APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Aug. 1994, p. 2958–2962
0099-2240/94/$04.00+0

Vol. 60, No. 8

Variable Colonization of Chickens Perorally Inoculated with *Escherichia coli* O157:H7 and Subsequent Contamination of Eggs

JEAN L. SCHOENI AND MICHAEL P. DOYLE*

Department of Food Microbiology and Toxicology and Department of Food Science, University of Wisconsin, Madison, Wisconsin 53706

Received 10 June 1993/Accepted 27 May 1994

Challenging 1-day-old White Leghorn chicks perorally with 2.6×10^1 to 2.6×10^5 *Escherichia coli* O157:H7 bacteria per chick resulted in cecal colonization at all levels. Two of six chicks inoculated with only 2.6×10^1 *E. coli* O157:H7 bacteria carried 10^3 to 10^4 *E. coli* O157:H7 bacteria per g of cecal tissue when sacrificed 3 months postinoculation. *E. coli* O157:H7 colonization persisted at least 10 to 11 months when chicks were administered 10^8 *E. coli* O157:H7 bacteria. Eggs from five hens that were fecal shedders of *E. coli* O157:H7 until the termination of the study (10 to 11 months) were assayed for *E. coli* O157:H7. The organism was isolated from the shells of 14 of 101 (13.9%) eggs but not from the yolks and whites. Considering that chicks can be readily colonized by small populations of *E. coli* O157:H7 and continue to be long-term shedders, it is possible that chickens and hen eggs can serve as vehicles of this human pathogen.

Escherichia coli O157:H7 has been associated with many outbreaks and cases of human hemorrhagic colitis and hemolytic-uremic syndrome (6). In North America, many of these outbreaks and cases have been epidemiologically linked to consumption of undercooked beef or raw milk (2, 4–6, 8–10, 14, 15). A survey of retail fresh meat and poultry from Madison, Wis., and Calgary, Alberta, Canada, revealed that *E. coli* O157:H7 was in 3.7% of beef, 1.5% of pork, 1.5% of poultry, and 2.0% of lamb samples (3). These data indicate that *E. coli* O157:H7 can be present in a variety of foods of animal origin.

Studies by Beery et al. (1) revealed that *E. coli* O157:H7 can colonize the ceca of young chicks. Studies by Stavric et al. (12, 13) verified these observations and further determined that colonization of *E. coli* O157:H7 in broilers and layers could be reduced by feeding anaerobic fecal bacteria of adult birds free from *E. coli* O157:H7 and *Salmonella* spp.

Although studies have revealed that chicks can be readily colonized by *E. coli* O157:H7, several questions remain unanswered. The purpose of this study was to (i) determine if small populations of *E. coli* O157:H7 would colonize chicks, (ii) determine if *E. coli* O157:H7 persists in the ceca for 8 to 10 months, and (iii) determine if *E. coli* O157:H7-colonized hens lay *E. coli* O157:H7-contaminated eggs.

MATERIALS AND METHODS

Colonization dose. *E. coli* O157:H7 strain 932NX (resistant to 30 μg of nalidixic acid per ml) (1) was grown in 50 ml of tryptic soy broth (Difco Laboratories, Detroit, Mich.) at 37°C for 16 to 18 h with agitation (100 gyrations per min). Cells were sedimented by centrifugation (6000 × *g*, 12 min) and then washed three times by alternate centrifugation and resuspension in 0.01 M phosphate-buffered saline (PBS; pH 7.2). Cells were adjusted to an optical density at 640 nm of 3.0 (ca. 2×10^9 CFU/ml). Cells were enumerated on MacConkey agar (Difco) plates after incubation at 37°C for 48 h.

The minimum colonization dose of strain 932NX was determined by administering by oral gavage with a 20-gauge ball-tipped cannula 0.5 ml of appropriately diluted bacterial suspension to six 1-day-old chicks per level of inoculum (ranging from 10^1 to 10^5 *E. coli* O157:H7 bacteria) or PBS. Each chick was housed individually in a stainless-steel wire cage (24.0 by 17.5 by 17.5 cm) to prevent cross-contamination. When the chicks outgrew these cages (ca. 3 weeks of age), they were transferred to larger stainless-steel cages (63.5 by 47 by 47 cm), where they remained for the duration of the study. The temperature, beginning at 37.8°C for 1-day-old chicks, was reduced 0.6°C each day until room temperature was reached. Lighting was cycled 12 h on and 12 h off. Relative humidity was increased for young chicks by placing pans of water near the heaters of the environmental chamber.

