Molecular Subtype Analyses of *Campylobacter* spp. from Arkansas and California Poultry Operations

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Campylobacter* isolates from diverse samples within broiler production and processing environments were typed by using *flaA* short variable region DNA sequence analysis. Sixteen flocks from four different farms representing two broiler producers in Arkansas and California were analyzed. Fourteen of the flocks (87.5%) were *Campylobacter*-positive; two remained negative throughout the 6-week rearing period. In general, multiple clones were present within a flock. Additionally, clones found within a flock were also present on the final product, although the diversity of *Campylobacter* spp. on the final product appeared to be reduced relative to that observed within the flock. Comparison of clones between flocks on the same farm revealed that some clones of *Campylobacter* persisted in multiple flocks. Furthermore, some clones were identified across the two farms that were under the same management. In two sampling periods, environmental isolates were positive for *Campylobacter* prior to flock shedding. Environmental samples associated with five additional flocks were positive for *Campylobacter* concomitantly with recovery of *Campylobacter* from the birds. Analysis of the environmental isolates that were positive prior to flock shedding demonstrated that in some instances the environmental isolates possessed genotypes identical to those of isolates originating from the flock, while in other cases the environmental isolates possessed genotypes that were distantly related to isolates obtained from the flock. Analyses of environmental isolates that tested positive concurrently with the positive isolates from the flocks demonstrated varied results; in some instances the environmental isolates possessed genotypes identical to those of isolates originating from the flock, while in other cases the environmental isolates possessed genotypes that were distantly related to isolates obtained from the flock. These data suggest that the external environment may contribute to *Campylobacter* contamination during poultry production and processing. However, environmental contamination with *Campylobacter* does not appear to be the sole contributing factor.

Campylobacter jejuni, a gram-negative, microaerophilic bacterium, is currently believed to be the leading bacterial etiological agent of acute gastroenteritis in the human population; the total number of *Campylobacter* enteritis cases in the United States is estimated at 2.4 million per year, or approximately 1 to 2% of the population per year (3, 28, 33). The majority of *C. jejuni* cases are enteric. Most episodes are confined to local acute gastroenteritis characterized by nausea, abdominal cramps, diarrhea, and fatigue. Infections are generally self-limited and are resolved within several days after initial onset. *Campylobacter* infections have also been associated with unnecessary appendectomies, reactive arthritis, and development of Guillain-Barré syndrome, although these complications are infrequent (4–6, 35).

Handling and consumption of poultry or poultry-related products are considered to be a primary source for *Campylobacter*-induced disease in humans (5, 18, 24). *Campylobacter* has been cultured from as many as 75% of the live broiler population and from as much as 80% of processed poultry meat samples sold commercially (13, 14, 19, 27). The high colonization prevalence of poultry and the resultant clinical infections in humans have prompted a number of investigations focused upon identifying and subsequently eliminating sources of *Campylobacter* contamination in chickens. However, the pathways involved in *Campylobacter* contamination of poultry flocks remain unclear. Several suspected sources or vectors of contamination have been studied and include transmission from parent to progeny through the egg, exposure of birds to contaminated water, a previously contaminated rearing environment, hatchery pads, litter, feed, personnel, small animals on the farm, flies, and rodents (10, 11, 16, 17, 20, 25, 26, 29).

In an effort to further elucidate the means by which poultry flocks are contaminated with *Campylobacter*, 16 flocks from four different farms, representing two broiler producers in the United States, were studied. Samples from both poultry production and poultry processing environments were collected and cultured for the presence of *Campylobacter*. All *Campylobacter* isolates obtained were genotyped by using *flaA* short variable region (SVR) DNA sequence analysis to determine the relationships between the isolates (22). The identification

TABLE 1. Positive *Campylobacter* isolates from the producer A high-performance (AH) site

Sample date (mo/day/yr)	Source	No. recovered
Spring		
04/06/98	Feces from poultry	13
04/07/98	Carcass rinse from poultry	1
04/07/98	Prescald water	3
04/07/98	Postscald water	3
04/07/98	Pretransport crate swab	5
Summer		
07/13/98	Feces from poultry	3
07/13/98	Drag swab from inside house	3
07/13/98	Cecal dropping	5
07/23/98	Carcass rinse from poultry	7
07/23/98	Pretransport crate swab	1
07/23/98	Post-transport crate swab	7
Fall		
11/24/98	Feces from poultry	6
11/24/98	Water line	1
11/24/98	Drag swab from inside house	2
11/24/98	Insect	1
11/24/98	Cecal dropping	3
11/26/98	Carcass rinse from poultry	10
11/26/98	Prescald water	1
11/26/98	Pretransport crate swab	3
11/26/98	Post-transport crate swab	7
Winter		
01/25/99	Feces from poultry	4
01/25/99	Drag swab from inside house	1
01/25/99	Cecal dropping	1
01/25/99	Postscald water	1
01/25/99	Post-transport crate swab	7

TABLE 2. Positive *Campylobacter* isolates from the producer A low-performance (AL) site

Sample date (mo/day/yr)	Source	No. recovered
Spring		
04/06/98	Feces from poultry	23
04/06/98	Drag swab from inside house	2
04/06/98	Mouse intestine	1
04/06/98	Wild bird feces	1
04/06/98	Animal feces	2
04/06/98	Insects	2
04/06/98	Boot swab	1
04/06/98	Fly strip	1
04/08/98	Carcass rinse from poultry	19
04/08/98	Postscald water	2
04/08/98	Pretransport crate swab	4
Summer		
07/13/98	Cecal dropping	3
07/27/98	Carcass rinse from poultry	1
07/27/98	Post-transport crate swab	5
Fall		
10/19/98	Wild bird feces	1
11/24/98	Feces from poultry	10
11/24/98	Drag swab from inside house	2
11/24/98	Mouse intestine	1
11/24/98	Wild bird feces	1
11/24/98	Cecal dropping	3
11/26/98	Prescald water	1
11/26/98	Postscald water	1
11/26/98	Pretransport crate swab	4
11/26/98	Post-transport crate swab	2
Winter		
01/11/99	Feces from poultry	19
01/11/99	Litter from house	2
01/11/99	Drag swab from inside house	3
01/11/99	Cecal dropping	1
01/25/99	Feces from poultry	7
01/25/99	Drag swab from inside house	2
01/25/99	Insects	1
01/25/99	Cecal dropping	1
01/26/99	Carcass rinse from poultry	2
01/26/99	Postscald water	1
01/26/99	Pretransport crate swab	5
01/26/99	Post-transport crate swab	7

of critical sources of *Campylobacter* contamination in poultry will allow for the development of intervention strategies that aggressively target specific locations. These interventions will facilitate the delivery of pathogen-free birds to the abattoir and consequently should reduce the incidence of human exposure.

MATERIALS AND METHODS

Broiler flocks. Participating producers were located in Arkansas and California. Each producer provided access to two rearing houses located on two different farms; one rearing house was associated with high broiler growth performance, while the other rearing house had a history of low broiler growth performance. High and low broiler growth performance evaluations were based upon feed conversion ratios, weight gain, and overall performance. For all sites, one separate broiler flock was sampled for each of the four seasons, spring, summer, winter, and fall. Additional information regarding husbandry practices as they relate to the sampled flocks was previously described (32).

Sample collection. Samples were collected in both the production environment and the processing environment for each of the flocks. Production samples were taken prior to flock placement, at delivery (delivery tray liners), and at 2-week intervals until slaughter (at 6 or 8 weeks of age). Production samples taken inside of the rearing facility at each sampling period included samples from broiler feces ($n = 25$), water lines ($n = 6$), water cups ($n = 6$), litter ($n = 6$), feed from the feed hopper ($n = 2$), feed from the feeder ($n = 2$), drag swabs ($n = 3$), wall swabs ($n = 2$), fan swabs ($n = 2$), mouse intestines (collected as trapped), insects (collected as observed), fly strips ($n = 2$), and cecal droppings ($n = 5$). Production-associated samples taken from the exterior of the rearing facility included samples from wild bird feces (collected as observed), animal feces (collected as observed), dirt ($n = 1$), standing water ($n = 1$), and boot swabs ($n = 3$).

Samples collected in the processing environment included samples from carcass rinses ($n = 25$), prechill water ($n = 5$), postchill water ($n = 5$), prescald water ($n = 5$), postscald water ($n = 5$), pretransport crate swabs ($n = 10$), and

post-transport crate swabs ($n = 10$). In general, samples were collected into sterile plastic bags and placed in insulated boxes containing ice packs for overnight transport to the laboratory.

Bacterial isolates. Fecal samples were weighed and diluted 1:3 (wt/vol) with Difco buffered peptone water (Becton Dickinson, Sparks, Md.). Serial dilutions were prepared and plated onto Campy-Cefex agar which was incubated at 42°C for 36 to 48 h in a microaerobic atmosphere (5% O_2, 10% CO_2, 85% N_2) (30). Following incubation, a representative number of presumptive *Campylobacter* colonies were confirmed by observation of the typical cellular morphology by using phase-contrast microscopy and with a commercial latex agglutination kit (Integrated Diagnostics, Inc., Baltimore, Md.).

Carcass rinse samples were taken as previously described (9). Briefly, carcasses were sampled as they exited the final chill tank. Each carcass was placed into a clean bag with 100 ml of sterile tap water and vigorously shaken for 1 min. A 0.1-ml aliquot was plated onto duplicate Campy-Cefex agar and incubated as described for fecal samples. In addition, a 10-ml aliquot was used to inoculate 90 ml of *Campylobacter* enrichment broth (Acumedia Manufacturers, Inc., Baltimore, Md.) plus *Campylobacter*-selective supplement (Bolton's; Medox Diagnostics, Ogdensburg, N.Y.) which was enriched at 37°C for 4 h followed by a 40-h incubation at 42°C. Following a total of 44 h of incubation, the enriched samples were plated onto Campy-Cefex agar plates and incubated as previously described

(32). Confirmation procedures for carcass rinse isolates were identical to those used for fecal isolates. All other samples were placed in *Campylobacter* enrichment broth plus *Campylobacter*-selective supplement and incubated as previously described (32).

Molecular subtype analysis. One *Campylobacter* isolate originating from each positive sample type during each sampling period was chosen for subtype analysis. Isolated colonies of *Campylobacter* were resuspended in 300 μl of sterile H_2O and placed at 100°C for 10 min. Ten microliters of each boiled cell suspension was used as the template for *flaA* SVR PCR with the primers FLA242FU (5′-CTA TGG ATG AGC AAT TWA AAA T-3′) and FLA625RU (5′-CAA GWC CTG TTC CWA CTG AAG-3′) (22). A 35-cycle reaction was used with 1 min of denaturing at 96°C, 1 min of annealing at 52°C, and a 1-min extension at 72°C. The resulting product was approximately 425 bp. Sequence data was generated by using either the FLA242FU primer or the FLA625RU primer with the Big Dye dye terminator cycle sequencing kit (Applied BioSystems Inc.-Perkin Elmer, Foster City, Calif.). Data were assembled with Sequencher version 4.1 (GeneCodes Corp., Ann Arbor, Mich.) and aligned by using ClustalX (34). The aligned sequences were compared, and dendrograms were generated by using the UPGMA algorithm with HKY85 distance measurements in version 4.0 of the Phylogenetic Analysis Using Parsimony (PAUP*) program (D. L. Swofford, Sinauer Associates, Sunderland, Mass., 1988).

