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Prevalence of Enterohemorrhagic *Escherichia coli* O157:H7 in a Survey of Dairy Herds

TONG ZHAO,[1] MICHAEL P. DOYLE,[1]* JACK SHERE,[2] AND LINDSEY GARBER[3]

Center for Food Safety and Quality Enhancement and Department of Food Science and Technology, University of Georgia, Griffin, Georgia 30223[1]; Animal and Plant Health Inspection Service, Veterinary Service, U.S. Department of Agriculture, Madison, Wisconsin 53719[2]; and U.S. Department of Agriculture Animal and Plant Health Inspection Service Centers for Epidemiology and Animal Health, Fort Collins, Colorado 80521[3]

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The prevalence of *Escherichia coli* O157:H7 in dairy herds is poorly understood, even though young dairy animals have been reported to be a host. From February to May 1993, 662 fecal samples from 50 control herds in 14 states, and from June to August 1993, 303 fecal samples from 14 case herds in 11 states were collected for isolation of *E. coli* O157:H7. Case herds were those in which *E. coli* O157:H7 was isolated from preweaned calves in a previous U.S. Department of Agriculture study, whereas control herds from which *E. coli* O157:H7 had not been isolated previously were randomly selected from the same states as case herds. Among the control herds, *E. coli* O157:H7 was isolated from 6 of 399 calves (1.5%) that were between 24 h old and the age of weaning and from 13 of 263 calves (4.9%) that were between the ages of weaning and 4 months. Eleven of 50 control herds (22%) were positive. Among the case herds, *E. coli* O157:H7 was isolated from 5 of 171 calves (2.9%) that were between 24 h old and the age of weaning and from 7 of 132 calves (5.3%) that were between the ages of weaning and 4 months. Seven of 14 case herds (50%) were positive. Sixteen of 31 isolates were obtained by direct plating, with populations ranging from 10^3 to 10^5 CFU/g. Fifteen of 31 isolates were isolated by enrichment only. Nineteen of the isolates produced both verocytotoxin 1 (VT-1) and VT-2, whereas 12 produced VT-2 only.

Enterohemorrhagic *Escherichia coli* O157:H7 was identified in 1982 as an important human pathogen causing hemorrhagic colitis and hemolytic uremic syndrome (HUS) and has been reported with increased frequency during the past decade as a cause of human illness (5, 8, 17). Dairy cattle, especially young animals, have been implicated as a principal reservoir of *E. coli* O157:H7, with undercooked ground beef and raw milk being the major vehicles of foodborne outbreaks (5, 8, 21, 22). The prevalence and mechanism of carriage of *E. coli* O157:H7 in dairy herds are poorly understood. Farm surveys have frequently detected verotoxin-producing *E. coli* in cattle, but these isolates were largely of serotypes that have not yet been associated with human disease (5, 8, 13). The number of serotypes of verotoxin-producing *E. coli* causing human disease is increasing, but *E. coli* O157:H7 continues to be the dominant cause of hemorrhagic colitis and HUS (5, 8, 9, 17).

Studies of cattle in the United States revealed that the prevalence of *E. coli* O157:H7 ranges from less than 1 to 5% (7, 11, 14, 21). The National Dairy Heifer Evaluation Project (NDHEP) of the U.S. Department of Agriculture (USDA) National Animal Health Monitoring System tested fecal samples from 6,894 preweaned calves in 1,068 herds in 28 states for *E. coli* O157:H7 between June 1991 and May 1992 (7). *E. coli* O157:H7 was isolated from fecal samples of 25 (0.36%) preweaned calves in 19 (1.8%) herds, with no regional, seasonal, or herd size clustering. The trend toward increased herd prevalence with increased number of samples per herd indicated that the true herd prevalence in the United States is likely considerably higher than 1.8% (7). Wells et al. (21) determined the prevalence of *E. coli* O157:H7 among cattle of different age groups during investigations of two sporadic cases of HUS associated with raw milk consumption and an outbreak of gastroenteritis and HUS caused by *E. coli* O157:H7. *E. coli* O157:H7 was isolated from 5 of 210 calves (2.3%) and 12 of 394 heifers (3.0%), but only 1 of 662 adult cows (0.15%).

However, the sensitivity and specificity of the methods used to isolate *E. coli* O157:H7 are continually improving, and hence the rates of finding *E. coli* O157:H7 will increase. As a follow-up to the 1991–1992 NDHEP, a study was done to determine the prevalence of *E. coli* O157:H7 among heifer calves up to 4 months of age in herds in which the agent was determined previously to exist.

