detection of *E. coli* O157:H7. Plates were incubated overnight at 42°C and examined for the presence of sorbitol-negative (i.e., white) colonies. A maximum of 15 sorbitol-negative colonies per sample were tested for the O157 antigen by using an O157 latex agglutination test (Oxoid, Basingstoke, England). Colonies that agglutinated were streaked onto MacConkey sorbitol agar, incubated overnight, and retested for the O157 antigen. Agglutination-positive isolates were then transferred to brain heart infusion (Difco) agar slants until biochemical and serological tests were conducted.

Sorbitol-negative and O157-positive colonies were confirmed biochemically as *E. coli* by using an API 20E biochemical test strip (bioMérieux Vitek, Inc., Hazelwood, Mo.). Isolates were also tested for β-glucuronidase activity by using 4-methylumbelliferyl-β-D-glucuronide (Sigma) (22) and for the presence of the H7 antigen by using antiserum as described by the manufacturer (Difco). A maximum of 12 confirmed colonies from each sample were stored in nutrient broth (Difco) containing 10% glycerol at −70°C for further analysis.

Genomic typing. The pulsed-field gel electrophoresis (PFGE) technique of contour-clamped homogeneous electric field (CHEF) electrophoresis was used for genomic typing of *E. coli* O157:H7 isolates as described previously (13, 17). Genomic DNAs were digested in agarose plugs with *Xba*I (Promega Corp., Madison, Wis.) as recommended by the manufacturer. The resulting fragments were resolved by CHEF-PFGE by using a CHEF-DRII apparatus (Bio-Rad Laboratories, Richmond, Calif.) at 200 V for 21 h at 18°C and switch times ramped from 1 to 40 s. Lambda concatamers (New England Biolabs, Inc., Beverly, Mass.) were used as DNA size standards.

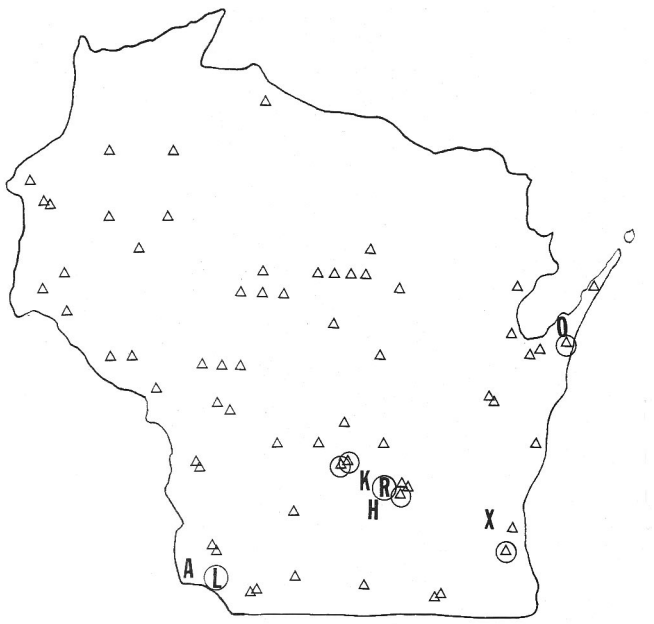

FIG. 1. Approximate locations of the 70 participating dairy farms: *E. coli* O157:H7-positive farms A, H, K, L, O, R, and X and negative farms (triangles) used in the prevalence and follow-up studies. Circles indicate that farms (L, R, and the circled locations) tested negative for *E. coli* O157:H7 in the prevalence study.

Detection of SLT genes. Two 20-bp oligonucleotide probes (14) were purchased from National Biosciences, Plymouth, Minn., and these probes were used to detect the presence of Shiga-like toxin (SLT) I and II sequences (17). The probes were labeled with digoxigenin, and hybridization was detected by using procedures described by the manufacturer (Boehringer Mannheim, Indianapolis, Ind.).

Data management and calculation of similarity indices. The presence or absence of macrorestriction fragments in each strain was converted to binary scores for analysis by ELBAMAP software, and the numbers of shared fragments in restriction endonuclease digestion profiles (REDP) were used to calculate Dice similarity indices as described by Brosch et al. (4).