RESULTS

Analyses of cultures. Sixteen flocks from four different farms, representing two U.S. broiler producers, were analyzed in this study. Flocks and isolates are designated such that the first letter indicates the producer code (A for Arkansas or C for California) and the second letter denotes whether the flock was reared on a high-performance (H) or low-performance (L) site. Isolates that were collected in the processing environment are further designated with a P as the third letter. All isolates analyzed in this investigation are presented in Tables 1 to 4. Fourteen (87.5%) of the flocks studied were *Campylobacter* positive; two flocks (CL-Summer and CL-Winter) remained negative throughout the 6-week rearing period. In 13 (92.9%) of the positive flocks, shedding of *Campylobacter* was detected at week 6; shedding was detected earlier, at week 4, in flock AL-Winter. In general, fecal samples, drag swabs from inside the house, cecal droppings, carcass rinses, pretransport crate swabs, and post-transport crate swabs comprised the majority of samples that were *Campylobacter* positive (Table 5). Samples from 12 of the 14 positive flocks included environmental samples that were *Campylobacter* positive; in 7 of these 12 flocks, samples taken from the external environment of the poultry house were *Campylobacter* positive. Only twice were environmental samples found to be positive prior to flock shedding (AL-Fall and CH-Winter) (Fig. 1 and 2).

Molecular subtype analyses of production-associated isolates. Molecular subtype analyses of *Campylobacter* isolated from individual flocks revealed that overall, multiple clones of *Campylobacter* were present within a single flock. As many as six distinct clones were isolated within a flock (AH-Spring) from samples representative of the broiler, fecal, and cecal samples (Fig. 3). The three exceptions observed in this investigation were the two positive flocks from the low-performance farm from producer C (CL-Spring and CL-Fall) (data not shown) and the samples obtained from producer A (AL-Spring) (Fig. 4). Only one predominant clone was observed for all related poultry samples collected during each of these specific investigations. Ten environmental samples were positive for *Campylobacter* during the AL-Spring analyses. Six of these isolates (two from drag swabs, AL48 and AL50; two from animal feces, AL63 and AL64; one from a fly strip, AL74; and

TABLE 3. Positive *Campylobacter* isolates from the producer C high-performance (CH) site

Sample date (mo/day/yr)	Source	No. recovered
Summer		
08/24/98	Feces from poultry	14
08/24/98	Drag swab from inside house	3
08/24/98	Cecal dropping	3
Fall		
10/25/98	Feces from poultry	2
10/25/98	Litter	3
10/25/98	Cecal dropping	1
10/26/98	Carcass rinse from poultry	23
10/26/98	Prechill water	2
10/26/98	Postscald water	1
10/26/98	Pretransport crate swab	5
10/26/98	Post-transport crate swab	9
Winter		
01/13/99	Mouse intestine	1
01/13/99	Wild bird feces	2
01/25/99	Standing water	1
02/28/99	Feces from poultry	2
03/01/99	Carcass rinse from poultry	1
Spring		
04/28/99	Feces from poultry	20
04/28/99	Litter pretransport crate swab	3
04/28/99	Drag swab from inside house	2
04/28/99	Fan swab	1
04/28/99	Wild bird feces	1
04/28/99	Animal feces	1
04/28/99	Cecal dropping	2

one from an insect sample, AL68) possessed *flaA* SVR DNA sequences identical to those from poultry, while three isolates (one from a mouse sample, AL56; one from an insect sample, AL66; and one from a boot swab, AL71) demonstrated 2.4% variability. The remaining isolate, AL62, from a wild bird, demonstrated 21.3% variability from poultry isolates. With respect to CL-Spring and CL-Fall, the same clone was detected

TABLE 4. Positive *Campylobacter* isolates from the producer C low-performance (CL) site

Sample date (mo/day/yr)	Source	No. recovered
Fall		
10/25/98	Feces from poultry	2
10/25/98	Litter	1
10/25/98	Drag swab from inside house	3
10/25/98	Boot swab	1
10/25/98	Cecal dropping	3
10/25/98	Carcass rinse from poultry	1
10/25/98	Post-transport crate swab	4
Spring		
04/28/99	Feces from poultry	21
04/28/99	Litter	2
04/28/99	Drag swab from inside house	2
04/28/99	Cecal dropping	2
04/28/99	Carcass rinse from poultry	7
04/28/99	Pretransport crate swab	1
04/28/99	Post-transport crate swab	5

TABLE 5. Percentage of *Campylobacter*-positive samples by sample type

Sample type	No. (%) of positive samples				
	AH $(n = 105)^a$	AL $(n = 144)$	CH $(n = 103)$	CL $(n = 55)$	Total $(n = 407)$
Fecal	26 (24.76)	59 (40.97)	38 (36.89)	23 (41.82)	146 (35.87)
Water line	1 (0.95)	0 (0)	0 (0)	0 (0)	1 (0.25)
Water cup	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Litter	0 (0)	2 (1.39)	6 (5.83)	3 (5.45)	11 (2.70)
Feed hopper	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Feeder	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Drag swab	6 (5.71)	9 (6.25)	5 (4.85)	5 (9.09)	25 (6.14)
Wall swab	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Fan swab	0 (0)	0 (0)	1 (0.97)	0 (0)	1 (0.25)
Mouse intestine	0 (0)	2 (1.39)	1 (0.97)	0 (0)	3 (0.74)
Wild bird feces	0 (0)	3 (2.08)	4 (3.88)	0 (0)	7 (1.72)
Animal feces	0 (0)	2 (1.39)	0 (0)	0 (0)	2 (0.49)
Insects	1 (0.95)	3 (2.08)	0 (0)	0 (0)	4 (0.98)
Dirt	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Standing water	0 (0)	0 (0)	1 (0.97)	0 (0)	1 (0.25)
Boot swab	0 (0)	1 (0.69)	0 (0)	1 (1.82)	2 (0.49)
Fly strip	0 (0)	1 (0.69)	0 (0)	0 (0)	1 (0.25)
Cecal dropping	9 (8.57)	8 (5.56)	6 (5.83)	5 (9.09)	28 (6.89)
Carcass rinse	18 (17.14)	22 (15.28)	24 (23.30)	8 (14.55)	72 (17.69)
Prechill water	0 (0)	0 (0)	2 (1.94)	0 (0)	2 (0.49)
Postchill water	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Prescald water	7 (6.67)	1 (0.69)	0 (0)	0 (0)	8 (1.97)
Postscald water	7 (6.67)	4 (2.78)	1 (0.97)	0 (0)	12 (2.95)
Pretransport crate swab	9 (8.57)	13 (9.03)	5 (4.85)	1 (1.82)	28 (6.89)
Post-transport crate swab	21 (20.00)	14 (9.72)	9 (8.74)	9 (16.36)	53 (13.02)

^{a} AH, Arkansas high-production site; AL, Arkansas low-production site; CH, California high-production site; CL, California low-production site. *n* is the total number of samples.

for all environmental and processing samples collected for each specific investigation. Interestingly, a pretransport crate swab sample (CLP48) collected during the CL-Spring investigation was the same clone that was seen within the corresponding flock and on the carcasses. Further subtype analyses demonstrated that all *Campylobacter* isolates obtained from the producer C low-performance farm, regardless of season, had identical *flaA* SVR DNA sequences and were therefore considered to be clonal.

As stated previously, environmental samples that were positive for *Campylobacter* prior to flock shedding were obtained from two flocks (AL-Fall and CH-Winter) in this investigation (Fig. 1 and 2). An isolate originating from a wild bird, AL61 10/19/98, was collected prior to the placement of flock AL-Fall. Subtype analysis demonstrated that this isolate was very closely related to 12 isolates representative of the broilers (fecal and cecal isolates, namely, AL01, AL10, AL14, AL16, AL18, AL20, AL23, AL24, AL77, AL78, AL79, and AL80). Four additional environmental isolates were collected from this farm once the flock was positive for *Campylobacter*. Only one of the isolates (AL56), from a mouse, was related to the *Campylobacter* that was observed in the broilers. Two of the three remaining isolates (drag swab isolates AL49 and AL50) were distinct from the broiler isolates; however, they matched isolates obtained from processing samples (pretransport crate swabs and post-transport crate swabs). The remaining isolate (AL62), from a wild bird, was distantly related (21.4% variability) to all other isolates obtained from the sampling of this flock.

Four environmental samples associated with the CH-Winter sampling were *Campylobacter* positive. Of these samples, three

were obtained prior to flock placement (one mouse sample, CH58, and two samples from wild bird feces, CH61 and CH62). The remaining environmental isolate (a sample from standing water, CH70) was positive 2 weeks later; however, flock shedding had not yet begun. Subtype analyses demonstrated that the three isolates obtained prior to flock placement were distinct from broiler-associated isolates (2.7 to 6.6% variability). The isolate from standing water, however, was identical to a fecal isolate.