* Corresponding author. Phone: (404) 228-7284. Fax: (404) 229-3216.

MATERIALS AND METHODS

Selection of calves. Calves were divided into three groups according to age. Group 1 comprised calves between 24 h old and the age of weaning (maximum of 15 per control herd and 25 per case herd). Group 2 comprised calves between the ages of weaning and 4 months (maximum of 15 per control herd and 25 per case herd). Group 3 comprised calves of more than 4 months of age. At 12 months after the initial test, sampling of four case herds was expanded to 15 calves in group 1, 15 calves in group 2, and 15 calves in group 3, not including the original positive calves.

Case herds. Herds in which *E. coli* O157:H7 was isolated previously in the NDHEP approximately 1 year previously were used as case herds. All *E. coli* O157:H7-positive calves identified in these herds were tested for *E. coli* O157:H7 at 2, 6, and 12 months after the first visit except for animals that died or were sold.

Control herds. Herds from which *E. coli* O157:H7 was not isolated in the NDHEP were randomly selected from the same states as the case herds served as control herds.

Time frame. Fecal samples from control herds were collected from February to May 1993, and fecal samples from case herds were collected from June to August 1993.

Feces. A sample of at least 10 g of feces was collected from each animal through rectum retrieval. Sterilized gloves were used for each animal to avoid cross-contamination during the sampling. The sample was placed in a tube containing 15 ml of Cary-Blair medium (Remel Co., Lenexa, Kans.) and kept

cold (ca. 5°C) with frozen ice packs. Samples were sent to the laboratory by Federal Express and tested within 24 to 72 h of rectal retrieval.

Direct plating. Upon arrival at the laboratory, 1.0 g of feces was serially (1:10) diluted in 9 ml of 0.85% NaCl to 10^{-4} CFU/g. Portions (0.1 ml) of 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} dilutions were plated in duplicate on the surface of sorbitol-MacConkey agar (SMA; Unipath, Oxoid Division, Ogdensburg, N.Y.) plates (11) containing 0.1% 4-methylumbelliferyl-β-D-glucuronide (MUG). Plates were incubated at 37°C for 18 to 24 h.

Selective enrichment. Approximately 10 g of fecal sample was added to 100 ml of modified Trypticase soy broth (mTSB), which contained 30 g of Trypticase soy broth (BBL, Cockeysville, Md.), 1.5 g of bile salts no. 3, 10 g of Casamino Acids, 1.35 g of KH_2PO_4, 6.0 g of Na_2HPO_4, 20 mg of novobiocin, and 10 mg of acriflavin per liter (15). The samples, in 250-ml Erlenmeyer flasks, were incubated at 37°C for 18 h with agitation (150 rpm).

ELISA. A sandwich enzyme-linked immunosorbent assay (ELISA) (15) was performed in 96-well polystyrene enzyme immunoassay-radioimmunoassay plates (GIBCO, Grand Island, N.Y.). Each well was coated with 1 μg of affinity-purified goat antibody against *E. coli* O157:H7 (Kirkegaard and Perry Laboratories, Gaithersburg, Md.) in 100 μl of 50 mM carbonate buffer, pH 9.6, and incubated at room temperature overnight with agitation (150 rpm). The plate was washed four times with 50 mM Tris, pH 7.4, containing 150 mM NaCl (TBS), and remaining binding sites were blocked with milk diluent-blocking solution (Kirkegaard and Perry) for 1 h at 37°C. The blocking agent was removed, and 200 μl of enrichment culture was added per well and incubated at 37°C for 1 h. After the wells were washed four times with TBS containing 0.05% Tween 20 (TBS-T), 100 μl of monoclonal antibody (MAb) 4E8C12 (16) (ascitic fluid diluted 1:2,000 in TBS-T) was added; the plate was then incubated at 37°C for 1 h. The wells were washed four times with TBS-T, and then 100 μl of goat anti-mouse immunoglobulin M (IgM) plus IgG conjugated to alkaline phosphatase (diluted 1:800 in TBS-T; Kirkegaard and Perry) was added and incubated at 37°C for 1 h. After the wells were washed four times with TBS-T, 100 μl of *p*-nitrophenyl phosphate in 1 M 2-amino-2-methyl-1-propanol (Sigma Chemical Company, St. Louis, Mo.), pH 9.9 (1 mg/ml), was added to each well. The plate was incubated at 37°C for 1 h, and the optical density at 405 nm was determined with an EL 312e microplate reader (Bio-Tek instruments, Winooski, Vt.). A reading of 0.15 above the negative control value was considered positive for detection of *E. coli* O157:H7.

