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Inhibition of *Campylobacter jejuni* Colonization in Chicks by Defined Competitive Exclusion Bacteria

JEAN L. SCHOENI AND AMY C. L. WONG*

Food Research Institute, Department of Food Microbiology and Toxicology, University of Wisconsin, Madison, Wisconsin 53706

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Campylobacter* enteritis in humans has been linked to consumption of chicken. Reducing *Campylobacter jejuni* colonization in chickens can potentially reduce *Campylobacter* infections in humans. In this study, the reduction of *C. jejuni* colonization in chicks by oral administration of defined competitive exclusion (CE) cultures, 2.5% dietary carbohydrates, or CE cultures and carbohydrates was examined. Prevention, elimination, or direct challenge of *Campylobacter* infection was simulated by administering treatments before, after, or at the same time as that of the *Campylobacter* inoculation. Additionally, the effect of maintaining high levels of protective bacteria was evaluated by administering CE cultures on days 1 and 4 (booster treatment). All treatments reduced *C. jejuni* colonization. Protection by aerobically grown CE cultures was not statistically different from that by anaerobically grown CE cultures. A combination of *Citrobacter diversus* 22, *Klebsiella pneumoniae* 23, and *Escherichia coli* 25 (CE 3) was the most effective CE treatment. Maintaining high numbers of CE isolates by administering CE boosters did not increase protection. The greatest reduction of *Campylobacter* colonization was observed in schemes to prevent or eliminate *C. jejuni* infection. *C. jejuni* was not detected in the ceca of birds receiving the prevention treatment, CE 3 with mannose, representing a 62% reduction in the colonization rate. The protection factor (PF), a value combining the colonization rate and the level of infection, for CE 3 with mannose was high (>13.2). Fructooligosaccharides alone strongly prevented *Campylobacter* colonization. Only 8% of the chicks in this group were colonized, with a PF of >14.3. Lactose and CE 3 greatly reduced established infections (PF, >20.1); *C. jejuni* was found in the ceca of only 5% of the chicks treated with this combination compared with 80% of the control chicks. While specific prevention and elimination treatments were highly protective, they were not statistically more protective than the best challenge and booster treatments. Thus, CE cultures and carbohydrates can be effectively administered to reduce *Campylobacter* colonization.

Campylobacter jejuni is recognized as a leading cause of acute bacterial gastroenteritis worldwide (4, 6, 12, 26, 31, 32, 38, 39). Surveys show that 30 to 100% of poultry carry the organism (11, 37). Investigations of outbreaks and sporadic cases of *Campylobacter* enteritis have revealed poultry as the principal vector of illness (5, 10, 13, 15, 16, 18, 23, 27). Case control studies in the United States indicate that 48 to 70% of sporadic cases of *C. jejuni* infection are due to consumption of chicken (10, 13). Surveys in The Netherlands (22) and Sweden (23) show a similar association. Infection usually occurs as a result of eating undercooked chicken or eating foods that have been cross-contaminated by raw chicken during storage and/or preparation.

Nurmi and Rantala (24) introduced the concept of competitive exclusion (CE) as a means to reduce *Salmonella* infections. Intestinal flora from adult chickens are introduced into young birds as suspensions of fecal droppings or cecal contents or as anaerobic subcultures of these materials. It is believed that anaerobic growth of CE bacteria is important to maintain their effectiveness (2). Studies using undefined bacterial mixtures obtained from the cecal content of adult chickens to reduce colonization of chicks by *C. jejuni* have shown different degrees of effectiveness (22, 29, 30, 32, 33, 36). In addition to variable efficacy, treatment with undefined CE cultures presents two major concerns. First, avian or human pathogens may be present, and second, treatment with undefined CE cultures is not acceptable to regulatory agencies in some countries (34).

These concerns can be addressed by defining the bacteria used in CE treatments. Schoeni and Doyle (28) excluded *C. jejuni* from colonizing chick cecal crypts with isolates that occupied the same niche and produced metabolites antagonistic to the organism. These aerobically maintained, defined bacteria protected 40 to 100% of the chicks from *Campylobacter* colonization and reduced the *C. jejuni* population in colonized birds. Aho et al. (1) were able to delay the onset of *C. jejuni* infection and consistently lower the level of colonization by administering organisms identified only as K-bacteria, grown under microaerobic conditions, combined with a commercial CE product (Broilact).

Another treatment which may reduce *C. jejuni* colonization is the administration of dietary carbohydrates. Dietary carbohydrates given with or without CE treatment have produced promising results in the inhibition of *Salmonella* colonization (3, 9). No work has been reported for *C. jejuni* in vivo, but in vitro studies conducted with INT 407 cells revealed that the adherence of *C. jejuni* was inhibited by certain carbohydrates, including mannose (7, 20).