Populations of *E. coli* O157:H7 per gram of feces were determined at 4, 8, and 12 weeks postinoculation. Fresh feces collected with sterile spatulas or cotton swabs from clean papers within 1 h of defecation were placed in preweighed sterile tubes. Feces were weighed, and PBS (1:10, wt/vol) was added. Each sample was mixed with a vortex mixer for 2 min and then serially (1:10) diluted in PBS. *E. coli* O157:H7 counts were done in duplicate by using pour plates of MacConkey agar plus 30 μg of nalidixic acid per ml. Plates were incubated at 37°C for 48 h, and red colonies typical of *E. coli* were counted. At least five colonies typical of *E. coli* O157:H7 from each plate of the highest dilution were selected and confirmed as *E. coli* O157:H7 by biochemical tests (API 20E miniaturized diagnostic kit; Analytab Products, Plainview, N.Y.), Vero cell cytotoxicity, and serology with O157 and H7 antisera (*E. coli* Reference Center, Pennsylvania State University, University Park).

At 3 months postinoculation, the chicks were sacrificed. The ventral trunk of each chick was soaked in 70% ethanol, the feathers were plucked, and the exposed skin was washed with 70% ethanol. The lower body cavity was aseptically surgically exposed, and the ceca were removed. Cecal tissue was homogenized in PBS (1:10, wt/vol) for 2 min with a Brinkmann

* Corresponding author. Present address: Center for Food Safety and Quality Enhancement and Department of Food Science and Technology, University of Georgia, Georgia Station, Griffin, GA 30223-1797. Phone: (404) 228-7284. Fax: (404) 229-3216.

TABLE 1. Colonization of chickens inoculated perorally with different populations of *E. coli* O157:H7

No. of *E. coli* O157:H7 inoculated	Chicken no.	No. of *E. coli* O157:H7 bacteria/g of feces			No. of *E. coli* O157:H7 bacteria/g of cecal tissue at 12 wk
		4 wk	8 wk	12 wk	
2.6×10^1	1	2.0×10^2	<10	<10	<10
	2	<10	1.1×10^3	<10	<10
	3	2.2×10^2	3.0×10^1	<10	<10
	4	<10	3.3×10^3	<10	7.3×10^3
	5	<10	<10	<10	<10
	6	1.4×10^2	<10	<10	3.4×10^4
2.5×10^2	1	3.0×10^1	1.0×10^3	<10	10
	2	1.8×10^3	<10	<10	<10
	3	1.0×10^1	<10	<10	7.7×10^2
	4	2.0×10^3	2.5×10^1	<10	1.8×10^3
	5	5.0×10^2	<10	<10	<10
	6	3.5×10^1	2.0×10^3	<10	<10
2.6×10^3	1	<10	<10	<10	<10
	2	<10	8.5×10	<10	<10
	3	2.0×10^1	<10	2.4×10^3	<10
	4	5.5×10^5	<10	<10	4.2×10^2
	5	1.2×10^5	<10	<10	<10
	6	2.0×10^1	<10	<10	<10
2.6×10^4	1	<10	<10	<10	<10
	2	<10	<10	2.8×10^2	<10
	3	1.3×10^2	<10	1.5×10^2	<10
	4	6.3×10^3	<10	2.9×10^3	3.5×10^1
	5	3.1×10^2	<10	3.6×10^2	<10
	6	1.0×10^2	<10	<10	<10
2.6×10^5	1	2.1×10^2	<10	4.2×10^2	2.8×10^2
	2	<10	<10	5.4×10^2	1.5×10^4
	3	<10	<10	1.6×10^3	<10
	4	3.0×10^1	4.5×10^2	1.0×10^1	<10
	5	3.0×10^1	3.5×10^2	1.6×10^2	<10
	6	2.0×10^1	1.7×10^2	2.0×10^1	2.1×10^3

Polytron homogenizer (model PT 10/35; Brinkmann Instruments, Westbury, N.Y.) and serially (1:10) diluted in PBS. *E. coli* O157:H7 counts were determined in duplicate by using the procedure described above.