RESULTS

Prevalence study. The survey of 70 Wisconsin farms revealed that 5 of these farms (herd prevalence, 7.1 ± 4.5%) and 10 of 560 weaned calves (animal prevalence, 1.8%) tested positive for *E. coli* O157:H7. Weaned calves were sampled because in previous studies workers found that these animals are more likely to shed *E. coli* O157:H7 than cattle of other ages (8, 11, 26). Figure 1 shows the distribution and approximate locations of the 70 herds tested and the five *E. coli* O157:H7-positive farms (farms A, H, K, O, and X). In addition, Fig. 1 shows the locations of two farms that tested negative in the prevalence study but positive in the follow-up study (farms L and R) (see below) and five farms that tested negative in both the prevalence and follow-up studies.

Follow-up study. The five farms that tested positive for *E. coli* O157:H7 in the prevalence study were all stratum 3 farms (based on the number of milking cows) and had herd sizes (total numbers of animals) ranging from 134 to 274 cattle (Table 1). However, 41 of the 70 farms tested (58.7%) were stratum 3 farms. Seven farms that tested negative for *E. coli* O157:H7 in the prevalence study were also examined in the follow-up study and were matched with the five positive farms

TABLE 1. Herd sizes and the numbers and locations of *E. coli* O157:H7-positive animals on farms tested in the follow-up study

Farm	Stratum[a]	Herd size[b]	No. positive/no. tested for *E. coli* O157:H7			Location of positive animals
			Cows and heifers[c]	Bulls and steers	Calves[d]	
Farms that were positive in the prevalence study						
A	3	204	3/13		0/16	Common pen
H	3	247	0/24	1/21	3/64	Common barn or pen[e]
K	3	134	0/17	0/1	0/33	
O	3	255			3/20	Common barn[f]
X	3	274	3/74	0/3	2/68	Common barn or pen[g]
Total			6/128 (4.7)[h]	1/25 (4.0)	8/201 (3.6)	
Farms that were negative in the prevalence study						
L	3	168	2/29	1/3	0/5	Common pen
LLL	3	216		0/7	0/13	
N	3	239			0/20	
O	3	122	0/12	0/6	0/3	
Q	3	232	0/6		0/16	
R	3	287	1/18	0/5		
W	3	103		0/5	0/15	
Total			3/65 (4.6)	1/26 (3.8)	0/72 (0)	

[a] Dairy farms were divided into four strata on the basis of the number of milking cows, as follows: 1 to 29 cows, stratum 1; 30 to 49 cows, stratum 2; 50 to 199 cows, stratum 3; and 200 or more cows, stratum 4.
[b] Total number of cattle present during the initial farm visit (prevalence study).
[c] Cows are female animals that have calved at least once. Heifers include female animals from 4 months of age to first calving.
[d] Weaned calves tested in the follow-up study.
[e] Of the four positive animals, two were calves that occupied the same pen (pen 2), although at different times. The remaining O157:H7-positive steer and calf were outside, but in close proximity to, the positive barn. In addition, two O157:H7-positive calves were identified in pen 1 during the initial farm visit (prevalence study). Both pen 1 and pen 2 were in the same barn that was previously used for hogs.
[f] The three positive calves were from pen 3 (two calves) and pen 4 (one calf), which shared drinking water. Also, during the initial visit we identified an O157:H7-positive calf in pen 1 of the same barn.
[g] The calves were from the same pen and shared drinking water, and the cows shared a barn and water.
[h] The values in parentheses are percentages.

by stratum and geographic location (Fig. 1). On subsequent visits to each of the five positive and seven negative farms an additional 517 fecal samples were obtained from cattle of various ages. A total of 15 animals from four of the five herds that previously tested positive and four animals from two of the seven previously negative herds tested positive for *E. coli* O157:H7. Animals that were O157:H7 positive on farms that were positive in the prevalence and follow-up studies were found at approximately the same frequency in each age and sex group, as follows: cows and heifers, 4.7%; bulls and steers, 4.0%; and calves, 3.6%. Similar percentages (ca. 4 to 5%) of cows and heifers and of bulls and steers were positive on farms that switched O157:H7 status from negative in the prevalence study to positive in the follow-up study, but none of the 72 calves tested were positive. However, 201 calves were tested on the previously positive farms (farms A, H, K, O, and X), whereas only 72 calves were tested on the farms that previously tested negative (farms L, LLL, N, O, Q, R, and W). Moreover, the two negative farms in the prevalence study that tested positive for *E. coli* O157:H7 in the follow-up study (farms L and R) had limited numbers of calves present at the time of sampling. Another observation made on O157:H7-positive farms was that positive animals shared water, inhabited the same barn or pen, occupied a pen that previously contained a positive animal, or were located in an area close to a positive barn or pen (Table 1). Additional studies should be performed to verify this observation.