The present study was conducted to optimize conditions for the use of defined CE cultures, with anti-*Campylobacter* activity, in reducing colonization of poultry by *C. jejuni*. This was done by the manipulation of CE culture composition and growth conditions and the use of dietary carbohydrates and different treatment administration schedules.

* Corresponding author. Mailing address: Food Research Institute, Department of Food Microbiology and Toxicology, University of Wisconsin, 1925 Willow Dr., Madison, WI 53706. Phone: (608) 263-1168. Fax: (608) 263-1114. Electronic mail address: amywong@macc.wisc.edu.

MATERIALS AND METHODS

Preparation of CE isolates, *C. jejuni*, and carbohydrates for peroral administration to chicks. Cecum-colonizing bacteria isolated from *Campylobacter*-free laying hens and previously shown by Schoeni and Doyle (28) to reduce *C. jejuni* colonization of chicks were used in this study. Aerobic cultures of *Citrobacter diversus* 22, *Klebsiella pneumoniae* 23, and *Escherichia coli* 25 were grown in 250-ml flasks containing 50 ml of tryptic soy broth (Difco Laboratories, Detroit, Mich.) at 37°C for 18 to 24 h. For anaerobic incubation, the isolates were inoculated into 250-ml sidearm flasks containing 50 ml of tryptic soy broth. The atmosphere in these flasks was exchanged four times with an anaerobic gas mixture (10% hydrogen, 10% carbon dioxide, 80% nitrogen). If washed CE cultures were required for treatment, the cells were sedimented by centrifugation (5,000 × *g*, 15 min), washed once in 0.1% peptone, sedimented, and then resuspended in 0.1% peptone. Cells were adjusted in 0.1% peptone to an optical density at 500 nm of 2.0 (ca. 10^9 CFU/ml). Unwashed cultures were adjusted to an optical density at 500 nm of 2.0 by adding tryptic soy broth to overnight cultures. Equal amounts of the appropriate strains were combined. Suspensions containing approximately equal numbers of *C. diversus* 22, *K. pneumoniae* 23, and *E. coli* 25 were designated CE 3. CE 2 contained *K. pneumoniae* 23 and *E. coli* 25. CE 1 contained only *E. coli* 25. Viable cell counts were determined on tryptic soy agar plates incubated at 37°C for 18 to 24 h. Unless stated otherwise, CE cultures were grown under aerobic conditions and washed before being combined and administered to chicks.

C. jejuni 108, a poultry isolate, was inoculated into 250-ml sidearm flasks containing 50 ml of *Campylobacter* growth broth (brucella broth [Difco] plus 0.3% sodium succinate and 0.01% cysteine-HCl [Sigma Chemical Co., St. Louis, Mo.]). The atmosphere in the flasks was exchanged four times with microaerobic gas (5% oxygen, 10% carbon dioxide, 85% nitrogen). *Campylobacter* broth cultures were incubated at 42°C for 18 to 24 h. Cells were sedimented and washed by the procedure described above and then adjusted in 0.1% peptone to an optical density at 500 nm of 0.5 (ca. 10^9 CFU/ml). *C. jejuni* was enumerated on brucella agar with 5% defibrinated sheep blood and incubated microaerobically at 42°C for 48 h.

Carbohydrate solutions (2.5%, wt/vol) were prepared in distilled water. α-Lactose (Sigma) was autoclaved at 121°C (15 lb/in^2) for 15 min. D-Mannose (Sigma) and fructooligosaccharides (FOS 50; ZeaGen, Broomfield, Colo.) were filter sterilized (cellulose acetate, 0.22-μm pore size; Corning Glass Works, Corning, N.Y.).