Long-term colonization. Fourteen 1-day-old chicks received perorally 1.3×10^8 *E. coli* O157:H7 strain 932NX bacteria per chick. The inoculum was prepared from a 16-h culture and administered by the procedures described above, with the exception that the cell suspension was adjusted to an optical density at 640 nm of 0.3 (ca. 2×10^8 CFU/ml). Ten 1-day-old chicks received 0.5 ml of 0.01 M PBS each as the negative control. Fecal samples were assayed at 7 and 14 days and at 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 months postinoculation to determine *E. coli* O157:H7 colonization. Sampling and *E. coli* O157:H7 counts were performed by using the procedures described above.

At 8 months postinoculation, the roosters (three control and seven inoculated) were sacrificed, and *E. coli* O157:H7 counts of cecal tissue with cecal contents were determined. The remaining hens (seven control and seven inoculated) were sacrificed at 10 months postinoculation, and the ceca with cecal contents, colons plus cloacae, small intestines (proximal, middle, and distal), gizzards, spleens, kidneys, livers, and hearts were assayed for *E. coli* O157:H7 counts. These tissues were prepared for analysis by homogenizing samples with appropriate minimal amounts of PBS to accommodate dilution and plating. Not all tissues were diluted 1:10 (wt/vol). The appropriate dilution factor was used in calculating *E. coli* O157:H7 counts per gram of tissue.

Egg screening. When hens from the long-term colonization study began laying eggs (ca. 5 months of age), the eggs were aseptically collected by persons wearing sterile latex gloves which were inverted around the eggs within 4 h of their being laid to determine contamination by *E. coli* O157:H7. Shells were assayed for the organism by placing each egg in an 18-oz (ca. 510-g) Whirl-Pak bag (11.5 by 23 cm; Nasco, Fort Atkinson, Wis.) containing 50 ml of lauryl tryptose broth (Difco). After the egg was soaked for 10 min at room temperature, the shell was manually rubbed with the broth for 1 min. The egg was removed from the bag, and the lauryl tryptose broth was incubated for 24 h at 37°C.

The bottom third of each eggshell was then treated by submersion in 70% ethanol for 2 min. The shell was opened (ca. 1.0 cm) with a sterile forceps, and the contents were added aseptically to 200 ml of lauryl tryptose broth in a sterile Stomacher Bag (17.75 by 33.5 cm; Seward Medical, London, England). The egg contents were mixed for 2 min by using a Stomacher 400 lab blender (Cooke Laboratory Products, Alexandria, Va.) and incubated for 24 h at 37°C. The presence of *E. coli* O157:H7 was determined with enrichment cultures of both eggshells and contents by streak plating culture medium onto MacConkey agar plus 30 μg of nalidixic acid per ml. Presumptive *E. coli* O157:H7 isolates were confirmed by the procedures described above.

Statistical analyses. The detection limit for *E. coli* O157:H7 in this study was 10 CFU/g. Statistical values were calculated by using PS-Plot for personal computers (Polysoft, Salt Lake City, Utah). Geometric means were calculated for the long-term colonization study by using log-transformed data which were back transformed for numerical consistency. The value 10 was log transformed to 1.00 for fecal counts of <10 CFU/g of feces to calculate means. If all counts within a sampling time were

