Journal of Food Protection, Vol. 73, No. 11, 2010, Pages 2093–2098

Research Note

Adherence, Invasion, Toxigenic, and Chemotactic Properties of Mexican *Campylobacter* Strains

ISMAEL MALAGÓN, SANTOS GARCÍA,* AND NORMA HEREDIA

Departamento de Microbiología e Inmunología, Facultad de Ciencias Biológicas, Universidad Autónoma de Nuevo León, Apdo. Postal 124-F, Ciudad Universitaria, San Nicolás de los Garza, N.L. 66451, México

MS 10-098: Received 8 March 2010/Accepted 15 July 2010

ABSTRACT

To determine the virulence factors of Mexican wild-type strains of *Campylobacter jejuni* and *Campylobacter coli,* 31 wild-type strains were isolated from food and from humans. The production of cytolethal distending toxin and the adherence and invasion capabilities of these strains were assayed in Vero cells. Hard agar plugs with repellents and attractants were used to examine chemotaxis. Mueller-Hinton agar with supplements was used for motility analysis and to measure hemolytic activity. Nine strains of *C. jejuni* and eight strains of *C. coli* exhibited motility, most within a diameter of 2 to 13 mm. Most of the strains reacted to the repellent compounds analyzed, and α- and β-like hemolysis and cytotoxicity in Vero cells were observed for all strains. Isolates adhered to and invaded Vero cells to various degrees. Although strains of *C. jejuni* exhibited stronger adherence but less invasion compared with strains of *C. coli,* the difference was not significant ($P > 0.05$). The strains of *C. jejuni* and *C. coli* isolated from food and from patients in Mexico could have major impacts on public health.

Campylobacter spp. is one of the most commonly reported bacterial causes of foodborne gastroenteritis in humans worldwide *(7).* The thermotolerant species *Campylobacter jejuni* and *Campylobacter coli* are frequent causes of acute bacterial enteritis due to consumption of contaminated food, primarily poultry *(24, 28).* The mechanism of pathogenesis is not well defined but involves factors such as motility, chemotaxis, colonization, adhesion, invasion, iron acquisition, and the formation of toxins *(6).* Motility is critical for many aspects of *Campylobacter* pathogenesis, as has been determined through in vitro studies of cell invasion and intestinal colonization *(24, 29).* Chemotaxis is probably important for the commensal and pathogenic aspects of *Campylobacter* behavior. The microorganism exhibits chemotactic motility toward amino acids that are found at high levels in the chick gastrointestinal tract and toward components of mucus *(29).*

C. jejuni strains associated with dysentery-like illness are more invasive and cytotoxic than other *Campylobacter* strains *(17).* The adherence to and invasion of host mucosal surfaces has been proposed as essential to *C. jejuni* pathogenesis *(20).* After passage through the stomach, these organisms colonize the ileum and colon, where they can interfere with the normal secretory or absorptive capacity of the intestine *(11).* The initial interaction between *Campylobacter* and its host could lead to the colonization of the mucous barrier and specific attachment to the mucosal cell surface. Many cytotoxic activities of *Campylobacter* also have been reported *(20),* but only the cytolethal distending toxin (CDT) has been defined.

Production of CDT by *Campylobacter* spp. was originally described by Johnson and Lior *(13, 14),* and it appears that CDT is produced by only some strains of *Campylobacter (21).* HeLa, Vero, and Chinese hamster ovary (CHO) cells are affected by exposure to CDT in culture supernatants of bacteria, leading to cell distention and disintegration *(14).* Experimental results suggest that this toxin also may assist in the spread of these bacteria in infected animals, particularly to the spleen *(21).* Conflicting results regarding the hemolytic activity of *Campylobacter* strains have been reported; hemolysin production has been related to virulence, this activity seems to depend on culture conditions *(19, 26).* Variations may exist in the virulence factors of different strains of *Campylobacter (1).* Phenotypic variations among variants of the same original *Campylobacter* strain also can arise after long-term in vitro passage or in response to varying culture conditions *(8).* The production of virulence factors also differs with the biotype, genotype, and host of origin *(20).* A high incidence of *Campylobacter* infection has been found in Mexican children *(2).* Because little information is available on the virulence factors of *Campylobacter* strains isolated in Mexico, in the present study the adherence, invasion, and toxigenic and chemotactic properties of Mexican *Campylobacter* strains were investigated. These wild-type strains of *C. jejuni* and *C. coli* were isolated from food, live animals, and humans in Mexico.

* Author for correspondence. Tel and Fax: +52 (818) 3763044; E-mail: santos@microbiosymas.com.