Evaluation of the protective effect of cecal isolates and carbohydrates on the colonization of chicks. Sixty-five to 85 1-day-old White Leghorn cockerel chicks were obtained from Sunnyside Inc. of Beaver Dam (Beaver Dam, Wis.) for each trial. Chicks were divided into groups of no fewer than five birds. Chicks were housed by treatment group in electric brooders with stainless-steel wire floors (model 6401; Brower Mfg. Co., Houghton, Iowa). The temperature was maintained at 35°C. Room lights were cycled 12 h on and 12 h off. Carbohydrates, CE cultures, or CE cultures with carbohydrates were assessed for efficacy in reducing *Campylobacter* colonization. A minimum of three trials per treatment was conducted. Each chick received 0.5 ml of CE suspension (10^8 CFU per chick) and/or 0.5 ml of *C. jejuni* 108 (10^8 CFU per chick) via a 20-gauge, ball-tipped cannula by the following treatment schedules: (i) for the colonization control, *C. jejuni* was administered on day 1 or 3; (ii) for the challenge studies, CE culture treatment was followed by *C. jejuni* administration on day 1; (iii) for the booster studies, CE cultures were administered on days 1 and 4 and *C. jejuni* was inoculated on day 1; (iv) for the prevention studies, CE cultures were inoculated on day 1 and administration of *C. jejuni* followed 2 days later; (v) for elimination studies, *C. jejuni* was given on day 1 and administration of CE cultures followed on day 3. Carbohydrate solutions were provided ad libitum beginning on day 1 or 3, alone or with CE cultures administered as described in the previous schedules. At 7 ± 1 days, chicks were sacrificed by carbon dioxide asphyxiation. The ventral trunk of the body was soaked with 70% ethanol, the lower intestine was surgically exposed, and the ceca were removed. One cecum was slit lengthwise and placed in a tube containing 2 ml of sterile deionized water. These samples were mixed for 30 s in a Vortex mixer, the tissue was removed, and the pH of the cecal contents in deionized water was determined with a Corning 240 pH meter with a general combination electrode. The other cecum was assayed for *C. jejuni*. The cecum was homogenized in 0.1% peptone (1:10, wt/vol) for 30 s with a Brinkmann homogenizer (model PT 10/35; Brinkmann Instruments Inc., Westbury, N.Y.). The cutting-blade assemblies were soaked in 95% ethanol and flame sterilized between each sample. The homogenate was serially (1:10) diluted in peptone. Ten 0.1-ml portions of the homogenate and duplicate 0.1-ml portions of the 1:10 dilutions were plated onto Campy BAP (brucella agar [Difco], 5% sheep blood, Campy selective supplement [SR 98; Oxoid U.S.A., Columbia, Md.]). Campy BAP plates were incubated microaerobically at 42°C for 48 h. Colonies typical of *C. jejuni* were counted, and random colonies were confirmed as *C. jejuni* by standard tests (17, 19). The lower limit of detection was 10 CFU of *C. jejuni* per g of cecum.

Reporting and analyses of data. Results are reported as percent colonization (%C), infection factor (IF), and protection factor (PF). %C was calculated for treatment groups by dividing the number of chicks with *C. jejuni* by the number of chicks tested and then multiplying the resulting number by 100. Pivnick et al. (25) introduced IF and PF to account for the level of infection per chick. The IF, which relates to the degree of infection, is the mean of the $\log_{10}$ values of the numbers of *C. jejuni* organisms per gram of cecal content per chick in a treatment group. When an entire treatment group had no detectable *C. jejuni*, the IF was arbitrarily assigned a value of <1 divided by the number of chicks in the group (35). The higher the number of *C. jejuni* per gram of cecum, the higher the IF. Efficacy of treatment is expressed by PF. PF is the ratio of IF for the untreated or control group of chicks to the IF for the treated group of chicks. High PF values denote high levels of protection. A PF of ≤1 signifies no protection.

IF and PF were calculated for each treatment in each trial by using the corresponding controls. Data from all trials for each treatment were grouped to provide a larger sample of the population. The combined results were averaged and presented with standard deviations. Control data presented in Tables 1, 2, 3, 5, and 6 are compilations of results from 21 trials where control chicks received *C. jejuni* on day 1; control results in Table 4 are compilations of data from 6 trials where *C. jejuni* was administered to control chicks on day 3 (prevention studies). These compiled numbers are considered an acceptable representation of the controls because no statistical differences ($P > 0.05$) were found between the controls of randomly selected treatment groups or between the mean and median.

The Conover multiple-comparison procedure and the Kruskal-Wallis nonparametric one-way rank sum analysis of variance were used to determine differences between treatment groups (8).

TABLE 1. Effect of growing CE cultures aerobically or anaerobically on inhibition of *C. jejuni* colonization

Treatment[a]	No. of trials	No. of chicks	%C	IF	PF
Control[b]	21	146	79.5 ± 14.3	3.5 ± 1.4	NA[c]
Aerobic growth of:					
CE 1	3	19	48.4 ± 45.1*[d]	3.1 ± 3.8	2.5 ± 1.6
CE 2	5	29	31.3 ± 32.4*	<1.3 ± 1.5*	>8.1 ± 8.5
CE 3	7	45	16.7 ± 23.9*	0.9 ± 1.0*	13.7 ± 11.9
Anaerobic growth of:					
CE 1	1	10	30.0*	1.2	1.7
CE 2	3	25	12.3 ± 2.2*	0.5 ± 0.2*	5.6 ± 0.9
CE 3	3	26	11.1 ± 11.1*	<0.5 ± 0.4*	>12.0 ± 12.2

[a] Chicks were given the indicated CE cultures, grown either aerobically or anaerobically, and then challenged with 10^8 *C. jejuni* organisms on day 1.
[b] Compilation of all control groups receiving *C. jejuni* alone on day 1.
[c] NA, not applicable. PF, by definition of the term, cannot be calculated for controls.
[d] An asterisk indicates a value significantly different ($P < 0.05$) from the control value.