TABLE 2. Long-term colonization of chickens by *E. coli* O157:H7

Chicken no.	Sex[a]	No. of *E. coli* O157:H7 bacteria/g of feces at postinoculation times:												No. of *E. coli* O157:H7 bacteria/g of cecal tissue at necropsy[b]	
		Spring				Summer			Fall			Winter		8 mo	10 mo
		7 days	14 days	1 mo	2 mo	3 mo	4 mo	5 mo	6 mo	7 mo	8 mo	9 mo	10 mo		
1	R	4.4×10^4	<10	2.8×10^2	1.6×10^4	<10	1.6×10^6	<10	<10	<10	<10			<10	
2	R	1.5×10^6	<10	1.7×10^2	<10	5.9×10^7	5.1×10^5	4.4×10^4	<10	<10	<10			<10	
3	R	2.5×10^7	1.5×10^5	8.5×10^4	6.9×10^5	2.5×10^3	4.7×10^5	5.2×10^4	<10	<10	<10			<10	
4	R	3.2×10^4	1.6×10^3	<10	6.8×10^5	4.8×10^2	9.3×10^4	2.2×10^6	<10	<10	9.5×10^1			9.5×10^1	
5	H	9.3×10^4	2.9×10^5	5.2×10^5	3.6×10^5	6.0×10^7	2.6×10^7	D[d]							6.5×10^1
6	H	2.4×10^6	9.3×10^4	4.0×10^5	2.3×10^5	2.3×10^7	6.8×10^5	1.8×10^5	<10	7.9×10^2	1.3×10^2	2.1×10^6	9.5×10^3	ND[e]	<10
7	H	4.6×10^3	2.3×10^4	4.4×10^4	2.1×10^7	7.9×10^3	1.4×10^6	3.3×10^3	<10	<10	1.4×10^2	<10	<10	ND	
8	R	4.6×10^3	1.1×10^5	7.4×10^3	<10	4.1×10^7	1.4×10^7	<10	<10	<10	<10			<10	<10
9	H	6.9×10^3	1.2×10^5	<10	2.0×10^7	2.7×10^5	1.7×10^7	6.8×10^3	2.9×10^4	9.3×10^6	4.1×10^4	<10	<10	ND	
10	H	8.9×10^5	2.0×10^3	1.1×10^3	D										
11	H	1.4×10^4	3.6×10^3	3.9×10^4	2.4×10^6	4.5×10^7	1.5×10^7	6.5×10^5	1.1×10^3	3.4×10^4	4.2×10^2	1.3×10^2	3.2×10^2	ND	3.5×10^2
12	R	1.1×10^4	2.1×10^4	9.5×10^4	2.9×10^7	5.6×10^6	4.8×10^7	1.3×10^3	<10	<10	<10			<10	
13	H	6.8×10^3	2.2×10^4	1.4×10^4	5.4×10^4	1.2×10^6	5.0×10^6	3.9×10^5	1.4×10^4	1.3×10^4	8.9×10^1	4.9×10^3	3.4×10^3	ND	4.8×10^1
14	R	3.6×10^6	1.2×10^4	2.3×10^4	<10	3.0×10^2	4.7×10^6	4.9×10^5	<10	<10	<10			<10	
Combined[f]	Median	2.3×10^4	2.2×10^4	1.9×10^4	3.6×10^5	1.2×10^6	4.7×10^6	2.9×10^4	<10	<10	5.0×10^1	1.3×10^2	3.2×10^2		
	Geometric mean[g]	9.5×10^4	8.3×10^{3c}	5.6×10^3	6.9×10^4	2.4×10^{5c}	3.2×10^{6c}	1.3×10^{4c}	5.2×10^{1c}	1.6×10^2	6.2×10^1	6.6×10^2	2.5×10^2		
Roosters	Median	4.4×10^4	1.2×10^4	7.4×10^3	1.6×10^4	2.5×10^3	1.6×10^6	4.4×10^3	<10	<10	<10	ND	ND		
	Geometric mean	1.9×10^5	2.5×10^{3c}	2.5×10^3	5.8×10^3	3.4×10^3	1.9×10^{6c}	4.4×10^{3c}	<10	<10	1.4×10^1	ND	ND		
Hens	Median	1.4×10^4	3.9×10^4	3.9×10^4	1.4×10^6	1.2×10^7	7.8×10^7	1.8×10^5	1.1×10^3	1.3×10^4	1.4×10^2	1.3×10^2	3.2×10^2		
	Geometric mean	4.8×10^4	2.8×10^4	1.3×10^4	1.3×10^{6c}	2.3×10^6	5.6×10^6	6.3×10^{4c}	5.4×10^{2c}	7.9×10^3	4.9×10^{2c}	6.6×10^2	2.5×10^2		

[a] R, rooster; H, hen.
[b] Roosters were necropsied at 8 months; hens were necropsied at 10 months.
[c] Significantly different ($P < 0.05$) from previous sampling time or season. Spring and winter counts were compared; however, winter sampling times did not precede summer sampling times.
[d] D, died.
[e] ND, not determined.
[f] Combined, roosters and hens.
[g] Geometric mean calculated by using 10 for all values denoted as <10 in the table, except in cases where all values within sampling time were <10.