In addition to fecal samples, a total of 302 environmental, feed, water, and nonfecal animal samples were examined for *E. coli* O157:H7 during the follow-up study. The numbers and types of samples examined were as follows: 7 alfalfa hay samples; 3 bedding material samples; 24 building, equipment, and pen swab samples; 21 colostrum samples; 37 feed grains samples; 38 samples of feed components (i.e., commercial mixes, calf starter, and feeds); 8 haylage samples; 5 manure pit samples; 9 meat and bone meal samples; 14 raw milk samples; 2 milk replacer samples; 18 samples of nonbovine feces (i.e., cat, dog, pig, rabbit, and raccoon feces); 6 cattle saliva samples; 9 silage samples; and 101 animal drinking water samples. Of these 302 samples, only 3 of the 101 water samples tested positive for *E. coli* O157:H7. Two of the positive animal drinking water samples were from farm R (O157:H7 negative in the prevalence study and positive in the follow-up study), and one animal drinking water sample was from farm H (O157:H7 positive in both the prevalence and follow-up studies).

CHEF-PFGE analyses of isolates from the prevalence study. Digestion of genomic DNA from *E. coli* O157:H7 isolates with *Xba*I and analysis by CHEF-PFGE produced 18 to 24 fragments that ranged from ca. 30 to 580 kb in length (Fig. 2). A single isolate from each of the 10 positive calves identified in the prevalence study was examined by CHEF-PFGE. Four distinct REDP were identified among the 10 isolates examined (Fig. 2). From these preliminary findings, it appeared that each farm had a distinct REDP and that farms A and H had the same REDP (REDP 33) despite being located approximately 75 miles (ca. 121 km) apart.

CHEF-PFGE analyses of isolates from the follow-up study. Genomic typing of the O157:H7 isolates obtained from the follow-up study demonstrated multiple REDP within a herd and established that the REDP present in a herd can change over time (Table 2). For example, farm H isolates displayed six REDP and farm X isolates displayed five REDP during the 8-month period of this study. On farm X, REDP 19 was found in all five positive animals identified from samples collected on

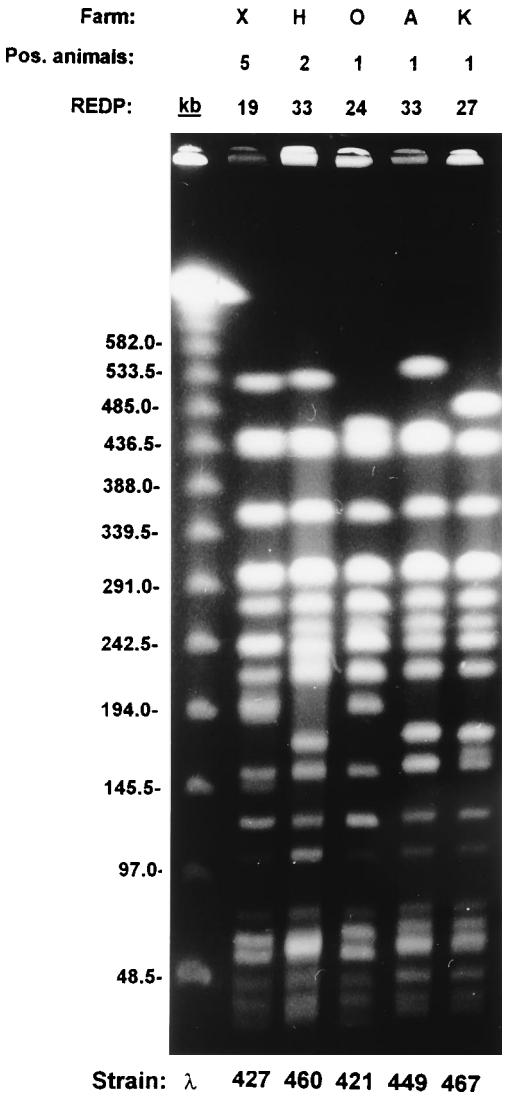

FIG. 2. Typical *Xba*I CHEF-PFGE gel for isolates obtained from *E. coli* O157:H7-positive animals identified during the prevalence study. The strain numbers are Food Research Institute-Kaspar culture collection numbers.