RESULTS

A total of 44 treatments were evaluated. Each trial included the appropriate *C. jejuni*, CE isolates, and uninoculated control groups. All chicks that remained uninoculated or received CE cultures only were negative for the presence of *C. jejuni* at necropsy. Approximately 80% of the 146 chicks receiving only *C. jejuni* 108 on day 1 were colonized by the organism. The %C by *C. jejuni* per treatment group ranged from 50 to 100. The mean IF was 3.6 ± 1.4 (range, 1.6 to 7.8). The mean pH of the cecal contents of chicks was 5.8 ± 0.2 (range, 5.5 to 6.2). Twenty-three of 37 (ca. 62%) birds receiving *C. jejuni* on day 3 had colonized ceca. %C ranged from 33 to 100 per treatment group. Infection factors (mean IF, 2.2 ± 1.6) ranged from 1.0 to 5.3. The pH of the cecal content was 5.7 ± 0.1 (range, 5.5 to 5.9). Although colonization measurements for the day 3 controls were lower than those for day 1 *C. jejuni* controls, no significant difference ($P > 0.05$) was found between the two groups. The mean cecal pH of chicks receiving lactose with or without CE cultures (mean, 5.3 ± 0.2; range, 4.4 to 6.0) or FOS (mean, 5.2 ± 0.2; range, 4.6 to 5.9) was significantly different ($P < 0.05$) from that of the controls. Significant pH changes were not observed with mannose.

Challenge studies. Sixteen of 21 trials involved test groups receiving CE cultures, CE cultures plus carbohydrates, or only carbohydrates followed immediately by *C. jejuni* challenge on day 1 to assess the effect of CE isolates in direct competition with *C. jejuni*. To determine whether atmospheric conditions during growth affected the ability of CE isolates to inhibit *C. jejuni* colonization, 1-day-old chicks were fed CE cultures grown aerobically or anaerobically. Introduction of washed, aerobically grown cultures followed immediately by *C. jejuni* resulted in PFs of 2.5, 8.1, and 13.7 for CE 1, CE 2, and CE 3, respectively (Table 1). The percentage of chicks colonized by *C. jejuni* was lower with anaerobically grown CE 1 and CE 2 test groups, as was IF. Slightly lower PFs of 1.7 and 5.6 were obtained when anaerobically cultured CE 1 and CE 2 were tested for the ability to inhibit *C. jejuni*. However, the differences in protective effect between aerobically and anaerobically grown CE cultures were not significantly different ($P > 0.05$). CE 3 appeared to be more effective in reducing colonization of *C. jejuni* than CE 2, and the results were significantly different ($P < 0.05$) from those of CE 1 when cultures were grown either aerobically or anaerobically.

Washing the CE 3 cultures before use appeared to increase protection (aerobic PF, 13.7; anaerobic PF, 12.0). The PFs for unwashed CE 3 treatments grown aerobically and anaerobically were 9.8 and 4.1, respectively (data not shown). However, the difference between these treatments was not statistically significant.

Stavric et al. (35) found that the protective activity of 50 pure cultures increased when the cultures were grown, mixed together, and reincubated before administration to 1-day-old chicks. Oral feeding of cocultured CE 3 isolates (CE 3T) was not as effective in reducing *C. jejuni* colonization as CE 3 grown separately and then combined for inoculation. Four CE 3T trials, totalling 25 chicks, were conducted. The %C ranged from 0 to 57. The mean IF was 1.1 ± 0.9, and the mean PF was 8.9 ± 11.9 (data not shown). Standard deviations greater than the mean could result when no colonization (%C, 0) was observed in at least one trial for a given treatment group.

Administration of lactose, mannose, or FOS with or without CE isolates. Carbohydrates alone and carbohydrates in combination with CE cultures provided protection against *C. jejuni* when treatment and *C. jejuni* were given on day 1 (Table 2). All PF values were >1.0. The use of only lactose, mannose, or FOS to inhibit *Campylobacter* colonization resulted in PF values of 1.6, 2.5, and 1.7, respectively. Combining CE 2 or CE 3 with lactose enhanced the efficacy of lactose. The PF values were increased to 7.6 and 9.5 ($P < 0.05$) for CE 2 and CE 3, respectively. No significant difference in protection was observed when CE 3 was administered with mannose (PF, 3.0) or FOS (PF, 1.8).