TABLE 3. Eggs laid by hens inoculated perorally with 1.3×10^8 *E. coli* O157:H7

Chicken no.[a]	No. of shells contaminated with *E. coli* O157:H7/no. of eggs laid at postinoculation times:						Total no. of shells contaminated with *E. coli* O157:H7/no. of eggs
	5 mo[b]	6 mo	7 mo	8 mo	9 mo	10 mo	
6	1/3	0/4	1/4	0/3	1/2	0/3	3/19 (15.8)[c]
7	0/2	0/2	0/2	0/2	0/2	0/2	0/12 (0)
9	0/4	1/4	1/5	1/5[d]	0/4	0/4	3/26 (11.5)
11	1/2	1/5	1/4	1/4	0/3	0/4	4/22 (18.2)
13	1/3	1/4	0/3	0/4	1/4	1/4	4/22 (18.2)
Total per month	3/14 (21.4)	3/19 (15.8)	3/18 (16.7)	2/18 (11.1)	2/15 (13.3)	1/17 (5.9)	14/101 (13.9)

[a] Numbers correspond to those shown in Table 2.
[b] Hens began laying eggs at ca. 5 months of age.
[c] Percentages are indicated in parentheses.
[d] *E. coli* O157:H7 isolated from a shell free of fecal soil; other *E. coli*-positive shells were soiled.

<10 CFU/g of feces, the mean was denoted as <10 (11). Significant differences between samplings and seasons were determined by conducting paired *t* tests at the $P < 0.05$ level.

RESULTS

Colonization dose. Eighty-three to 100% of chicks administered 2.6×10^1 to 2.6×10^5 *E. coli* O157:H7 bacteria were colonized by *E. coli* O157:H7 at some time during the 12 weeks of examination. *E. coli* O157:H7 was not detected (<10 CFU/g) at 3 months postinoculation in fecal samples of chickens receiving 2.6×10^1 or 2.6×10^2 *E. coli* O157:H7 bacteria but was recovered from cecal tissue of two of six chickens (average, 2.1×10^4 or 1.3×10^3 CFU/g, respectively) (Table 1). *E. coli* O157:H7 was isolated at 3 months postinoculation from the cecal tissue of only one of six chickens receiving either 2.6×10^3 or 2.6×10^4 *E. coli* O157:H7 bacteria. Interestingly, four of six chickens receiving 2.6×10^4 CFU of *E. coli* O157:H7 bacteria per g shed in their feces an average of 9.2×10^2 *E. coli* O157:H7 bacteria per g at 3 months postinoculation, but *E. coli* O157:H7 (35 CFU/g) was detected in the cecal tissue of only one chicken. At the highest inoculum level, 2.6×10^5 *E. coli* O157:H7 bacteria, all birds excreted *E. coli* O157:H7 in their feces at 3 months (average, 4.6×10^2 CFU/g), but only three of six birds carried a detectable level in their ceca (average, 5.8×10^3 CFU/g).

Long-term colonization. Fecal shedding of *E. coli* O157:H7 in 3 of 14 chickens perorally challenged with 1.3×10^8 *E. coli* O157:H7 bacteria per chick continued throughout the 10-month test period. A significant increase ($P < 0.05$) in fecal shedding was observed during the summer months (Table 2). Maximum shedding (median, 4.7×10^6 CFU/g; geometric mean, 3.2×10^6 CFU/g) occurred at 4 months of age. Populations of *E. coli* O157:H7 increased significantly ($P < 0.05$) in hen feces at 2 months and remained high through 4 months. In roosters, *E. coli* O157:H7 populations increased slowly from the 14-day sampling time and peaked at 4 months with a significant increase from the 3-month sampling. *E. coli* O157:H7 counts in feces of male birds decreased to <10 CFU/g at 6 months, but counts of 95 CFU/g were detected in one rooster at the 8-month sampling time. The population of *E. coli* O157:H7 bacteria per g of feces in hens decreased significantly ($P < 0.05$) after 4 months but were not as low as the populations shed by roosters. Mean *E. coli* O157:H7 counts in hens at 6, 8, and 10 months were 5.4×10^2, 4.9×10^2, and 2.5×10^2 CFU/g, respectively. At 10 months, three of the remaining five hens were shedding *E. coli* O157:H7, and the pathogen was recovered from the samples of colon plus cloaca (5.0×10^1, 3.0×10^2, and 3.0×10^3 CFU/g; data not shown) and the cecal tissue of the positive birds when they were necropsied (Table 2). *E. coli* O157:H7 was not recovered from the samples of gizzard, spleen, kidneys, liver, heart, or small intestine of any of the chickens at the time of necropsy.