1 March 1994; animal 17 also carried a strain displaying REDP 20. However, 1 month later, REDP 19 was found in only two of four positive animals (animals 22 and 23); the remaining two animals contained *E. coli* O157:H7 strains that produced REDP 21 and 22 (animal 21) and only REDP 22 (animal 24). On the final visit (1 October 1994) an REDP 29 isolate was recovered from animal 25 on farm X. In contrast, an REDP 23 *E. coli* O157:H7 strain was isolated from fecal samples collected on farm O 7 months apart (on 1 March 1994 and 5 October 1994).

CHEF-PFGE analyses of multiple isolates from the same animal. To determine if different *E. coli* O157:H7 strains were present in a given animal, a maximum of 12 colonies from fecal samples were analyzed by CHEF-PFGE. Some of the fecal samples initially yielded only a single or few *E. coli* O157:H7 colonies following selective enrichment and plating. To obtain additional isolates, portions from retained fecal samples stored at −20°C were again enriched and plated. Despite these attempts, the fecal samples from animals 3 and 9 each yielded only a single colony, while 4 to 12 isolates were obtained from the remaining 27 fecal samples (Table 2). Genomic typing of the 160 isolates obtained revealed that an animal may harbor *E. coli* O157:H7 strains that display different REDP. For example, 7 of the 29 positive animals (24%) harbored O157:H7 strains with different REDP, and animals 5 (9 isolates) and 28 (9 isolates) each displayed three different REDP. With the exception of animal 7 on farm H, one of the REDP from animals shedding multiple types of O157:H7 was also found in at least one other animal on the same farm. Figure 3 shows that REDP 30, 31, and 32 were found in a cow (animal 28) and REDP 30 was found in a bull (animal 26) and in a cow (animal 27) on farm L. This suggests that each herd has a unique REDP, although another closely related REDP may also be present. In all, 19 REDP were found in dairy cattle in Wisconsin (Table 2) and one additional REDP was found in an animal drinking water sample (farm R).

Comparison of the REDP of bovine and water isolates. The discriminatory power of CHEF-PFGE analyses of *E. coli* O157:H7 isolates was further illustrated when the REDP of water isolates were compared with the REDP of bovine isolates (Fig. 4). Six visits to farm R resulted in one positive heifer sample and two positive water samples. Both water isolates displayed REDP 28, whereas the heifer isolate displayed REDP 25. In contrast, on farm H, an O157:H7 REDP 33 isolate was obtained from the water and from five of six positive animals identified on three visits during the period from 3 May 1994 to 19 September 1994. Only two isolates from animals 5 and 7 and one isolate from animal 6 were not REDP 33 isolates. These results indicate that *E. coli* O157:H7 may be disseminated or maintained within a herd by contaminated water. However, since one-third of the environmental samples tested were water samples, it is possible that testing a greater number of other environmental samples would reveal other niches that this pathogen occupies.

REDP similarities. The Dice similarities for the 20 *E. coli* O157:H7 REDP identified in the Wisconsin isolates ranged from 64 to 98% (Table 2). The similarities of strains obtained from the same herd were 78 to 100%, and the different REDP recovered from a single animal were ≥85% similar. The strain (FRIK 783) which displayed the most dissimilar REDP was from a heifer (animal 24) on farm R and was 64 to 73% similar to the other isolates (mean similarity index, 67%) (Table 2 and Fig. 4).