Booster studies. CE cultures were administered on days 1 and 4 to determine whether an additional dose of CE isolates would enhance the protective effect of CE isolates in the intestinal tract of the chicks by maintaining high CE numbers. Since Schoeni and Doyle (28) found that *E. coli* 25 was dominant in the ceca of chicks 3 weeks after they received CE 3 or CE 1, the CE 3M test group (containing approximately equal numbers of *C. diversus* 22 and *K. pneumoniae* 23) was included among the booster treatments in the study presented here to examine whether a booster of nondominant CE (i.e., *C. diversus* 22 and *K. pneumoniae* 23) would increase protection. An additional dose of CE 3 was found to be more protective than boosting with nondominant CE (i.e., CE 3M). PF values ranging from 1.7 to >3.7 were obtained when booster treatments, with or without lactose, were used to reduce *C. jejuni* colonization (Table 3). CE 3, gavaged on days 1 and 4, was ranked, by Kruskal-Wallis analysis, the most effective booster treatment (PF, 3.4 ± 4.8; IF, 1.4 ± 1.3; %C, 31.6 ± 42.3). CE 3M protected at a lower level (PF, 1.7 ± 1.1; IF, 2.6 ± 2.1; %C, 39.3 ± 27.8). No significant difference ($P > 0.05$) was detected between administering a single dose of CE 2 or CE 3 on day 1 (Table 1) and the CE 2 or CE 3 booster treatments.

TABLE 2. Challenge studies: the effect of administering carbohydrate, CE culture, and *C. jejuni* on day 1

Treatment[a]	No. of trials	No. of chicks	%C	IF	PF	pH[b]
Control[c]	21	146	79.5 ± 14.3	3.5 ± 1.4	NA[d]	5.8 ± 0.2
Lactose with:						
No CE[e]	5	32	61.7 ± 29.8	3.3 ± 1.6	1.6 ± 0.7	5.5 ± 0.4*[f]
CE 1	3	18	66.7 ± 16.7	3.5 ± 1.3	1.2 ± 0.4	5.4 ± 0.4*
CE 2	5	29	42.7 ± 33.6*	<1.6 ± 1.3*	>7.6 ± 10.3	5.5 ± 0.3*
CE 3	5	29	26.2 ± 22.6*	<1.0 ± 0.7*	>9.5 ± 9.8	5.4 ± 0.1*
Mannose with:						
No CE	4	34	42.6 ± 13.1*	2.1 ± 1.0	2.5 ± 1.3	5.6 ± 0.2
CE 3	5	51	30.4 ± 9.9*	1.4 ± 0.3	3.0 ± 1.0	5.8 ± 0.3
FOS with:						
No CE	4	31	52.1 ± 23.9	2.7 ± 0.9	1.7 ± 0.4	5.2 ± 0.3*
CE 3	3	27	51.9 ± 3.2	2.3 ± 0.2	1.8 ± 0.7	5.3 ± 0.4*

[a] Chicks were given the indicated carbohydrate (2.5%), ad libitum beginning day 1, with or without CE cultures and immediately challenged with 10^8 *C. jejuni* organisms.
[b] Mean pH of cecal homogenates.
[c] See Table 1, footnote *b*.
[d] See Table 1, footnote *c*.
[e] No CE, carbohydrate administered alone.
[f] See Table 1, footnote *d*.

Prevention studies. The ability of CE 3 and carbohydrates to inhibit subsequent colonization of *C. jejuni* was examined by administering CE cultures on day 1 and then *C. jejuni* on day 3 (Table 4). As a preventative treatment, CE 3 with no carbohydrates provided a PF of >2.7 ± 1.0. *Campylobacter* colonization was reduced from 62% (controls) to approximately 20% of the CE 3-treated chicks. Protection by CE 3 was increased when 2.5% mannose or 2.5% FOS was provided. *C. jejuni* was not detected in the 25 chicks that received mannose and CE 3 (%C, 0; PF, >13.2). *Campylobacter* colonization was reduced to approximately 15% by administering FOS and CE 3. When 2.5% lactose, mannose, or FOS solutions were supplied as the sole protective measure, the highest PF (>14.3 ± 15.8) was obtained with FOS. Approximately 8% of the chicks receiving FOS beginning on day 1 were colonized by *C. jejuni* administered on day 3. Since %C is used in the Kruskal-Wallis rank analysis, the FOS treatment ranked second to CE 3 plus mannose (%C, 0.0; PF, >13.2) in protection efficacy. However, no statistical difference between these groups was found by the Conover multiple-comparison procedure.