Egg screening. The shells and contents of 101 eggs were tested for *E. coli* O157:H7. Fourteen shells (13.9%) were positive, and all but one shell were soiled with feces (Table 3). All eggs with positive shells were laid by hens with detectable levels of *E. coli* O157:H7 in their feces. All contaminated eggs were laid by four hens (no. 6, 9, 11, and 13; Table 2), with three or four contaminated eggs laid by each. No more than one contaminated egg was laid by each hen per month, and the lowest fecal count of *E. coli* O157:H7 of a hen laying a shell-contaminated egg was 4.2×10^2 CFU/g. One hen (no. 7) laid two eggs per month, but none was contaminated with *E. coli* O157:H7. Hens 5 and 10 died before laying any eggs. *E. coli* O157:H7 was not recovered from egg contents.

DISCUSSION

Beery et al. (1) demonstrated by bacterial enumeration and histologic examination that *E. coli* O157:H7 colonizes the ceca of chickens. The bacteria attach to and erode the cecal epithelium, thereby allowing the bacteria to penetrate to connective tissue (1). In our study, fecal shedding was observed in three hens throughout the 10-month period of evaluation. *E. coli* O157:H7 was detected in the feces of roosters only through 5 months of sampling but was detected at 8 months in the cecal tissue of one of seven previously colonized roosters. *E. coli* O157:H7 was detected in both the samples of colon plus cloaca and the cecal tissues of three of five hens at the termination of the study, i.e., at 10 months postinoculation. *E. coli* O157:H7 also was detected in the feces and cecal tissue of an 11-month-old hen retained from a previous experiment and necropsied with this group. The ability of *E. coli* O157:H7 to adhere to and penetrate the epithelia of ceca appears to be the mechanism for prolonged fecal shedding of the bacteria. The rate of cecal colonization and the population of *E. coli* O157:H7 shed in feces were higher in hens than in roosters.

E. coli O157:H7 is transient in the chicken intestinal tract (1). Hence, the surface of eggs could be contaminated by *E. coli* O157:H7 when eggs pass through the cloaca. Unlike *Salmonella enteritidis*, which can contaminate the content of eggs by the transovarian route, our limited study of 101 eggs indicates that *E. coli* O157:H7 does not contaminate the yolks or whites of eggs by this mechanism. Stavric et al. (12) challenged chicks with 10^3 to 10^9 *E. coli* O157:H7 strain 932NX bacteria as controls in studies on competitive exclusion with fecal cultures. The ceca of 50% of chicks receiving 10^3 *E. coli*

O157:H7 bacteria were colonized by the pathogen at 6 days postinoculation, whereas 89% of chicks administered 10^5 *E. coli* O157:H7 bacteria were colonized. Hence, these investigators also determined that *E. coli* O157:H7 could readily colonize chicks.

Interestingly, the long-term colonization study revealed that maximum fecal shedding of *E. coli* O157:H7 occurred at 4 months of age, which also was during the summer month of July when peak rates of *E. coli* O157:H7 infection of humans occur (7). After July, there was a decline in the *E. coli* O157:H7 population shed in feces through the fall and winter months. *E. coli* O157:H7 populations also were higher in the spring than in the fall months.