Plating after enrichment. After enrichment, 1 ml of culture medium from *E. coli* O157:H7 ELISA-positive samples was serially (1:10) diluted in 0.85% NaCl to 10^{-8}, and 0.1 ml of 10^{-5}, 10^{-6}, 10^{-7}, and 10^{-8} dilutions was inoculated onto SMA-MUG plates in duplicate. Plates were incubated at 37°C for 18 to 24 h.

Selection of *E. coli* O157:H7 colonies. Each sorbitol-negative and MUG-negative colony was streaked onto an SMA-MUG plate and incubated for 18 h at 37°C. Sorbitol-negative and MUG-negative colonies were tested for agglutination by the *E. coli* O157 latex agglutination assay (Unipath, Oxoid Division, Ogdensburg, N.Y.). Colonies positive by the agglutination assay were replated one to two times on SMA-MUG plates. The bacteria were confirmed as *E. coli* by biochemical tests with the API 20E miniaturized diagnostic test (Analytab Products, Division of Sherwood Medical, Plainview, N.Y.). Presumptive *E. coli* O157 isolates were further identified as *E. coli* O157:H7 by Bacto *E. coli* O157 and H7 antisera (Difco Laboratories, Detroit, Mich.) according to the procedures described by the manufacturer and Farmer and Davis (6).

Determination of verotoxin production. A sandwich ELISA in which MAb 9C9, specific for verocytotoxin 1 (VT-1) (18, 20), or MAb BC5BB12, specific for VT-2 (4), was used as the capture antibodies and rabbit polyclonal antibodies raised against each toxin individually were used as the detection antibodies was developed for determination of verotoxin production. The ELISA plate was first coated with an MAb specific for VT-1 or VT-2 at a concentration of 3 μg of IgG in 50 mM carbonate buffer, pH 9.6, per well and held overnight at room temperature. The MAbs used in the assay were purified on a protein A affinity column (Bio-Rad Laboratories, Hercules, Calif.). Bovine serum albumin (5%; Sigma Chemical Company) in TBS was added to block nonbinding sites, and 100 μl of culture medium was added to each well. After washing, 1.0 μg of rabbit polyclonal antibody to VT-1 or VT-2 was added to each well as the detection antibody, and then 100 μl of alkaline phosphatase-labeled goat anti-rabbit IgG (Kirkegaard & Perry Laboratories) was added and incubated at 37°C for 1 h. After washing, 100 μl of *p*-nitrophenyl phosphate in 1 M 2-amino-2-methyl-1-propanol (Sigma Chemical Company) was added to each well. The plate was incubated at 37°C for 1 h, and the optical density at 405 nm was determined.

RESULTS

A total of 662 fecal specimens from 50 control herds in 14 states were assayed for *E. coli* O157:H7. The bacterium was isolated from 19 calves in 11 herds from February to May 1993 (Table 1). Six of 399 calves (1.5%) in group 1 (24 h to weaning) and 13 of 263 (4.9%) in group 2 (weaned to 4 months) were *E. coli* O157:H7 positive. Eleven of the 50 control herds (22%) were *E. coli* O157:H7 positive.

A total of 303 fecal specimens from 14 case herds in 11 states were tested for *E. coli* O157:H7 (Table 2). The bacterium was isolated from 12 calves in seven herds from June to August 1993. Five of 171 calves (2.9%) in group 1 and 7 of 132 (5.3%) in group 2 were *E. coli* O157:H7 positive. Seven of 14 case herds (50%) were positive.

The methods used to isolate *E. coli* O157:H7 included both direct plating and selective enrichment. *E. coli* O157:H7 was isolated from all 31 positive calves by the enrichment method, whereas the bacterium was isolated from only 16 of the 31 (51.6%) positive calves by direct plating. *E. coli* O157:H7 was present in populations of 10^3 CFU/g in 2 calves, 10^4 CFU/g in 11 calves, and 10^5 CFU/g in 3 calves. Fifteen of 31 calves (48.4%) were positive by enrichment culture only, at populations of $<10^2$ *E. coli* O157:H7 CFU/g. Nineteen of the 31 isolates (61.3%) produced both VT-1 and VT-2, whereas 12 (38.7%) produced VT-2 only.