Presence of SLT genes. At least one isolate representing each of the 20 REDP identified was examined by using digoxigenin-labeled oligonucleotide probes for SLT I and SLT II. A total of 13 strains were positive for both SLT I and SLT II, while 1 and 6 strains were positive for only SLT I and only SLT II, respectively (data not shown). Isolates that had the same REDP had the same toxin profile, but isolates that had the same toxin profile had different REDP. It is noteworthy that when multiple REDP were found in a single animal, the strains had the same toxin profile. These data indicate that although toxin typing can provide insight concerning the virulence potential of isolates, genomic fingerprinting is a more discriminating typing method.

DISCUSSION

A majority of food-borne *E. coli* O157:H7 outbreaks involve beef products, particularly ground beef (10), which suggests that bovines are a primary source of this pathogen. Most of the ground beef produced in the United States is from dairy cattle, and approximately 8.6% of the 2.8×10^6 cattle slaughtered in federally inspected plants each month are dairy cattle (20a). As

TABLE 2. Numbers and *Xba*I REDP of *E. coli* O157:H7 isolates found in herds and animals during multiple visits to positive farms

Farm	Collection date (mo-day-yr)	Animal no.[a]	No. of isolates examined[b]	*Xba*I REDP (no. of isolates)	% Similarity among isolates[c]
A	3-1-94	1[d]	5	33 (5)	97
	10-1-94	2	5	26 (5)	
	10-1-94	3	1	26 (1)	
	10-1-94	4	3	26 (3)	
H	5-3-94	5[d]	9	33(6), 34 (2),35 (1)	78–98
	5-3-94	6[d]	6	33 (5), 36 (1)	
	5-18-94	7	5	37 (3), 38 (2)	
	5-18-94	8	1	33 (1)	
	5-18-94	9	4	33 (4)	
	9-19-94	10	8	33 (8)	
K	6-13-94	11[d]	4	27 (4)	100
O	3-1-94	12[d]	12	23 (11), 24 (1)	95
	10-5-94	13	6	23 (6)	
	10-5-94	14	5	23 (5)	
	10-5-94	15	5	23 (5)	
X	3-1-94	16[d]	5	19 (5)	80–95
	3-1-94	17[d]	4	19 (3), 20 (1)	
	3-1-94	18[d]	6	19 (6)	
	3-1-94	19[d]	5	19 (5)	
	3-1-94	20[d]	11	19 (11)	
	4-1-94	21	4	21 (2), 22 (2)	
	4-1-94	22	4	19 (4)	
	4-1-94	23	4	19 (4)	
	4-1-94	24	4	22 (4)	
	10-1-94	25	5	29 (5)	
L	9-19-94	26	7	30 (7)	94–96
	9-19-94	27	6	30 (6)	
	9-19-94	28	9	30 (6), 31 (2), 32 (1)	
R	10-12-94	29	7	25 (7)	100
Total			160		64–98

[a] Includes all age and sex classifications of dairy cattle (i.e., calves, cows, heifers, and steers).
[b] Number of colonies recovered following enrichment and plating and then analyzed by CHEF-PFGE.
[c] Similarities among REDP of O157:H7 isolates from each farm.
[d] Animal tested positive for O157:H7 during the prevalence study.

a prominent dairy state, Wisconsin is an important contributor to the ground beef supply, with ca. 1.5×10^6 dairy cattle and approximately 15.4×10^6 lb (6.99×10^6 kg) of ground beef produced per year (20a). Thus, one of the goals of this study was to determine the prevalence of *E. coli* O157:H7 in Wisconsin dairy herds.

Our survey of Wisconsin farms revealed that 7.1% of the dairy herds were positive for *E. coli* O157:H7. Although the prevalence in Wisconsin herds was similar to data for the state of Washington (11), our findings probably underestimate the actual prevalence because our study based the number of calves tested on each farm on the results of Garber et al. (8), who reported that 5% of weaned calves shed O157:H7, and ca. 1.8% of the weaned calves tested positive in this study. The change in the O157:H7 status of the herds tested in the prevalence and follow-up studies supports the view that the number of calves tested per farm was too low, although intermittent shedding may also affect the detection of O157:H7 in a herd (6, 21). The prevalence in weaned calves determined in this study (1.8%) is lower than the prevalence in a national survey (5% [8]) but higher than the prevalence in a Washington state study (0.65%) (11). The higher prevalence values found in our study and the national survey compared with the Washington state study were probably due to the size of the fecal samples collected and analyzed in the former studies.