Elimination studies. CE cultures with or without carbohydrate treatments successfully reduced established *C. jejuni* infections. Administration on day 3 of CE 2 or CE 3 to birds which had received 10^8 *C. jejuni* on day 1 resulted in PF values of >14.9 and >14.3, respectively (Table 5). When 2.5% lactose was provided on day 1 and then CE 3 treatment was provided on day 3, the PF was increased to >20.1 and the %C by *C. jejuni* was reduced to 4.8 ± 8.3. The mean cecal content pH of this group (5.2 ± 0.1) was approximately 0.5 pH unit lower than the cecal pH of the control birds. The treatment with lactose (day 1) and CE 3 (day 3) was found to be significantly more effective ($P < 0.05$) than all other elimination and challenge treatments (Table 2) in terms of %C, IF, and PF. When lactose was withheld until day 3, the PFs for elimination treatments with or without CE were reduced more than 10-fold compared with treatments providing lactose beginning on day 1. Similarly, treatment with mannose or FOS (day 1 or 3) with or without CE 3 eliminated *C. jejuni* at much lower levels than the treatment with lactose (day 1) and CE (day 3; data not shown). PFs for these treatment groups ranged from 1.4 to 2.5, and the %C ranged from 38.4 to 55.6.

Evaluation by rank. The IF and %C values obtained for all 44 treatments evaluated in this study were compared and ranked for effectiveness by using the Kruskal-Wallis rank sum analysis of variance. All treatment and control groups can be compared by %C and IF, while PFs, by definition, are not calculated for controls. The ranks established by looking at %C and IF were averaged to determine the best treatments for reducing *C. jejuni* colonization in chicks (Table 6). The best

TABLE 3. Booster studies: effect of administering *C. jejuni* on day 1 and CE treatment on days 1 and 4

Treatment[a] (day 1; day 4)	No. of trials	No. of chicks	%C	IF	PF	pH[b]
Control[c]	21	146	79.5 ± 14.3	3.5 ± 1.4	NA[d]	5.8 ± 0.2
CE 2; CE 2	4	25	45.2 ± 35.0*[e]	2.0 ± 1.5*	2.4 ± 2.1	5.7 ± 0.2
CE 3; CE 3	4	25	31.6 ± 17.4*	1.4 ± 1.3*	3.4 ± 2.1	5.8 ± 0.2
CE 3; CE 3M	4	25	39.3 ± 27.8*	2.6 ± 2.1	1.7 ± 1.1	5.8 ± 0.2
Lactose with:						
CE 2; CE 2	4	25	39.9 ± 20.9*	2.1 ± 1.5	2.2 ± 1.9	5.2 ± 0.3*
CE 3; CE 3	4	26	40.5 ± 42.3*	<2.3 ± 2.3*	>3.7 ± 4.8	5.3 ± 0.3*

[a] Chicks were given the indicated CE treatment on days 1 and 4. Lactose (2.5%) was provided ad libitum beginning on day 1, and 10^8 *Campylobacter* organisms were administered on day 1.
[b] See Table 2, footnote *b*.
[c] See Table 1, footnote *b*.
[d] See Table 1, footnote *c*.
[e] See Table 1, footnote *d*.

TABLE 4. Prevention studies: ability of CE cultures and carbohydrate treatments administered on day 1 to prevent colonization by *C. jejuni* administered on day 3

Treatment[a]	No. of trials	No. of chicks	%C	IF	PF	pH[b]
Control[c]	6	37	61.6 ± 23.2	2.2 ± 1.6	NA[d]	5.7 ± 0.1
CE 3	5	27	20.2 ± 13.3*[e]	<1.1 ± 0.7	>2.7 ± 1.0	5.8 ± 0.1
Lactose with:						
No CE[f]	4	25	31.1 ± 5.5	1.5 ± 1.1	2.0 ± 0.5	5.1 ± 0.6*
CE 3	4	27	40.8 ± 24.9	2.2 ± 1.8	1.4 ± 0.3	5.4 ± 0.1*
Mannose with:						
No CE	4	25	12.9 ± 10.6*	<0.3 ± 0.1*	>5.0 ± 2.8	5.7 ± 0.1
CE 3	3	25	0.0 ± 0.0*	<0.2 ± 0.1*	>13.2 ± 5.2	5.8 ± 0.1
FOS with:						
No CE	4	25	7.7 ± 9.0*	<0.2 ± 0.1*	>14.3 ± 15.8	5.4 ± 0.2*
CE 3	4	25	14.9 ± 20.2*	<0.9 ± 1.3*	>6.4 ± 3.7	5.3 ± 0.6*

[a] Chicks were given the indicated CE treatment on day 1 and 10^8 *C. jejuni* organisms on day 3. Carbohydrate (2.5%) was provided ad libitum beginning on day 1.
[b] See Table 2, footnote *b*.
[c] See Table 1, footnote *b*.
[d] See Table 1, footnote *c*.
[e] See Table 1, footnote *d*.
[f] See Table 2, footnote *e*.

treatment against each infection scheme tends to rank substantially higher than the second-best treatment.