The prevalence of *E. coli* O157:H7 was substantially higher

TABLE 1. Distribution of dairy calves from control herds with *E. coli* O157:H7 in feces

Herd origin	Group 1[a]		Group 2[b]	
	No. tested	No. positive for *E. coli* O157:H7	No. tested	No. positive for *E. coli* O157:H7
California	41	1	44	6
Colorado	40	0	15	0
Florida	6	0	6	1
Idaho	18	0	13	0
Maryland	28	1	28	0
Minnesota	28	0	23	0
New England	43	0	25	0
Nebraska	19	2	2	0
New York	48	1	32	2
Ohio	21	1	6	0
Oregon	36	0	23	0
Tennessee	18	0	15	1
Washington	36	0	16	0
Wisconsin	17	0	15	3
Total	399	6	263	13

[a] Group 1, 24 h to weaning.
[b] Group 2, weaned to 4 months.

TABLE 2. Distribution of dairy calves from case herds with *E. coli* O157:H7 in feces

Herd origin	Group 1[a]		Group 2[b]	
	No. tested	No. positive for *E. coli* O157:H7	No. tested	No. positive for *E. coli* O157:H7
California	25	0	25	1
Colorado	25	0	25	0
Maryland	10	0	13	0
Minnesota	5	2	0	0
Nebraska	7	0	0	0
New York	31	0	23	4
Ohio	4	1	4	0
Oregon	16	0	4	0
Tennessee	9	0	11	0
Vermont	14	0	10	1
Washington	25	2	17	1
Total	171	5	132	7

[a] Group 1, 24 h to weaning.
[b] Group 2, weaned to 4 months.

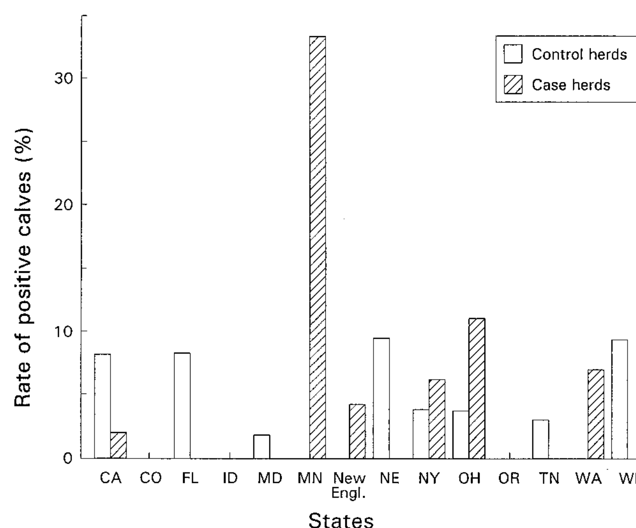

FIG. 1. Distribution of *E. coli* O157:H7 among dairy calves in herds from different origins.

in case herds (50%) than in control herds (22%) ($P = 0.05$, Fisher's exact test, two-tailed). The isolation rates for group 2 calves from both case (5.3%) and control (4.9%) herds were not significantly different ($P = 0.9$, Fisher's exact test, two-tailed). The isolation rates for group 1 calves were higher in case herds (2.9%) than in control herds (1.5%) but there was no statistically significant difference ($P = 0.3$, Fisher's exact test, two-tailed).

The geographic distribution of calves carrying *E. coli* O157:H7 was diverse, with calves from the states of California (8 of 135), Minnesota (2 of 56), Ohio (2 of 35), and Wisconsin (3 of 32) having the highest rates of carriage (Fig. 1).

Most of the 12 calves in case herds from which *E. coli* O157:H7 was isolated on the first sampling were retested at approximately 2 months, 6 months, and 1 year after the initial sampling. *E. coli* O157:H7 was not isolated from any of seven positive calves sampled at 2 months, but was isolated from one of eight calves sampled at 6 months. The population of *E. coli* O157:H7 in the fecal specimen was 10^5 CFU/g. Three calves were sampled approximately 1 year after the initial sampling; *E. coli* O157:H7 was not isolated from any of these calves. However, expanded sampling of four case herds revealed that *E. coli* O157:H7 still existed in two of these herds (Table 3). Of 15 calves from case herds from which *E. coli* O157:H7 was isolated during the initial NDHEP study, the fecal sample from 1 was positive at a population of 10^4 *E. coli* O157:H7 CFU/g after approximately 1 year. Five of these 15 calves, including the 1 confirmed positive, were retested 2 months after the 1-year sampling date. All were *E. coli* O157:H7 negative. Two of the 15 calves were tested 4 months after the 1-year follow-up sampling, and 1 that was *E. coli* O157:H7 negative at the 1-year sampling date and 2-month follow-up was positive for *E. coli* O157:H7, at a population of 10^2 CFU/g.