In the seven remaining flocks where environmental samples were positive, three flocks were from the high-performance site of producer C (CH-Spring, CH-Summer, and CH-Fall) (Fig. 5, 6, and 7), three were from the producer A high-performance site (AH-Summer, AH-Fall, and AH-Winter) (Fig. 8, 9, and 10), and one was from the producer A low-performance site (AL-Winter) (Fig. 11). A total of four distinct *Campylobacter* clones were observed from the broiler-associated samples from the AL-Winter investigation (Fig. 11). One of the subtypes, comprising isolates AL01B, AL23B, and AL24B, was present only during week 6 of sampling; all other subtypes were present at both week 4 and week 6 of sampling. Eight *Campylobacter*-positive environmental samples were isolated from this flock; five were isolated in week 4, when flock shedding began (two are litter isolates, AL38 01/11/99 and AL40 01/11/99, and three are drag swab isolates, AL48 01/11/99, AL49A 01/11/99, and AL49B 01/11/99), while three were isolated in week 6 (two are drag swab isolates, AL49B 01/25/99 and AL50B 01/25/99, and one is an insect isolate, AL66 01/25/99). All eight of the environmental *Campylobacter* isolates matched broiler-associated isolates. Additionally, all environ-
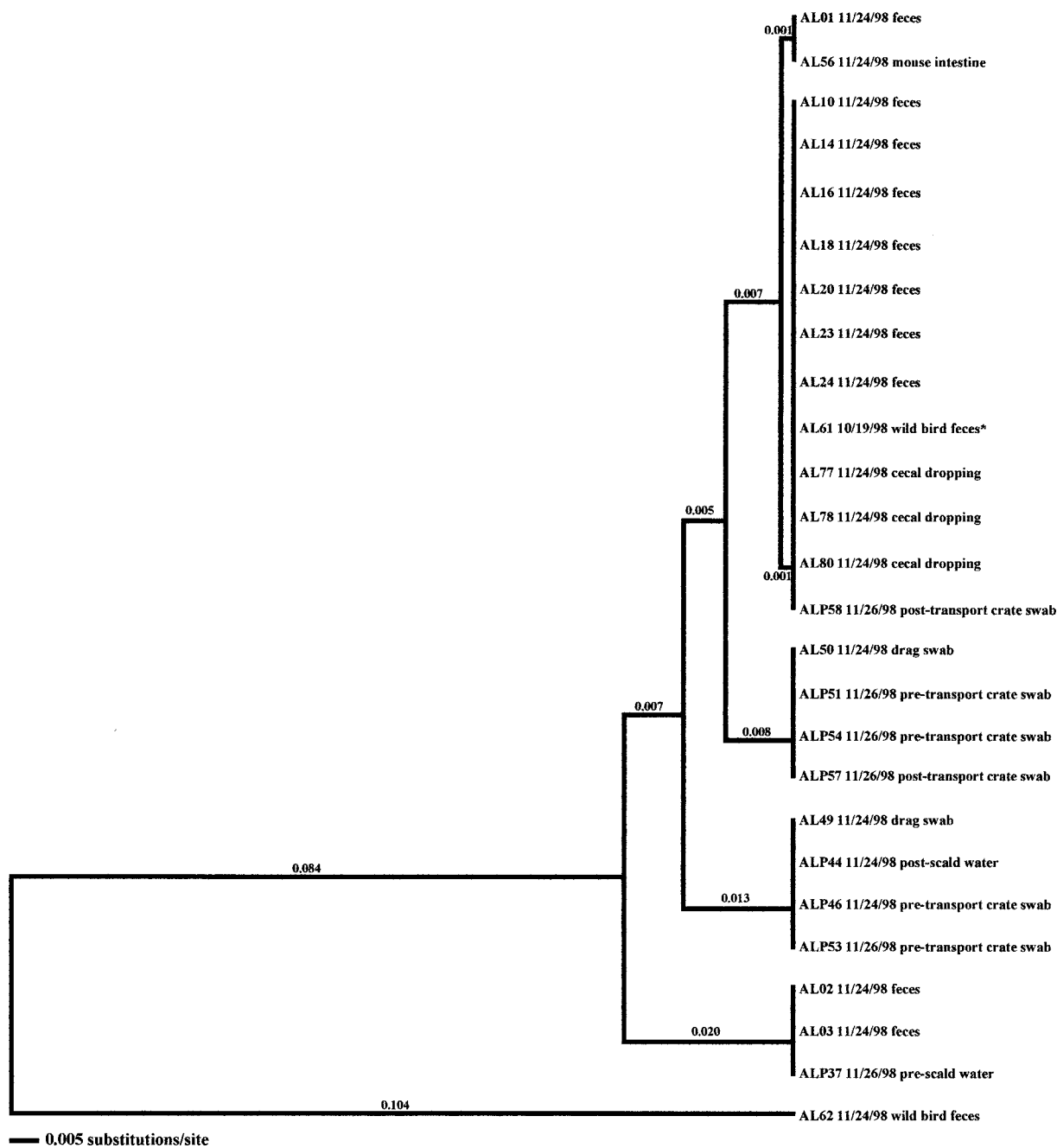
FIG. 1. Relationships derived from comparison of the SVR DNA sequences of the *flaA* genes from *Campylobacter* organisms isolated from the Arkansas low-performance farm in the fall. The dendrogram was generated as described in Materials and Methods, and the isolates are labeled as described in Results. The dates of isolation and the sources of the isolates are also provided.

mental isolates were comprised of the four distinct subtypes associated with the broilers.

In the six other flocks where environmental isolates were *Campylobacter* positive, all environmental-associated isolates were obtained during the same week as the broiler-associated samples. Four of these flocks possessed environmental isolates that all had *flaA* SVR DNA sequences identical to those of the respective fecal and/or cecal isolates. These environmental samples included three drag swabs (AH 48, AH49, and AH50)

from AH-Summer (Fig. 8), one drag swab (AH50) from AH-Winter (Fig. 10), three litter samples (CH38, CH39, and CH43) from CH-Fall (Fig. 7), and a variety of samples from CH-Spring that included three litter samples (CH39, CH42, and CH43), two drag swabs (CH49 and CH50), one fan swab (CH53), one sample from wild bird feces (CH62), and one sample from animal feces (CH63) (Fig. 5). Subtype analysis of environmental isolates from AH-Fall (Fig. 9) and CH-Summer (Fig. 6) revealed that some isolates possessed subtypes that are

FIG. 2. Relationships derived from comparison of the SVR DNA sequences of the *flaA* genes from *Campylobacter* organisms isolated from the California high-performance farm in the winter. The dendrogram was generated as described in Materials and Methods, and the isolates are labeled as described in Results. The dates of isolation and the sources of the isolates are also provided. Asterisks denote isolates that were obtained prior to flock shedding.
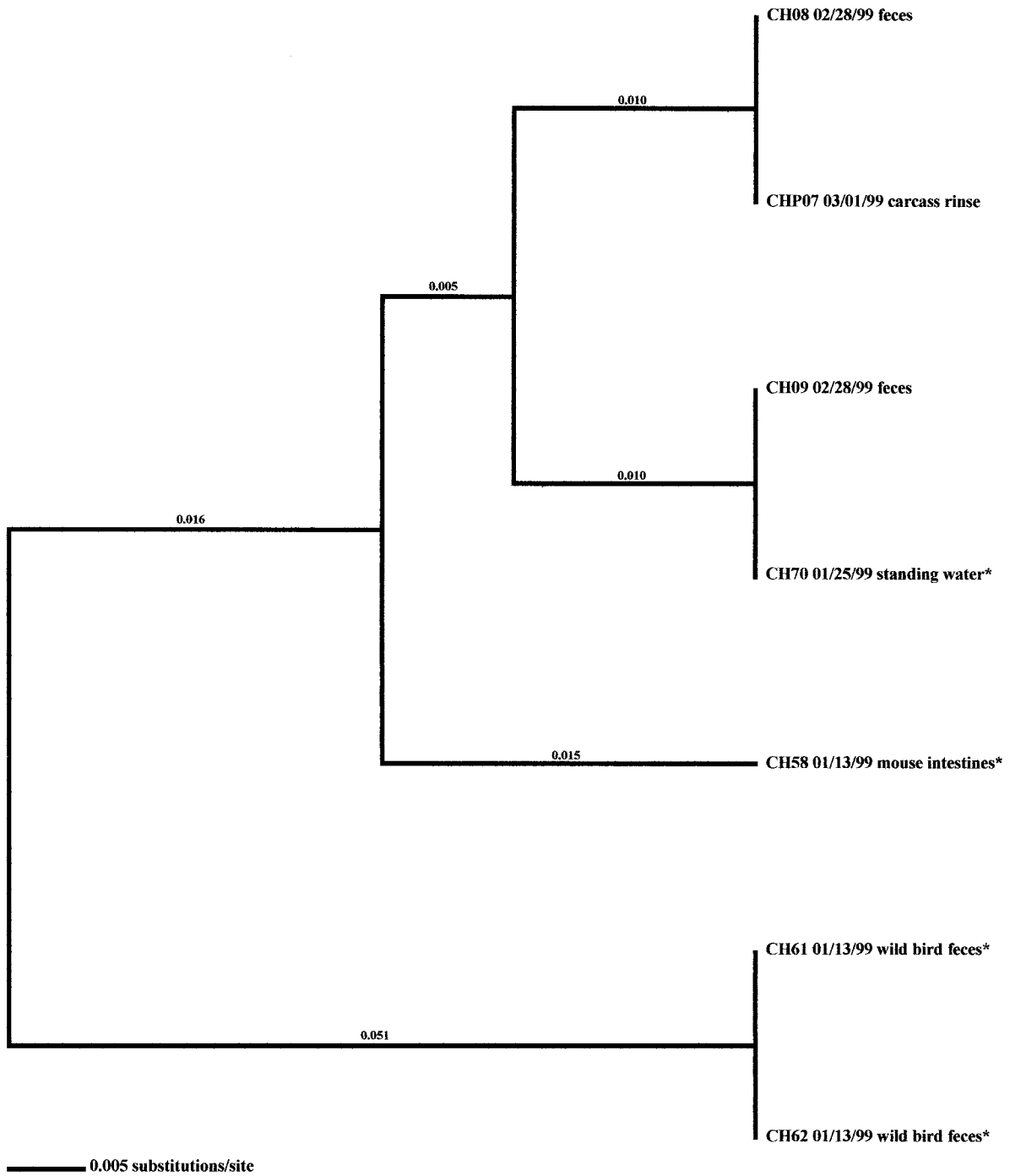
FIG. 3. Relationships derived from comparison of the SVR DNA sequences of the *flaA* genes from *Campylobacter* organisms isolated from the Arkansas high-performance farm in the spring. The dendrogram was generated as described in Materials and Methods, and the isolates are labeled as described in Results. The dates of isolation and the sources of the isolates are also provided.

identical to the respective fecal and/or cecal isolates, while other environmental isolates did not. Three isolates from AH-Fall (one water line isolate, AH28, one drag swab isolate, AH49, and one insect isolate, AH66) had *flaA* SVR DNA sequences that were identical to each other as well as to those of broiler-associated isolates; one isolate, a drag swab isolate (AH50), was distantly related to all other isolates obtained from this flock. Three drag swab isolates were obtained from CH-Summer; two isolates (CH49 and CH50) had subtypes identical to those of broiler-associated isolates, while the third

isolate (CH48) was distantly related to all other isolates (5.5% variability).

Molecular subtype analyses of processing-associated isolates. Comparison of *Campylobacter* subtypes between production- and processing-associated isolates of the same flock demonstrated that in general, clones found within the flock during production were the same clones that were recovered from the carcasses, although the number of distinct clones was reduced on the carcasses. Four of the 14 *Campylobacter*-positive flocks were *Campylobacter* negative with respect to carcass rinse sam-

AL01 04/06/98 feces
AL02 04/06/98 feces
AL03 04/06/98 feces
AL04 04/06/98 feces
AL05 04/06/98 feces
AL06 04/06/98 feces
AL07 04/06/98 feces
AL08 04/06/98 feces
AL09 04/06/98 feces
AL10 04/06/98 feces
AL11 04/06/98 feces
AL12 04/06/98 feces
AL13 04/06/98 feces
AL14 04/06/98 feces
AL15 04/06/98 feces
AL18 04/06/98 feces
AL19 04/06/98 feces
AL20 04/06/98 feces
AL21 04/06/98 feces
AL22 04/06/98 feces
AL23 04/06/98 feces
AL24 04/06/98 feces
AL25 04/06/98 feces
AL48 04/06/98 drag swab
AL50 04/06/98 drag swab
AL63 04/06/98 animal feces
AL64 04/06/98 animal feces
AL68 04/06/98 insect
AL74 04/06/98 fly strip
ALP02 04/08/98 carcass rinse
ALP03 04/08/98 carcass rinse
ALP05 04/08/98 carcass rinse
ALP07 04/08/98 carcass rinse
ALP08 04/08/98 carcass rinse
ALP09 04/08/98 carcass rinse
ALP10 04/08/98 carcass rinse
ALP11 04/08/98 carcass rinse
ALP12 04/08/98 carcass rinse
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ALP14 04/08/98 carcass rinse
ALP15 04/08/98 carcass rinse
ALP16 04/08/98 carcass rinse
ALP17 04/08/98 carcass rinse
ALP18 04/08/98 carcass rinse
ALP19 04/08/98 carcass rinse
ALP20 04/08/98 carcass rinse
ALP22 04/08/98 carcass rinse
ALP25 04/08/98 carcass rinse
ALP44 04/08/98 post-scald water
ALP45 04/08/98 post-scald water
ALP46 04/08/98 pre-transport crate swab
ALP48 04/08/98 pre-transport crate swab
ALP49 04/08/98 pre-transport crate swab
ALP50 04/08/98 pre-transport crate swab
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AL66 04/06/98 insect
AL71 04/06/98 boot swab
AL62 04/06/98 wild bird feces