DISCUSSION

Limited research using defined CE cultures to inhibit *C. jejuni* colonization in poultry has been conducted. This study confirms that CE cultures defined by Schoeni and Doyle (28) by niche and ability to produce anti-*C. jejuni* metabolites can significantly reduce ($P < 0.05$) colonization of chicks by *C. jejuni*. The effects of atmospheric conditions on growth, dietary carbohydrates, and variation of treatment schedule on *C. jejuni* colonization were explored. All treatments in this study reduced the %C of *C. jejuni*. CE 2 and CE 3 also reduced the level of infection (IF). CE 1 did not significantly ($P > 0.05$) reduce IF and was removed from testing after three trials. In prevention trials, CE 3 reduced *C. jejuni* colonization to 20.2%, a value comparable to 15.1% found in studies by Schoeni and Doyle (28), where chicks received the same CE isolates on day 1 and *C. jejuni* on day 8. Some of the differences may be due to the age of chicks at the times of treatment, challenge, and necropsy. CE 2 was not included in prevention trials because CE 3 appeared to provide more protection in schemes tested earlier in the study.

It is believed that anaerobic growth of CE bacteria, prior to treatment, is important to maintain their effectiveness (2). No statistical difference ($P > 0.05$) was found between protection provided by aerobically and anaerobically grown CE cultures in this study. Combinations of aerobically grown *C. diversus* 22, *K. pneumoniae* 23, and *E. coli* 25 were effective. In other defined CE culture studies, Schoeni and Doyle (28) used aerobically grown bacteria, while Aho et al. (1) combined microaerophilic K-bacteria and the facultatively anaerobic bacteria of Broilact to exclude *C. jejuni*. Facultative anaerobes such as the CE 3 cultures or the Broilact organisms appear to be effective in reducing *Campylobacter* colonization, and anaerobic growth conditions are unnecessary. This is a major advantage for cultivation, storage, handling, and commercial applications.

TABLE 5. Elimination studies: ability of CE treatment administered on day 3, with or without carbohydrates, to reduce colonization by *C. jejuni* administered on day 1

Treatment[a]	No. of trials	No. of chicks	%C	IF	PF	pH[b]
Control[c]	21	146	79.5 ± 14.3	3.5 ± 1.4	NA[d]	5.8 ± 0.2
CE 2	3	25	38.9 ± 41.9*[e]	<2.2 ± 2.9*	>14.9 ± 21.7	5.7 ± 0.3
CE 3	3	25	20.4 ± 26.3*	<1.2 ± 1.7*	>14.3 ± 16.6*	5.8 ± 0.2
Lactose (day 1) with:						
No CE[f]	5	32	61.7 ± 29.8	3.3 ± 1.6	1.6 ± 0.7	5.5 ± 0.4*
CE 2	3	25	18.9 ± 20.1*	1.0 ± 0.8*	11.4 ± 15.3	5.0 ± 0.4*
CE 3	3	25	4.8 ± 8.3*	<0.4 ± 0.5*	>20.1 ± 18.5*[g]	5.2 ± 0.1*
Lactose (day 3) with:						
No CE	3	27	74.1 ± 16.9	2.2 ± 0.5	1.3 ± 0.9	5.2 ± 0.4*
CE 3	3	27	59.3 ± 28.0	1.9 ± 0.8	1.4 ± 0.0	5.2 ± 0.1*

[a] Chicks were given 10^8 *C. jejuni* organisms on day 1. Lactose (2.5%) was provided ad libitum beginning on day 1 or 3, as indicated. CE treatment was administered on day 3.
[b] See Table 2, footnote *b*.
[c] See Table 1, footnote *b*.
[d] See Table 1, footnote *c*.
[e] See Table 1, footnote *d*.
[f] See Table 2, footnote *e*.
[g] Value significantly different ($P < 0.05$) from those for all other elimination treatments, including mannose and FOS, with or without CE (data not shown).

TABLE 6. Best-ranked treatment groups for each treatment scheme[a]

Scheme	Treatment	Rank avg[b]
Control[c]	*C. jejuni* (day 1)	42.5
	C. jejuni (day 3)	37.5
Challenge[d]	CE 3	8
	CE 3 + lactose	17.5
Booster[e]	CE 3	18
	CE 3 + lactose	26.5
Prevention[f]	CE 3 + mannose	1
	FOS	2.5
Elimination[g]	CE 3 + lactose (day 1)	2.5
	CE 2 + lactose (day 1)	9

[a] Treatments were ranked by using the Kruskal-Wallis nonparametric one-way rank sum analysis of variance. Lower rank indicates lower IF and %C but is not related to PF.

[b] Average rank was calculated by adding the IF and %C ranks for each treatment and dividing by 2. PF rank was not included because PF cannot be calculated for the control groups.