The fact that low populations of *E. coli* O157:H7 can colonize ceca, grow in the intestinal tract, and be excreted in feces for several months suggests that chickens could be a source of this human pathogen. Surveys of feces and ceca of chickens, using sensitive procedures to detect and isolate *E. coli* O157:H7, are needed to determine whether chickens naturally carry *E. coli* O157:H7 and serve as a reservoir of the pathogen.

REFERENCES

1. **Beery, J. T., M. P. Doyle, and J. L. Schoeni.** 1985. Colonization of chicken cecae by *Escherichia coli* associated with hemorrhagic colitis. Appl. Environ. Microbiol. **49:**310–315.
2. **Borczyk, A. A., M. A. Karmali, H. Lior, and L. M. C. Ducan.** 1987. Bovine reservoir for verotoxin-producing *Escherichia coli* O157: H7. Lancet **i:**98.
3. **Doyle, M. P., and J. L. Schoeni.** 1987. Isolation of *Escherichia coli* O157:H7 from retail fresh meats and poultry. Appl. Environ. Microbiol. **53:**2394–2396.
4. **Lamothe, F., C. Gaudreau, D. Bernard, and S. Gill.** 1983. Hemorrhagic colitis following the consumption of hamburger—Quebec. Can. Dis. Weekly Rep. **9:**50–51.
5. **Martin, M. L., L. D. Shipman, J. G. Wells, M. E. Potter, K. Hedberg, I. K. Wachsmuth, R. V. Tauxe, S. P. Davis, J. Arnoldi, and J. Tilleli.** 1986. Isolation of *Escherichia coli* O157:H7 from dairy cattle associated with two cases of haemolytic uraemic syndrome. Lancet **ii:**1043.
6. **Padhye, N. V., and M. P. Doyle.** 1992. *Escherichia coli* O157:H7: epidemiology, pathogenesis, and methods for detection in food. J. Food. Prot. **55:**555–565.
7. **Pai, C. H., N. Ahmed, H. Lior, W. M. Johnson, H. V. Sims, and D. E. Woods.** 1988. Epidemiology of sporadic diarrhea due to verocytotoxin-producing *Escherichia coli*: a two-year prospective study. J. Infect. Dis. **157:**1054–1057.
8. **Pai, C. H., R. Gordon, H. V. Sims, and L. E. Bryon.** 1984. Sporadic cases of haemorrhagic colitis associated *Escherichia coli* O157:H7. Ann. Intern. Med. **101:**738–742.
9. **Riley, L. W., R. S. Remis, S. D. Helgerson, H. B. McGee, J. G. Wells, B. R. Davis, R. J. Herbert, E. S. Olcott, L. M. Johnson, N. T. Hargrett, P. A. Blake, and M. L. Cohen.** 1983. Hemorrhagic colitis associated with a rare *Escherichia coli* serotype. N. Engl. J. Med. **308:**681–685.
10. **Ryan, C. A., R. V. Tauxe, G. W. Hosek, J. G. Wells, P. A. Stoesz, N. W. McFaddin, Jr., P. W. Smith, G. F. Wright, and P. A. Blake.** 1986. *Escherichia coli* O157:H7 diarrhea in a nursing home: clinical epidemiological, and pathological findings. J. Infect. Dis. **154:**631–638.
11. **Snedecor, G. W., and G. Cochran.** 1980. Statistical methods, 7th ed. The Iowa State University Press, Ames.
12. **Stavric, S., B. Buchanan, and T. M. Gleeson.** 1992. Competitive exclusion of *Escherichia coli* O157:H7 from chicks with anaerobic cultures of faecal microflora. Lett. Appl. Microbiol. **14:**191–193.
13. **Stavric, S., B. Buchanan, and T. M. Gleeson.** 1993. Intestinal colonization of young chicks with *Escherichia coli* O157:H7 and other verotoxin-producing serotypes. J. Appl. Bacteriol. **74:**557–563.
14. **Stavric, S., and J. I. Speirs.** 1989. *Escherichia coli* associated with hemorrhagic colitis. Can. Inst. Food Sci. Technol. J. **22:**205–208.
15. **Stewart, P. J., W. Desormeaux, and J. Chene.** 1983. Hemorrhagic colitis in a home for the aged—Ontario. Can. Dis. Weekly Rep. **9:**29–32.