On subsequent visits to previously positive and negative farms, the *E. coli* O157:H7 status of three herds changed. Other researchers (8, 28) have also reported changes in the O157:H7 status of farms. As mentioned above, a change in the O157:H7 status of a herd may be due to an inadequate number of animals tested and/or intermittent shedding of the pathogen (6, 16, 21). Our findings support the conclusion of Garber et al. (8) that the O157:H7 status of a herd cannot be ascertained from a single test involving a limited number of cattle in a herd.

During the follow-up study, we observed that O157:H7-positive animals shared the same barn, pen, or water, occupied a pen that previously contained a positive animal, or were located in an area that was close to a positive barn or pen. Grouping of preweaned calves has been associated with the *E. coli* O157:H7 status of herds in another study (8). Similarly, in a study performed with sheep, *E. coli* O157:H7 was transmitted from inoculated lambs to mothers (16). Collectively, the results of these studies suggest that transmission among animals and contact with areas previously contaminated by animals shed-

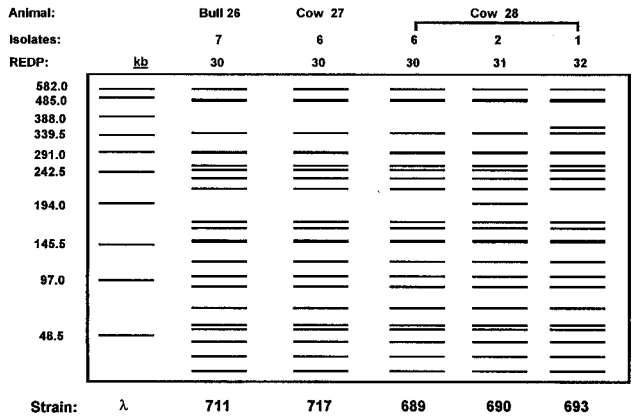

FIG. 3. Diagram generated by ELBAMAP software of *Xba*I REDP of multiple *E. coli* O157:H7 isolates obtained from positive animals on farm L. The strain numbers are Food Research Institute-Kaspar culture collection numbers.

ding *E. coli* O157:H7 are important factors in disseminating this pathogen in a herd. Likewise, the detection of *E. coli* O157:H7 in animal drinking water and the failure to detect this pathogen in a limited number of other environmental samples indicate that contaminated water may also be an important source of this pathogen on farms.

With the exception of two recent investigations (17, 19), in previous farm surveys and traceback studies of *E. coli* O157:H7 workers did not utilize genomic typing methods that have the discriminatory capacity of CHEF-PFGE. In this study, *Xba*I was used in combination with CHEF-PFGE for analysis of O157:H7 strains because this enzyme was more discriminatory than several other enzymes tested (3, 13, 17). A total of 20 REDP were identified among the 163 O157:H7 isolates recovered from fecal samples and animal drinking water samples. One to six REDP O157:H7 isolates were found on each farm during the 8-month study period, but the REDP were generally similar and distinct for each farm. Only REDP 33 was found on more than one farm (farms A and H). It is noteworthy that the only REDP found on more than one farm was obtained from

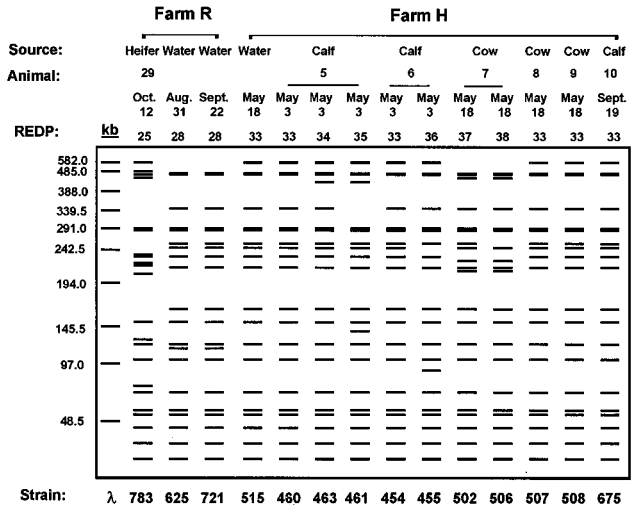

FIG. 4. Comparison of REDP of *E. coli* O157:H7 strains isolated from water and animals on farms R and H. The diagram was generated by using ELBAMAP software. The strain numbers are Food Research Institute-Kaspar culture collection numbers.