0.012
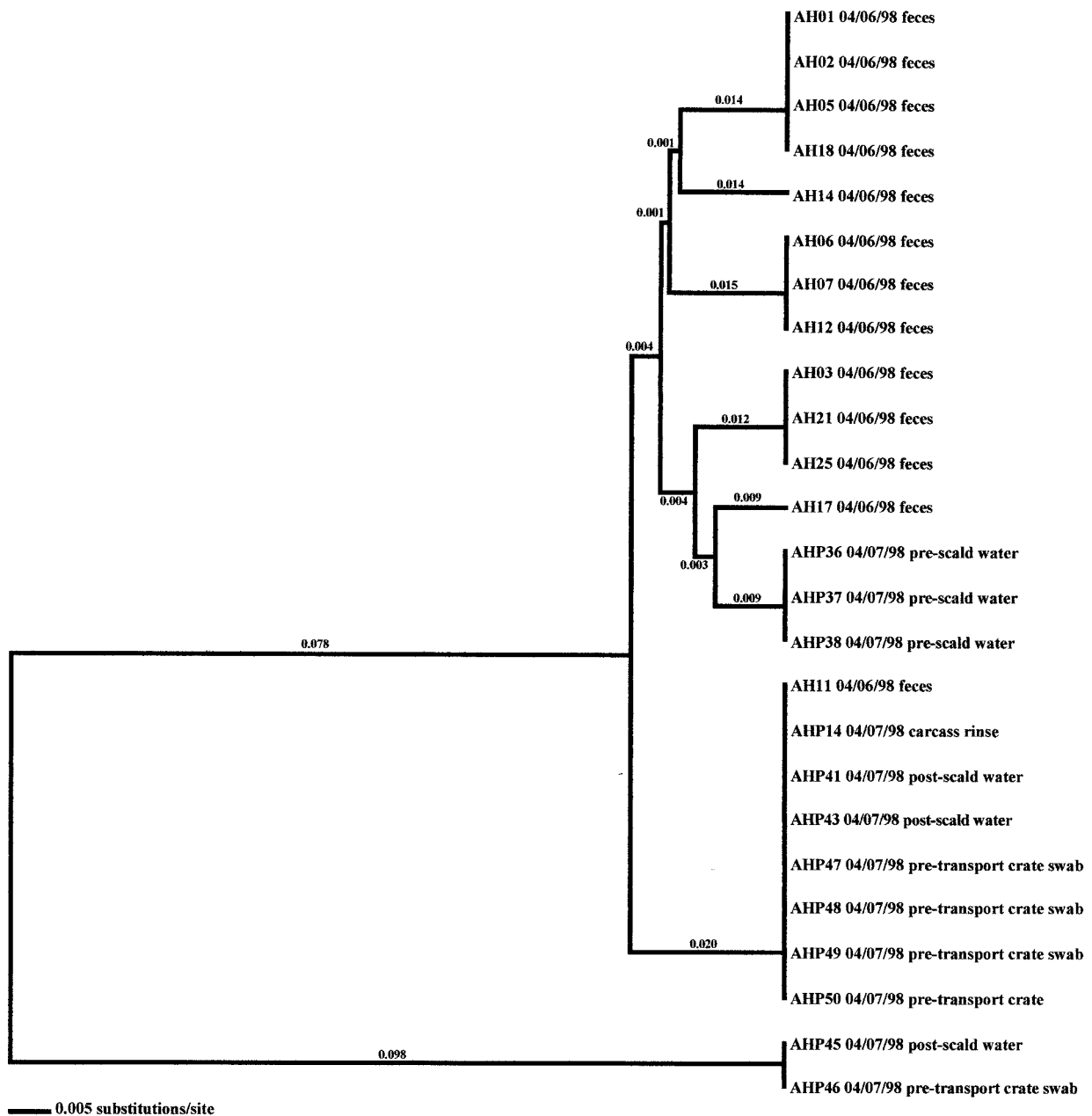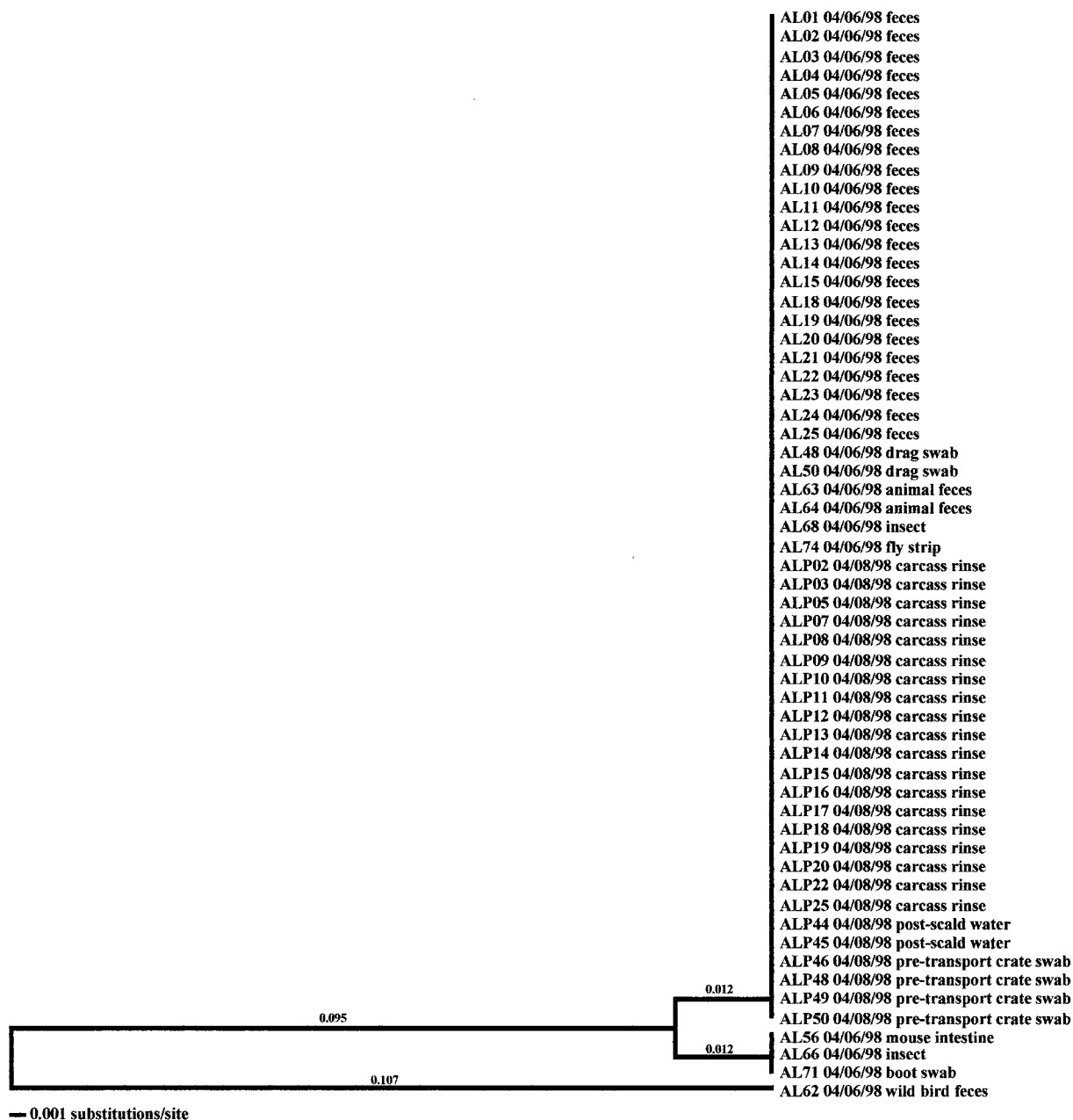0.095
0.012
0.107

— 0.001 substitutions/site

FIG. 4. Relationships derived from comparison of the SVR DNA sequences of the *flaA* genes from *Campylobacter* organisms isolated from the Arkansas low-performance farm in the spring. The dendrogram was generated as described in Materials and Methods, and the isolates are labeled as described in Results. The dates of isolation and the sources of the isolates are also provided.

ples and therefore no comparison could be made. In 9 of the remaining 10 *Campylobacter*-positive flocks sampled, at least one subtype recovered from production-associated samples was also recovered from the carcass rinse samples. The last flock, AL-Summer (Fig. 12), had one carcass rinse isolate with no apparent matches to production isolates; however, the number of isolates recovered from this flock sampling was low.

Post-transport crate swab isolates were recovered from 9 of the 14 *Campylobacter*-positive flocks. In eight of these flocks, the post-transport crate isolates matched the isolates from the broiler production samples. Furthermore, the subtypes of post-transport crate isolates also matched the subtypes found in the