TABLE 3. Distribution of dairy calves from four case herds with *E. coli* O157:H7 in feces at approximately 1 year after initial sampling

Herd origin	Group 1[a]		Group 2[b]		Group 3[c]	
	No. tested	No. positive for *E. coli* O157:H7	No. tested	No. positive for *E. coli* O157:H7	No. tested	No. positive for *E. coli* O157:H7
California	15	0	15	0	17	1
New York[d]	30	0	25	2	29	2
Ohio	10	0	5	0	5	0
Total	55	0	45	2	51	3

[a] Group 1, 24 h to weaning.
[b] Group 2, weaned to 4 months.
[c] Group 3, more than 4 months.
[d] Two herds were tested.

DISCUSSION

Since the first two outbreaks of *E. coli* O157:H7 infection, which were associated with undercooked ground beef, cattle have been suspected as an important source of the pathogen (1–3, 11, 13, 14). Subsequent studies of cattle have revealed that *E. coli* O157:H7 is carried in the intestinal tract. Wells et al. (21) isolated *E. coli* O157:H7 from fecal specimens of cattle while investigating dairy herds associated with two human cases of *E. coli* O157:H7 infection. The pathogen was isolated from 5 of 210 calves, 12 of 394 heifers, and 1 of 662 cows. This and later studies (7, 14, 23, 24) revealed that *E. coli* O157:H7 is more frequently carried by calves and heifers than by adult cattle. Our study revealed that weaned calves (4.9 to 5.3%) more frequently shed *E. coli* O157:H7 in feces than preweaned calves (1.5 to 2.9%). Similar results have been observed by others (2, 23, 24). A study of 1,131 cows and 659 calves less than 3 months of age randomly selected from 100 dairy farms in southern Ontario revealed that calves greater than 2 weeks of age had a significantly greater chance of carriage of verotoxin-producing *E. coli* than those under 2 weeks of age (23, 24). Synger and Hopkins (19) in 1992 tested more than 2,000 fecal samples from a variety of animals of different ages, including 1,247 cattle, 450 sheep, 114 pigs, 113 birds, and 209 miscellaneous species, for *E. coli* O157. Of these, the bacterium was isolated only from five calves (age range, 2 days to 3 weeks) from different farms. Chapman et al. (2) isolated *E. coli* O157 from 84 (4%) of 2,103 bovine rectal swabs taken from cattle at a local abattoir, with the positive cattle coming from diverse sources within England.

The methods used for isolating *E. coli* O157:H7 greatly influence the isolation rates. Our studies revealed that only 52% of the calves determined to be shedding *E. coli* O157:H7 in feces were identified by directly plating dilutions of fecal specimens onto selective agars. The bacterium was isolated from 48% of the positive calves only by the enrichment procedure, likely because the population of *E. coli* O157:H7 in the feces was less than 10^2 CFU/g (the minimum level of sensitivity of the direct plating procedure). Most previously reported studies of the carriage of *E. coli* O157:H7 by cattle did not use an enrichment procedure or the ELISA procedure employed in our study for initial presumptive detection of *E. coli* O157:H7. The ELISA procedure enabled us to focus on specific samples and test, if necessary, all colonies typical of *E. coli* O157:H7 on SMA-MUG plates (sorbitol negative and MUG negative). *E. coli* O157:H7 colonies are often in the minority among sorbitol-negative, MUG-negative colonies. As better detection procedures are developed, greater prevalence of *E. coli* O157:H7 is likely to be identified (5, 17).

Two of 15 cattle were *E. coli* O157:H7 positive more than 1 year after the bacterium was originally isolated from the same animals. The bacterium was not consistently isolated at intermediate sampling periods, suggesting that the methods used for detection may not consistently isolate the pathogen, that *E. coli* O157:H7 is not consistently shed in feces at detectable

populations, or that the animals were reinfected by *E. coli* O157:H7 after an initial infection. Comparison of isolates from the same animal by genomic DNA fingerprinting indicated that the isolates had different DNA profiles, suggesting that the animals either were reinfected by *E. coli* O157:H7 or carried more than one strain of this bacterium (12).

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