water (REDP 33). In our previous study (17) of 26 isolates (18 REDP) of *E. coli* O157:H7 obtained from 19 farms in 16 states, O157:H7-positive farms also had distinct REDP (Table 2). Our results suggest that there is limited farm-to-farm transmission and that there is probably more than one source of *E. coli* O157:H7 on each farm. However, a comparison of the REDP of the 26 O157:H7 strains obtained from 16 states with the REDP of the strains isolated during this study revealed levels of similarity ranging from 57 to 95%. REDP 33 obtained from Wisconsin farms A and H was 95% similar to the REDP of an O157:H7 isolate obtained from a calf on a Colorado farm (data not shown). Thus, most O157:H7-positive herds in Wisconsin contain a distinct strain(s), but there are exceptions. Studies are in progress to determine if REDP 33 is common among outbreak strains of *E. coli* O157:H7 and to expand our repository of O157:H7 isolates and REDP for additional comparisons among isolates.

An analysis of multiple isolates obtained from fecal samples by using CHEF-PFGE revealed that more than one REDP was present in 7 of 29 positive animals (24%), although the REDP were very similar (Dice similarity indices, ≥85%). The high levels of similarity among O157:H7 strains found in the same animal indicate that there may have been genetic changes, such as the loss of a plasmid or an SLT-encoding phage, which resulted in minor changes in the REDP. Karch et al. (15) suggested that a genetic change(s) or clonal turnover was the likely cause of REDP deviation in sequential isolates obtained from patients infected with *E. coli* O157. Although the isolates from four of the seven positive farms produced two or more REDP, on farm O REDP 23 was detected in strains that were collected 7 months apart. Furthermore, repeated analyses of standard reference strains of *E. coli* O157:H7 indicated that REDP are stable during laboratory storage (16a); however, it is possible that clonal turnover is faster in situ than in the laboratory.

Dice similarity indices were calculated to compare strains and to determine the degrees of relatedness. The levels of similarity for the 20 O157:H7 REDP found in Wisconsin ranged from 64 to 98%. However, the levels of REDP similarity within a herd ranged from 78 to 98%, and the levels of similarity for isolates obtained from the same animal were ≥85%. As another example, REDP 33, 34, and 35 found in animal 5 were 93 to 98% similar. In comparison, the levels of similarity for 26 *E. coli* O157:H7 isolates obtained from 16 farms across the United States ranged from 49 to 89% (17). In a related study of *Yersinia* strains, the levels of similarity for *Yersinia enterocolitica* serotype O:3 strains obtained from a restricted geographic region were 91 to 98% (5). Thus, isolates with REDP that have a high degree of similarity could be associated with an outbreak or an index strain despite minor differences in profiles.

Water samples were the only nonfecal samples that tested positive for *E. coli* O157:H7. Water has been implicated in human outbreaks (24), and this study demonstrated that water may be an important source of O157:H7 on farms. The results of CHEF-PFGE confirmed that the isolate found in a common water tank was identical to the strain found in five of six animals on farm H. In contrast, on farm R the O157:H7 isolate obtained from a water tank (REDP 28) was different from the calf isolate (REDP 25). Moreover, the REDP of the water and bovine isolates on farm R were only 65% similar, suggesting that these isolates are separate clones that probably originated from different sources.

The results of this study indicated that contaminated animal drinking water may contribute to the dissemination and/or maintenance of *E. coli* O157:H7 on farms. Also, horizontal

transmission contributed to the O157:H7 status of a farm, but additional studies are needed to verify these observations. Genomic typing of O157:H7 isolates revealed that a herd or animal may contain isolates that have multiple but similar REDP that can change over time. In this regard, Dice similarity indices of REDP were useful in determining levels of relatedness among isolates. The use of CHEF-PFGE resulted in identification of several clonal types of serotype O157:H7 in Wisconsin and the detection of a source of this pathogen on dairy farms.

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