majority of the respective carcass rinse isolates. Pretransport crate swab isolates were recovered from eight positive flocks. Five of these eight flocks (AH-Spring, AL-Spring, AL-Fall, AL-Winter, and CL-Spring) (Fig. 3, 4, 9, 11, and data not shown, respectively), demonstrated that the subtypes of the pretransport crate swab isolates were identical to the subtypes that were found in the production sample isolates from the respective flock. In two of the eight flocks, CH-Fall and AH-Fall (Fig. 7 and 9), two *flaA* SVR DNA sequence subtypes were observed. In each flock, one subtype was unique, while the second subtype matched isolates from carcass rinse samples only. Pretransport crate isolates from flock AH-Summer
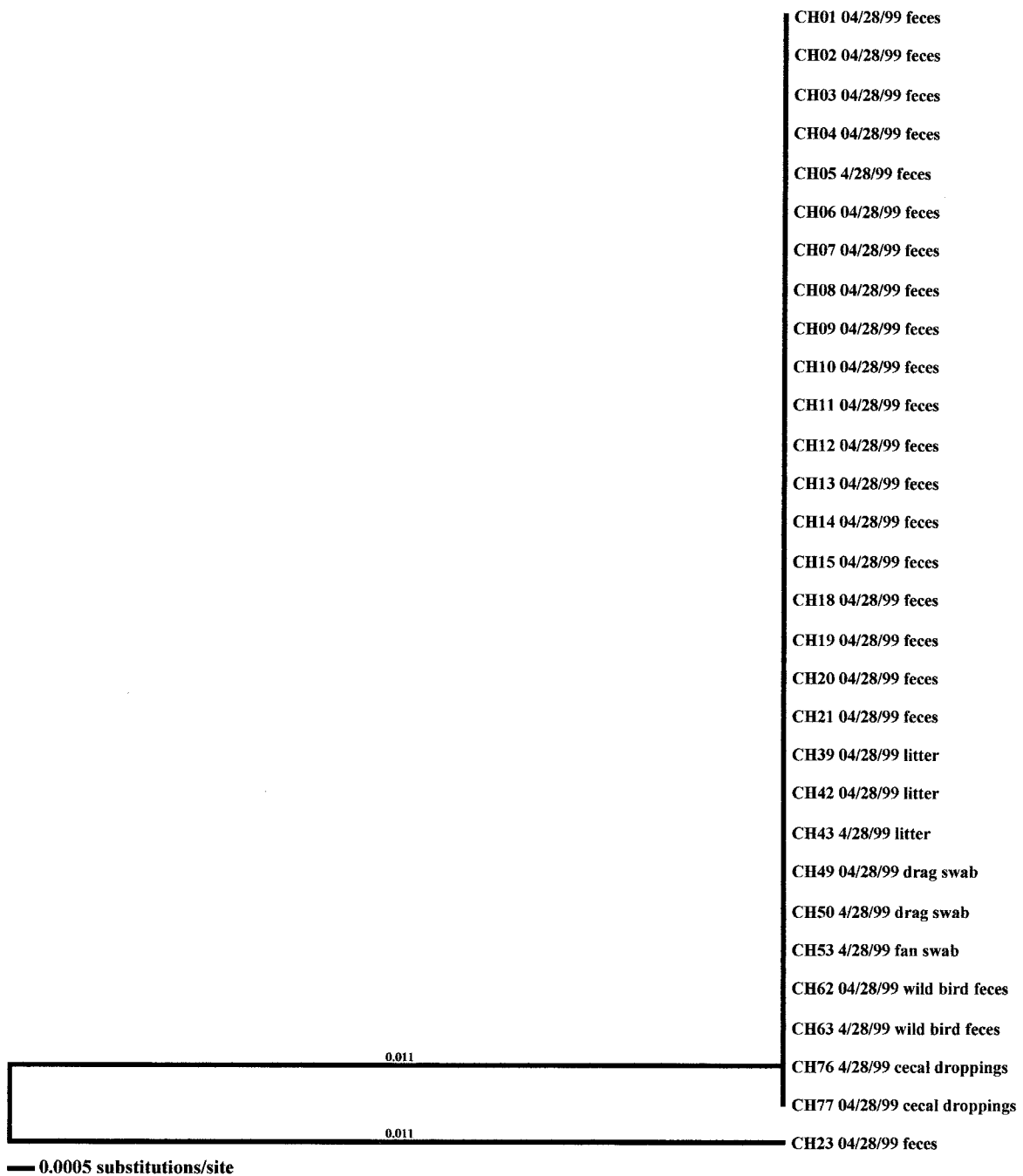
FIG. 5. Relationships derived from comparison of the SVR DNA sequences of the *flaA* genes from *Campylobacter* organisms isolated from the California high-performance farm in the spring. The dendrogram was generated as described in Materials and Methods, and the isolates are labeled as described in Results. The dates of isolation and the sources of the isolates are also provided.

(Fig. 8) possessed subtypes that were unique and matched no other isolates.

Analysis of the remaining processing samples (from prescald water, postscald water, prechill water, and postchill water) revealed that a greater number of *Campylobacter*-positive samples were obtained from producer A than from producer C. Additionally, more *Campylobacter* isolates were collected from pre- and postscald water (14 isolates) than from pre- and postchill water (2 isolates). Molecular subtype analysis dem-

onstrated that the prescald water isolates were comprised of both unique subtypes and subtypes that matched fecal isolates and carcass rinse isolates from the respective flocks. The postscald water isolates possessed *flaA* SVR DNA sequence subtypes that matched those of the respective production-associated isolates. The two prechill water isolates (CHP28 and CHP30) from CH-Fall had subtypes that matched carcass rinse isolates and pretransport crate isolates.

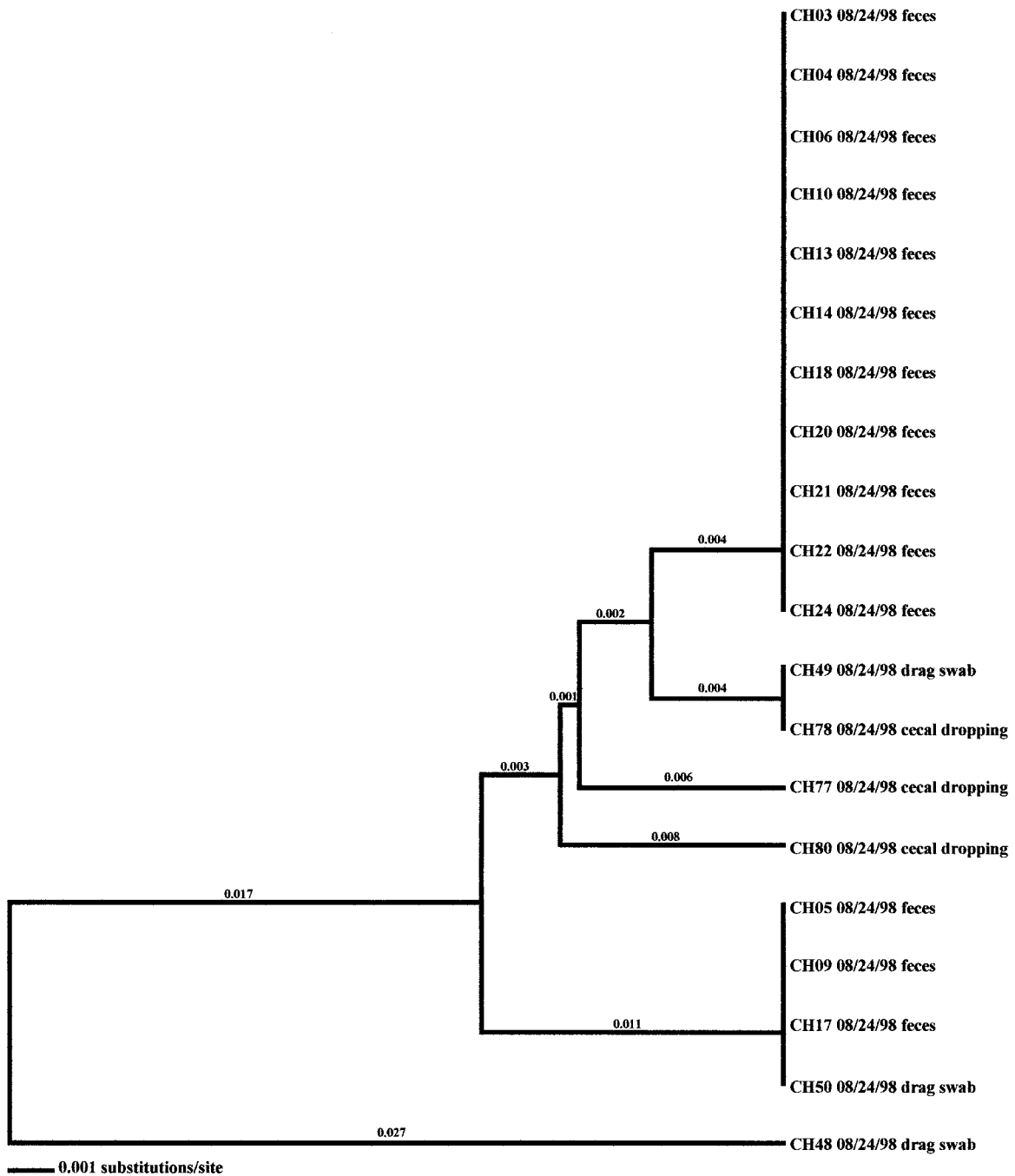**Molecular subtype analyses of *Campylobacter* isolates be-**

FIG. 6. Relationships derived from comparison of the SVR DNA sequences of the *flaA* genes from *Campylobacter* organisms isolated from the California high-performance farm in the summer. The dendrogram was generated as described in Materials and Methods, and the isolates are labeled as described in Results. The dates of isolation and the sources of the isolates are also provided.

tween flocks. As stated previously, molecular subtype analysis of all *Campylobacter* isolates obtained from the producer C low-performance farm, regardless of season, had identical *flaA* SVR DNA sequences and were therefore considered to be clonal. Further analysis demonstrated that this subtype was also present in all flocks sampled from the high-performance farm of producer C; however, additional subtypes were also present. Analysis of all *Campylobacter* isolates obtained from the producer C high-performance

farm during all seasons revealed that several of the same subtypes were present in different flocks. A similar observation, namely, that particular subtypes persist throughout the year on different farms, was made for producer A. Subtype analysis of all *Campylobacter* isolates obtained in this study from both producer A and producer C revealed that closely related subtypes were present within the operations of the two individual producers over all of the seasons investigated.

CH09 10/25/98 feces
CH13 10/25/98 feces
CH38 10/25/98 litter
CH39 10/25/98 litter
CH43 10/25/98 litter
CH76 10/25/98 cecal dropping
CHP04 10/26/98 carcass rinse
CHP07 10/26/98 carcass rinse
CHP10 10/26/98 carcass rinse
CHP13 10/26/98 carcass rinse
CHP14 10/26/98 carcass rinse
CHP17 10/26/98 carcass rinse
CHP28 10/26/98 pre-chill water
CHP44 10/26/98 post-scald water
CHP58 10/26/98 post-transport crate swab
CHP64 10/26/98 post-transport crate swab
CHP01 10/26/98 carcass rinse
CHP02 10/26/98 carcass rinse
CHP03 10/26/98 carcass rinse
CHP05 10/26/98 carcass rinse
CHP08 10/26/98 carcass rinse
CHP09 10/26/98 carcass rinse
CHP12 10/26/98 carcass rinse
CHP15 10/26/98 carcass rinse
CHP16 10/26/98 carcass rinse
CHP18 10/26/98 carcass rinse
CHP20 10/26/98 carcass rinse
CHP21 10/26/98 carcass rinse
CHP22 10/26/98 carcass rinse
CHP23 10/26/98 carcass rinse
CHP24 10/26/98 carcass rinse
CHP25 10/26/98 carcass rinse
CHP62 10/26/98 post-transport crate swab
CHP65 10/26/98 post-transport crate swab
CHP06 10/26/98 carcass rinse
CHP30 10/26/98 pre-chill water
CHP48 10/26/98 pre-transport crate swab
CHP49 10/26/98 pre-transpot crate swab
CHP51 10/26/98 pre-transport crate swab
CHP55 10/26/98 pre-transport crate swab
CHP57 10/26/98 post-transport crate swab
CHP59 10/26/98 post-transport crate swab
CHP60 10/26/98 post-transport crate swab
CHP61 10/26/98 post-transport crate swab
CHP53 10/26/98 pre-transport crate swab
CHP63 10/26/98 post-transport crate swab