[c] Controls received only *C. jejuni* on the designated day.

[d] Challenge indicates treatment plus *C. jejuni* administered on day 1.

[e] CE was inoculated on days 1 and 4, *C. jejuni* was administered on day 1, and carbohydrate was provided ad libitum beginning on day 1.

[f] CE was inoculated on day 1, carbohydrate was provided ad libitum beginning on day 1, and *C. jejuni* was administered on day 3.

[g] *C. jejuni* was inoculated on day 1, lactose was provided ad libitum beginning on day 1, and CE was inoculated on day 3.

Carbohydrates may enhance CE protection by inhibiting adherence, decreasing cecal pH, or influencing bacterial populations of the intestine (7, 9, 14, 20, 21). In this study, select carbohydrate treatments increased protection by CE cultures in prevention and elimination trials. Mannose with CE 3 was found significantly more protective ($P < 0.05$) than CE 3 alone (prevention studies; Table 4). The %C from CE 3 trials was 20.2 (PF, >2.7). This value was decreased to 0.0% (PF, >13.2) when mannose was provided. In this study, mannose reduced *C. jejuni* colonization in vivo. Other researchers found that mannose inhibited the adherence of *Campylobacter* spp. to INT 407 cells in vitro (7, 20). The CE isolates used in this study were originally selected on the basis of their ability to occupy the same niche as *C. jejuni* in the mucus layer of cecal crypts of chickens (28). These isolates could have filled the niche normally occupied by *C. jejuni*, while mannose prevented the adherence of incoming *C. jejuni* to remaining sites. FOS, used with or without CE cultures, successfully prevented *Campylobacter* colonization. The PF (>14.3) was greater than that of CE 3 with mannose (>13.2), but 7.7% of the birds in the FOS treatment group were colonized by *C. jejuni*. *C. jejuni* was not detected in the birds receiving mannose with CE 3. Bailey et al. (3) showed that in chicks challenged with 10^6 salmonellae, colonization was reduced ca. 30% when 0.75% FOS and partially protective CE cultures were provided. With FOS alone, a 12% reduction in *Salmonella* colonization was observed. FOS has been shown to influence intestinal bacterial populations by enhancing the growth of *Bifidobacterium* (14) and *Lactobacillus* (21) spp. Since both FOS and CE 3 plus mannose efficiently reduce *C. jejuni* colonization in prevention schemes, manipulation of intestinal flora by carbohydrate stimulation or addition of CE cultures may play a role in CE protection.

Administering lactose ad libitum beginning on day 1 profoundly affected the efficacy of CE 3 when used against established infections. This treatment was found to be significantly more protective ($P < 0.05$) than all other elimination treatments. Interestingly, lactose provided beginning on day 3 combined with CE 3 was not significantly protective. The protective effect of CE 3 appears to be enhanced when introduced into an environment with a lower pH. Corrier et al. (9) showed that when lactose was administered, the bacteriostatic action of volatile fatty acids was increased as the cecal pH decreased. In the present study, lactose significantly ($P < 0.05$) reduced cecal pH, which might have provided the added protection. Carbohydrates were less effective in reducing *C. jejuni* colonization in challenge situations. Treatment with CE 3 alone was more effective than when combined with a carbohydrate in both challenge and booster treatment schemes. Booster treatments (CE cultures administered on days 1 and 4) were less effective than challenge treatments (CE cultures administered on day 1 only), indicating that early establishment of CE cultures may be beneficial.

CE cultures protected against colonization by *C. jejuni* whether chicks were challenged on day 1 or 3 and reduced established *C. jejuni* populations. Treatments ranking 1, 2, and 3 were prevention and elimination treatments. Contrary to other reports, anaerobically grown CE isolates were not more protective than aerobically grown CE isolates. Protection by CE cultures grown and stored aerobically was maintained throughout the course of this study. This suggests that CE cultures can be used as a prophylactic or therapeutic treatment.

Defined CE 3 cultures used in this study appear to be most effective when used with carbohydrates to prevent (PF, >13.2) or eliminate (PF, >20.1) *C. jejuni* infection. However, carbohydrates alone were also able to reduce *Campylobacter* colonization. The PF for FOS alone, in prevention studies, was >14.3 (%C, 7.7). No statistical difference was found between the best-ranked treatments from each administration scheme. Larger test groups may be required to obtain such a difference. Although CE bacteria with and without carbohydrates can substantially reduce colonization of chicks by *C. jejuni*, additional research is needed to further improve the efficacy of these bacteria and to understand their role in CE. Parameters such as age of chicks, mixture composition, inoculum level, duration of protection, effectiveness toward other *C. jejuni*, and prophylactic and therapeutic uses of CE cultures need to be evaluated to fully assess the usefulness of these defined protective bacteria.

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