0.034

0.007

0.006

0.007

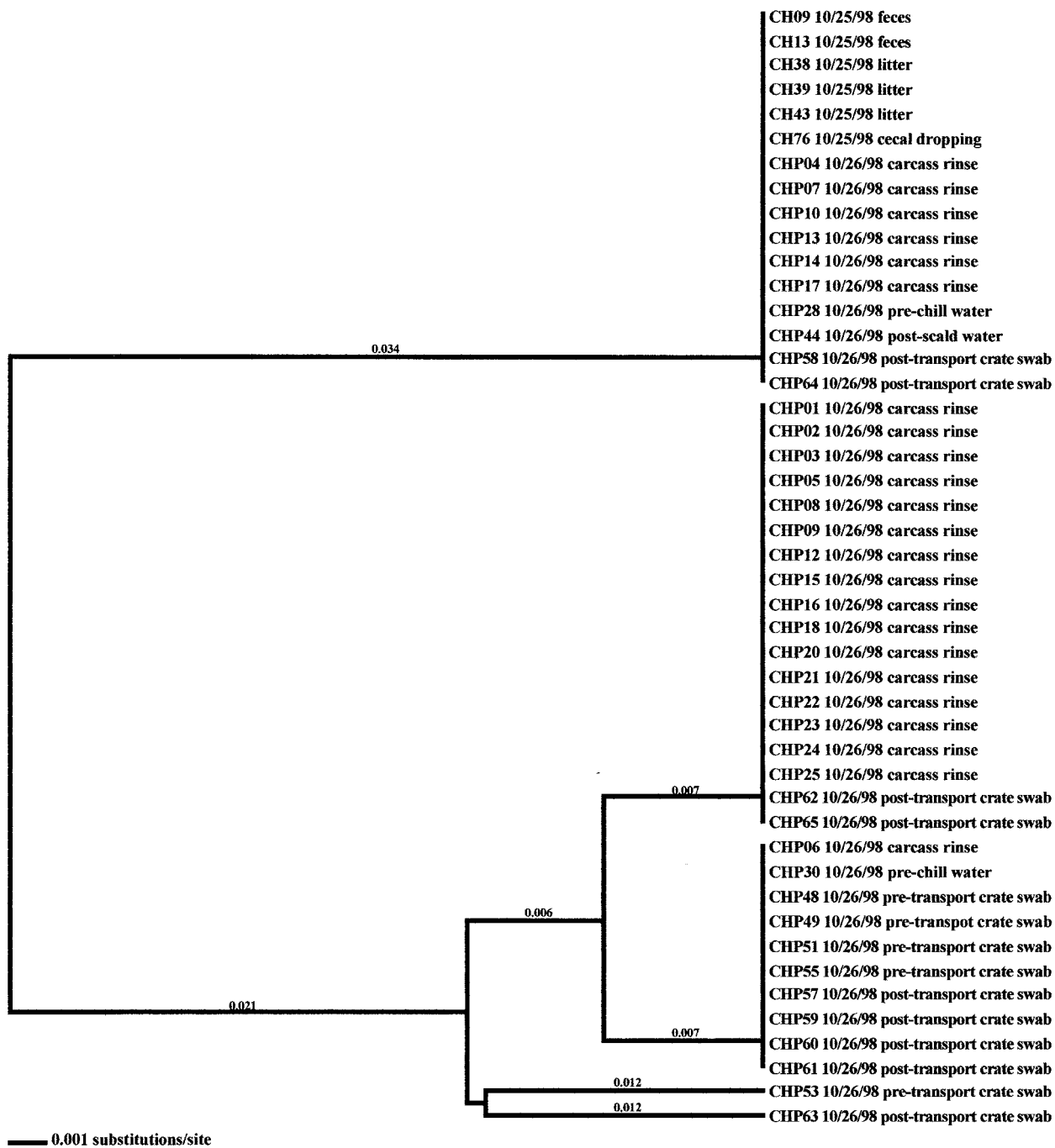0.012

0.012

0.021

■■■ 0.001 substitutions/site

FIG. 7. Relationships derived from comparison of the SVR DNA sequences of the *flaA* genes from *Campylobacter* organisms isolated from the California high-performance farm in the fall. The dendrogram was generated as described in Materials and Methods, and the isolates are labeled as described in Results. The dates of isolation and the sources of the isolates are also provided.

DISCUSSION

The Centers for Disease Control and Prevention estimates that *Campylobacter* enteritis is a multibillion-dollar disease and that the consumption of poultry is a primary source for the corresponding clinical infections in humans. Therefore, an understanding of the pathways involved in *Campylobacter* contamination and transmission in poultry flocks is critical for the development of intervention strategies and for the subsequent

reduction of *Campylobacter* in poultry. These data demonstrate that, in general, multiple clones of *Campylobacter* are present within a flock and that the clones that are present within a flock are also present on the final product, although the number of distinct clones is reduced. These findings concur with what has been observed in the United Kingdom and elsewhere (1, 2, 21). One explanation for the decrease in the diversity of *Campylobacter* clones recovered from production relative to process-
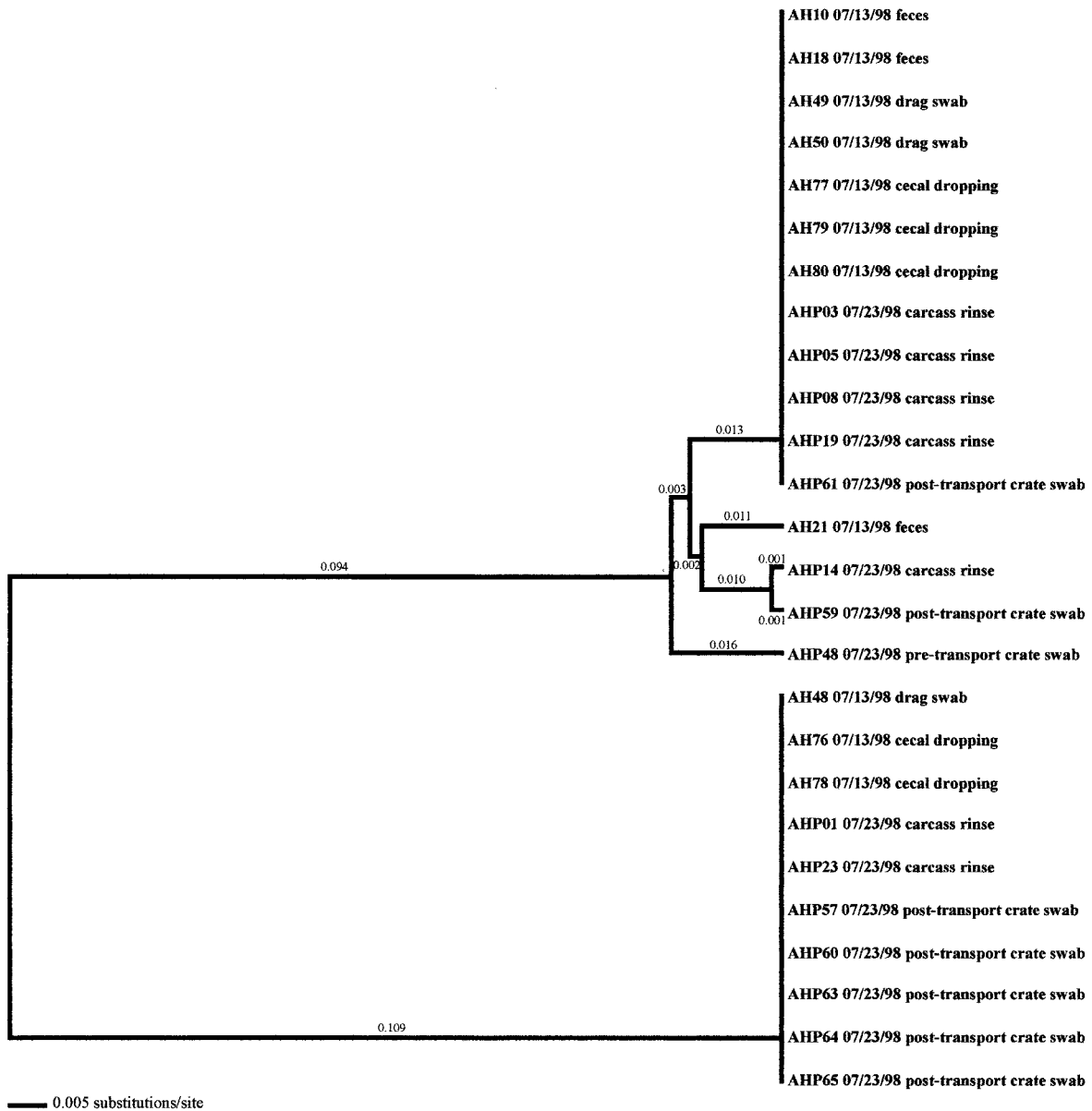
FIG. 8. Relationships derived from comparison of the SVR DNA sequences of the *flaA* genes from *Campylobacter* organisms isolated from the Arkansas high-performance farm in the summer. The dendrogram was generated as described in Materials and Methods, and the isolates are labeled as described in Results. The dates of isolation and the sources of the isolates are also provided.

ing is that during processing, exposure to adverse conditions (chlorination, heat treatment, cold treatment, exposure to oxygen, etc.) reduces the total number of *Campylobacter* organisms, thus reducing the diversity (23). A second possible explanation is that the processing-associated isolates were recovered by using an enrichment methodology, while the fecal isolates were recovered by using direct plating. It has been previously demonstrated that the use of enrichment media for the recovery of *Campylobacter* may result in the preferential selection of certain subtypes (23).

Our findings also demonstrate that closely related clones are present throughout the poultry industry, regardless of the producer and location. It is not believed that this observation is a

result of a media preference, because direct plating, rather than enrichment, was used for the recovery of *Campylobacter* from broiler fecal samples. However, it should be noted that all culture methodologies possess an inherent bias in that only a finite number of colonies can be picked and subsequently typed. Consequently, this limitation allows for only the most predominant clones to be detected. A second explanation is that some subtypes of *Campylobacter* may be better adapted for survival in different niches than are others. It may well be that the environmental stresses associated with having a chicken as a niche have led to the preferential survival of specific *Campylobacter* clones. A third, but related, explanation may be the transmission of *Campylobacter* from breeder hens

AH02 11/24/98 feces
AH03 11/24/98 feces
AH04 11/24/98 feces
AH08 11/24/98 feces
AH21 11/24/98 feces
AH28 11/24/98 water line
AH49 11/24/98 drag swab
AH66 11/24/98 insect
AH76 11/24/98 cecal dropping
AH79 11/24/98 cecal dropping
AH80 11/24/98 cecal dropping
AHP05 11/26/98 carcass rinse
AHP06 11/26/98 carcass rinse
AHP08 11/26/98 carcass rinse
AHP09 11/26/98 carcass rinse
AHP10 11/26/98 carcass rinse
AHP11 11/26/98 carcass rinse
AHP12 11/26/98 carcass rinse
AHP14 11/26/98 carcass rinse
AHP17 11/26/98 carcass rinse
AHP39 11/26/98 pre-scald water
AHP56 11/26/98 post-transport crate swab
AHP59 11/26/98 post-transport crate swab
AHP60 11/26/98 post-transport crate swab
AHP63 11/26/98 post-transport crate swab
AHP65 11/26/98 post-transport crate swab
AH05 11/24/98 feces
AHP61 11/26/98 post-transport crate swab
AHP46 11/26/98 pre-transport crate swab
AHP49 11/26/98 pre-transport crate swab
AH50 11/24/98 drag swab
AHP16 11/26/98 carcass rinse
AHP52 11/26/98 pre-transport crate swab
AHP58 11/26/98 post-transport crate swab

0.018
0.003
0.005
0.003
0.002
0.007
0.001
0.009
0.003
0.003
0.007
0.003
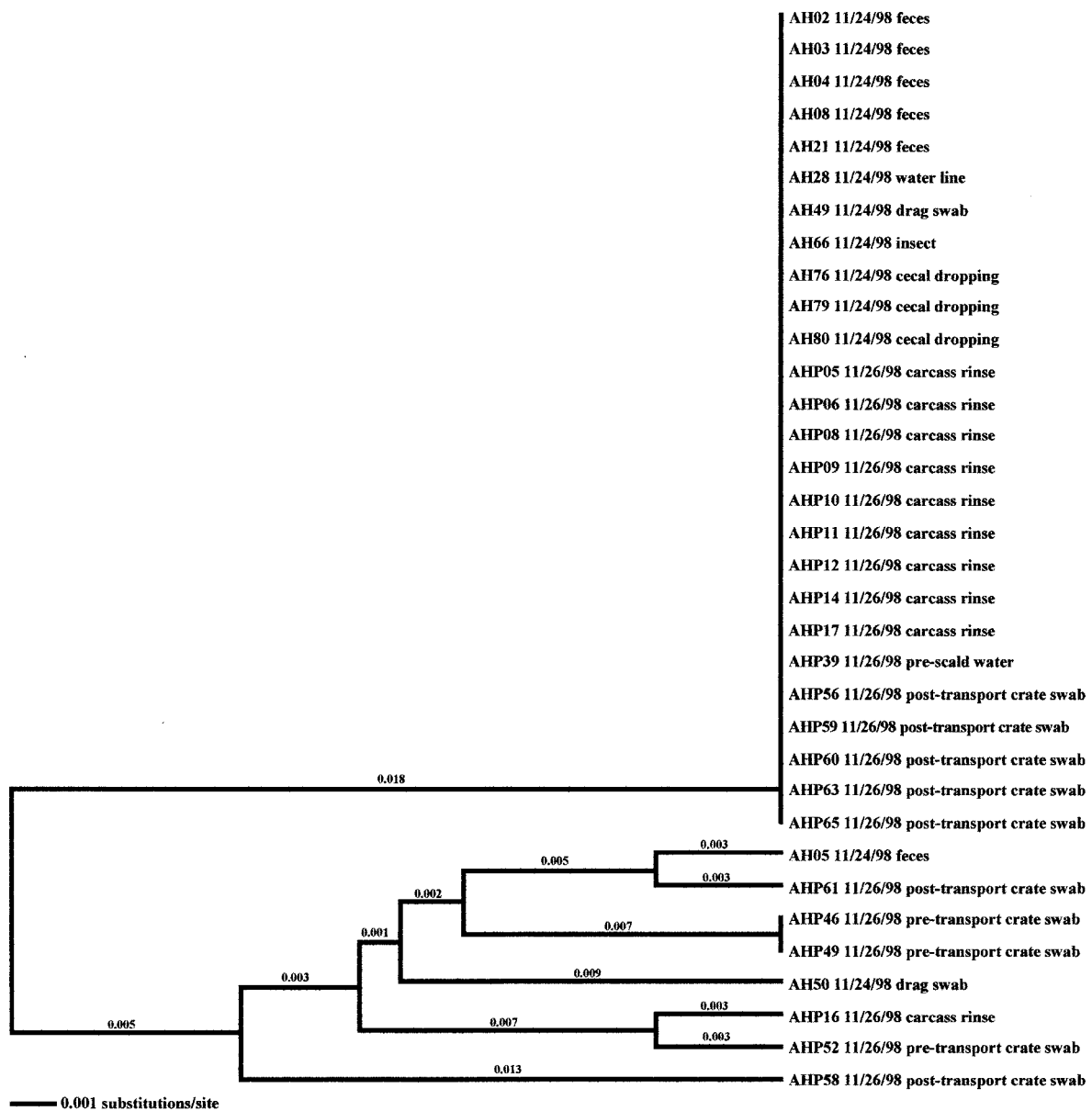0.005
0.013

0.001 substitutions/site

FIG. 9. Relationships derived from comparison of the SVR DNA sequences of the *flaA* genes from *Campylobacter* organisms isolated from the Arkansas high-performance farm in the fall. The dendrogram was generated as described in Materials and Methods, and the isolates are labeled as described in Results. The dates of isolation and the sources of the isolates are also provided.

to the broiler offspring (7–9, 12, 15, 20). A limited number of *Campylobacter* clones may be present within the population of breeder grandparents as well as the subsequent breeder hens and roosters. These clones may be passed on to the broiler offspring, where they proliferate and become the predominant clones. Should either of the two scenarios occur, new intervention strategies directed against the predominant clones or focused on the breeder hens and roosters will need to be developed.

Production-associated environmental samples were positive in 12 of the 16 flocks sampled. In 10 of these 12 flocks, environmental samples turned positive during the same week in which flock shedding began. In general, environmental isolates

obtained from within the rearing facility (samples from drag swabs, litter, water line swabs, and fan swabs) possessed subtypes identical to those of the fecal and cecal isolates from the respective flocks. Considering that most of these samples were negative for *Campylobacter* prior to flock shedding, it is likely that shedding of *Campylobacter* from the broilers led to the contamination of the rearing-facility samples rather than the reverse. Analyses of external environmental isolates demonstrated varied results. In four of the five flocks where samples from the external environment of the rearing facility turned positive with flock shedding, environmental isolates had *flaA* SVR DNA sequences identical to those of the fecal and cecal isolates of the broilers. The exception, AL-Spring, revealed
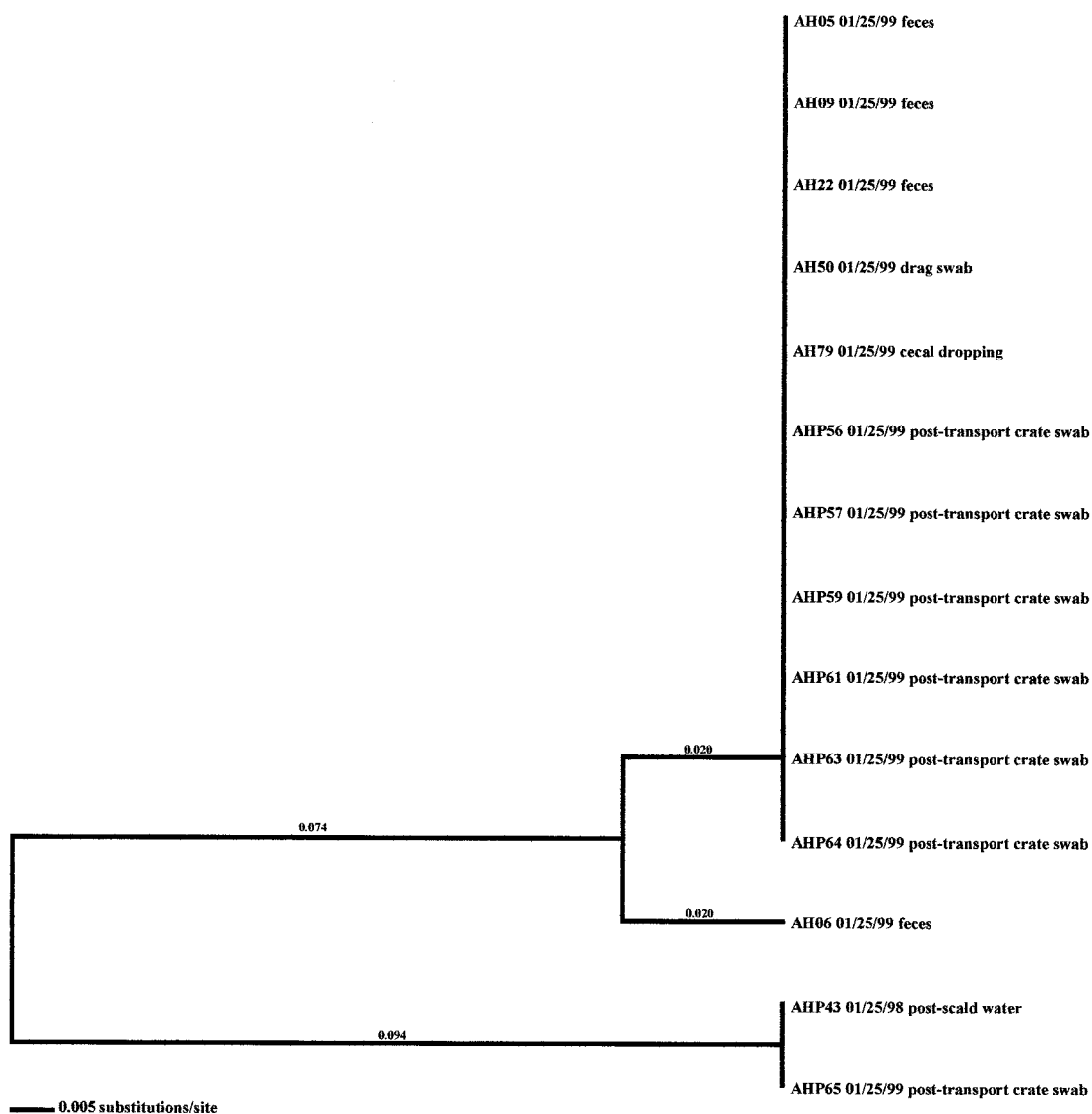
FIG. 10. Relationships derived from comparison of the SVR DNA sequences of the *flaA* genes from *Campylobacter* organisms isolated from the Arkansas high-performance farm in the winter. The dendrogram was generated as described in Materials and Methods, and the isolates are labeled as described in Results. The dates of isolation and the sources of the isolates are also provided.

that some environmental isolates possessed genotypes identical to those of isolates originating from the flock, while other environmental isolates possessed genotypes that were distantly related to isolates obtained from the flock. Considering the free access that some vectors (mice, insects, and wild birds) have inside and outside of the rearing facility, coupled with the fact that positive isolates were obtained concurrently from all samples, it is difficult to determine which vector, poultry or environment, served as the source of the observed *Campylobacter* contamination.

Analysis of *Campylobacter* isolates from the two investigations where positive samples were obtained prior to flock shedding, namely, the AL-Fall and CH-Winter investigations, demonstrated that particular *Campylobacter* subtypes from these previously isolated environmental samples were identical to some of the *Campylobacter* subtypes observed within the flocks

once shedding began. An isolate originating from a wild bird, AL61, had a *flaA* SVR DNA sequence identical to that of a subset of isolates collected from the broiler-associated samples. Additional subtypes were also present within this flock. A standing-water isolate, CH70, obtained from the CH-Winter investigation, had a subtype identical to that of a subsequently isolated broiler isolate. It should be noted that the number of *Campylobacter*-positive isolates obtained from this particular investigation was quite low. The above observations suggest that the environment can be a contributing factor to *Campylobacter* contamination of broiler flocks. Certain husbandry practices, such as opening the rearing facility to the outside environment for cooling, may lead to this contamination. Conversely, practices such as the reuse of litter and limited disinfection of the rearing facility between flocks appeared to have little effect on contamination of flocks. The presence of addi-

AL01A 01/11/99 feces
AL04 01/11/99 feces
AL05 01/11/99 feces
AL06 01/11/99 feces
AL07 01/11/99 feces
AL08 01/11/99 feces
AL10A 01/11/99 feces
AL10B 01/25/99 feces
AL11A 01/11/99 feces
AL12 01/11/99 feces
AL13 01/11/99 feces
AL16A 01/11/99 feces
AL18 01/11/99 feces
AL19 01/11/99 feces
AL20 01/11/99 feces
AL22 01/11/99 feces
AL23A 01/11/99 feces
AL24A 01/11/99 feces
AL48 01/11/99 drag swab
AL49A 01/11/99 drag swab
AL50B 01/25/99 drag swab
AL78 01/11/99 cecal dropping
AL79 01/25/99 cecal dropping
ALP47 01/26/99 pre-transport crate swab
ALP48 01/26/99 pre-transport crate swab
ALP51 01/26/99 pre-transport crate swab
ALP55 01/26/99 pre-transport crate swab
AL50A 01/11/99 drag swab
AL16B 01/25/99 feces
AL25 01/11/99 feces
AL38 01/11/99 litter
AL40 01/11/99 litter
AL01B 01/25/99 feces
AL23B 01/25/99 feces
AL24B 01/25/99 feces
AL49B 01/25/99 drag swab
ALP12 01/26/99 carcass rinse
ALP59 01/26/99 post-transport crate swab
ALP64 01/26/99 post-transport crate swab
ALP57 01/26/99 post-transport crate swab
AL02B 01/25/99 feces
AL02A 01/11/99 feces
AL11B 01/25/99 feces
AL66 01/25/99 insect
ALP10 01/26/99 carcass rinse
ALP44 01/26/99 post-scald water
ALP56 01/26/99 post-transport crate swab
ALP58 01/26/99 post-transport crate swab
ALP60 01/26/99 post-transport crate swab
ALP61 01/26/99 post-transport crate swab
ALP54 01/26/99 pre-transport crate swab
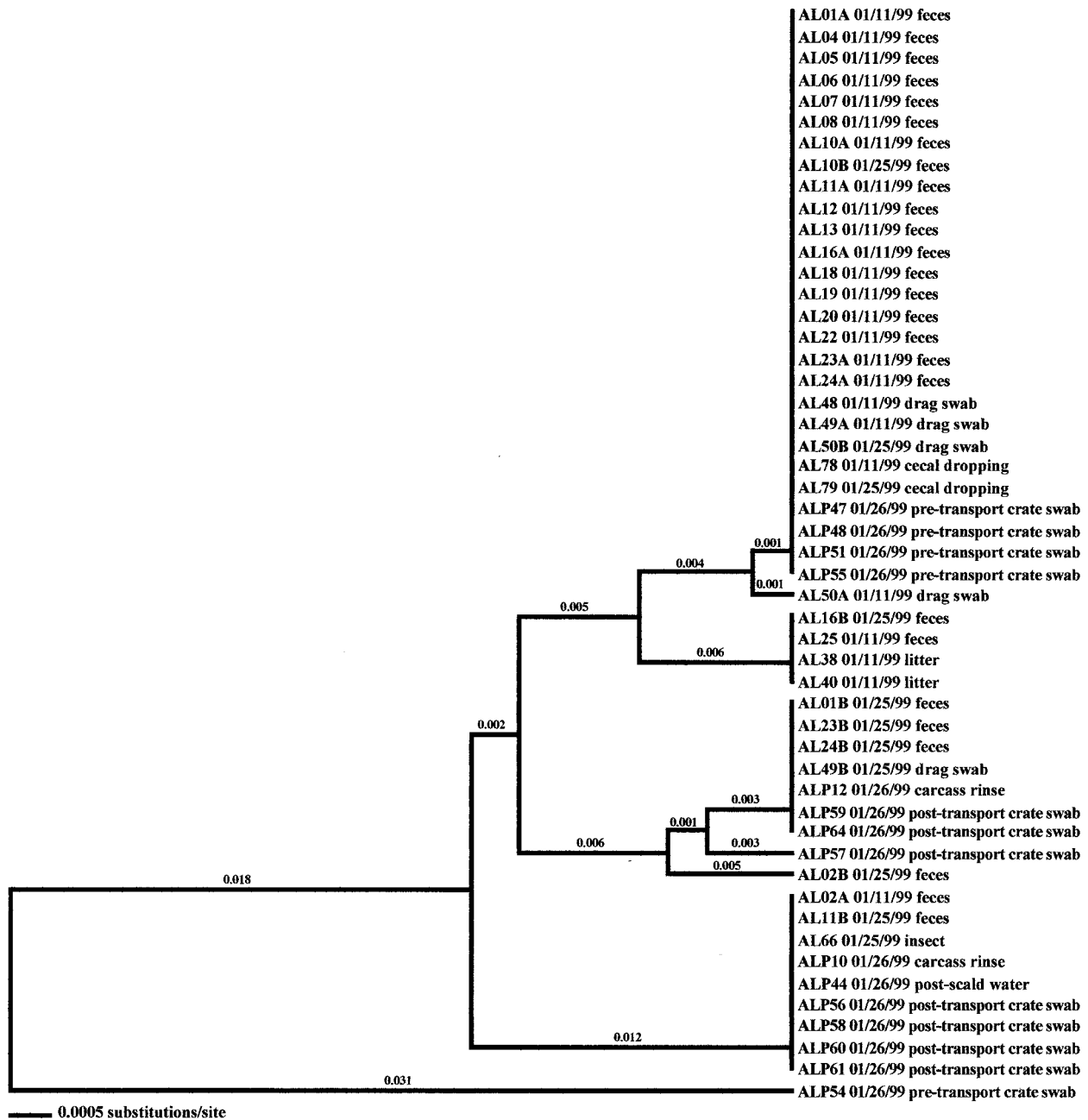
0.0005 substitutions/site

FIG. 11. Relationships derived from comparison of the SVR DNA sequences of the *flaA* genes from *Campylobacter* organisms isolated from the Arkansas low-performance farm in the winter. The dendrogram was generated as described in Materials and Methods, and the isolates are labeled as described in Results. The dates of isolation and the sources of the isolates are also provided.

tional subtypes within broiler flocks, coupled with the previous observation that environmental samples are rarely positive prior to flock shedding, indicates, however, that the environment may not be the sole source for *Campylobacter* contamination of broiler flocks. Alternatively, *Campylobacter* organisms that were sublethally injured from environmental exposure may not have been adequately recovered in this investigation. Improved methods of culture and molecular detection would greatly facilitate epidemiologic investigations.

Analysis of *Campylobacter* isolates recovered from the pro-

cessing environment revealed that contamination of the final product (carcass) originated primarily from the intestinal contents of the broiler flock. This may have occurred during processing or, more likely, transpired during transport of the birds (31). However, additional sources within the processing environment, such as pretransport crates and residual *Campylobacter* from previously processed flocks, also contributed to contamination of the final product. These observations suggest that more robust cleaning of crates and of the processing facilities may result in a further decline of *Campylobacter* con-
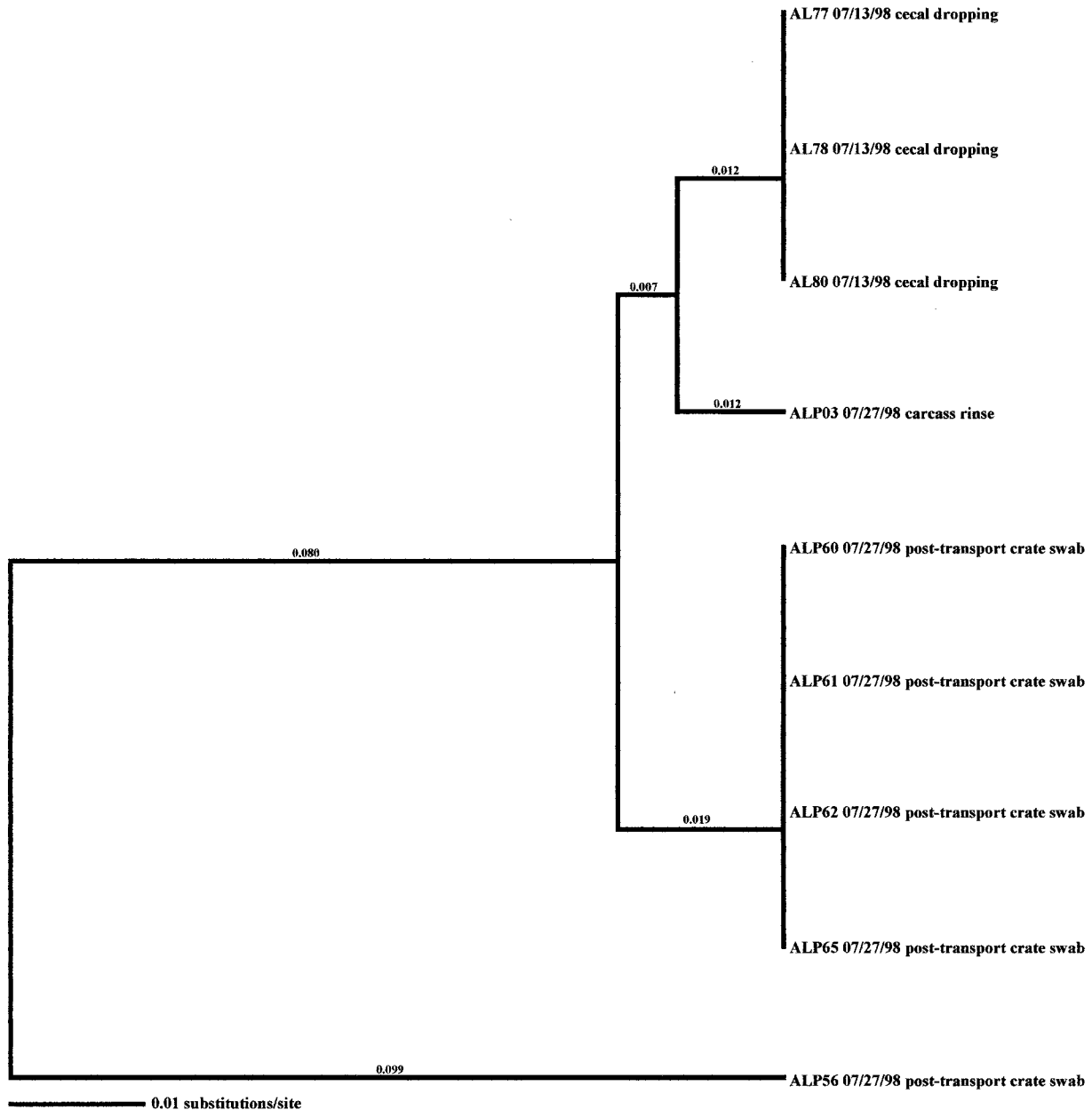
FIG. 12. Relationships derived from comparison of the SVR DNA sequences of the *flaA* genes from *Campylobacter* organisms isolated from the Arkansas low-performance farm in the summer. The dendrogram was generated as described in Materials and Methods, and the isolates are labeled as described in Results. The dates of isolation and the sources of the isolates are also provided.

tamination of the final market product. Improved detection methods and additional epidemiologic investigations are needed to further elucidate the means by which broiler flocks become contaminated with *Campylobacter*.

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