MATERIALS AND METHODS

Bacterial strains. Thirty-one native *Campylobacter* strains (16 *C. coli* and 15 *C. jejuni* strains) were analyzed. Six *C. jejuni* strains were isolated from human samples (kindly provided by Dr. Guillermo Ruiz-Palacios, National Institute of Medical Sciences and Nutrition, Mexico City): four from diarrheic persons and two from healthy persons. Five isolates (four *C. coli* and one *C. jejuni*) were obtained from poultry, and one was obtained from a pig. We isolated 18 strains (11 *C. coli* and 7 *C. jejuni*) from raw chicken meat. All animal and food strains were collected in Monterrey, Mexico, and all had been identified by biochemical assays and PCR following the method of Cloak and Fratamico *(3)*. *C. jejuni* NADC 5653 (Dr. Irene Wesley, U.S. Department of Agriculture, Agricultural Research Service, National Animal Disease Center, Ames, IA) was used as a reference strain.

All *Campylobacter* strains were stored at −80°C in 20% glycerol brain heart infusion broth (Difco, BD, Sparks, MD) supplemented with 0.6% yeast extract. Activated cultures were obtained from fresh medium and incubated under microaerobic conditions (42°C in a chamber with 10% CO_2) *(10)*. When necessary during the experiments, counts of viable bacteria were determined on Mueller-Hinton (MH) agar (Difco, BD) supplemented with 5% (vol/vol) defibrinated horse blood *(5)* and incubated at 42°C under microaerobic conditions.

Motility assay. The motility assays were performed according to the method described by Szymanski et al. *(25)*, with modifications. Plates were filled with MH soft agar, containing MH broth plus 0.4% agar and 0.5 μl/ml 2,3,5-triphenyltetrazolium chloride (CTT; Sigma Aldrich, Toluca, Mexico). Aliquots (70 μl) of bacterial culture (1.0×10^7 CFU) were inoculated into a hole (7 mm in diameter) made in the center of the plate. Plates were incubated under microaerobic conditions (10% CO_2, 42°C for 24 h). The diameter of the red area around the hole (the consequence of CTT transformation to formazan) was measured.

Chemotaxis assay. The effects of L-aspartic acid, L-cysteine hydrochloride, sodium citrate, L-serine, taurocholic acid, chenodeoxycholic acid, and deoxycholic acid on chemotaxis of native *Campylobacter* strains were determined using the hard agar plug (HAP) assay described by Hugdahl et al. *(12)*. HAPs were prepared as follows. The test compound was dissolved in phosphate-buffered saline (PBS) at twice the required concentration (0.1 M) and filter sterilized (0.45-μm-pore-size filter; Acrodisc, Gelman Sciences, Ann Arbor, MI), and the pH was adjusted to 7.0 with HCl or NaOH. HAPs were prepared by adding 10 ml of compound solution to 10 ml of dissolved agar (0.4% Bacto agar [Difco, BD] in PBS) at 70°C. The mixture was then poured into petri dishes and allowed to solidify. Cylindrical plugs (7 mm in diameter) were made using an inverted culture tube (6 by 50 mm).

The chemotaxis assay was performed as follows. A bacterial suspension (10 ml) adjusted to 9×10^8 CFU/ml (in PBS at pH 7.0) was mixed with 10 ml of PBS with 0.8% agar at 50°C. The mixture was then poured into petri plates. After solidification, 5 HAPs containing test compounds were placed in the plate containing the inoculated soft agar in a circular arrangement. Plates were incubated under microaerobic conditions (42°C for 4 h). The presence of zones of bacterial accumulation around a plug was considered a positive result for chemotaxis (+, strain was attractant); bacterial clearing in the region around the HAP was a negative result for chemotaxis (−, strain was repellent), and when neither bacterial accumulation nor clearing around a HAP was observed, the result was recorded as no chemotaxis (0). Monosodium L-glutamate was used as a positive control, and cholic acid was used as a negative control.

Hemolytic-like activity assay. For analysis of the hemolytic-like activity (HLA), the method described by Misawa et al. *(19)* was followed with some changes. All the strains were initially cultured on blood agar base no. 2 plates (Oxoid, Basingstoke, UK) containing 5% horse blood and incubated microaerobically (37°C for 48 h). A loopful of bacteria of each strain was inoculated onto MH agar with 5% horse blood at pH 6.5 for α-hemolysis or at pH 7.5 for β-hemolysis and incubated at 37°C for 48 h (α-hemolysis) or 7 days (β-hemolysis) under a high-CO_2 atmosphere (33%). After incubation, strains that produced a red-pink zone around the colony were judged to have α-HLA, and those with a transparent zone were considered to have β-HLA. *Streptococcus sanguinis* ATCC 10556 (α-HLA) and *Streptococcus pyogenes* ATCC 19615 (β-HLA) were used as controls.

Adherence and invasion assay. For mammalian cell culture, a stock culture of Vero ATCC CCL-81 cells was grown (5% CO_2 at 37°C) in monolayers in Dulbecco's minimal essential medium (DMEM; Invitrogen, Carlsbad, CA) with 1% (vol/vol) 200 mM glutamine (Invitrogen) and 3% (vol/vol) fetal bovine serum (FBS; Invitrogen). For experimental assays, cells were harvested by trypsinization and seeded into 96-well tissue culture trays at 2×10^5 cells per well and incubated for 24 h under 5% CO_2 at 37°C.

To evaluate adherence and invasion, the method of Elvers and Park *(5)* was followed with modifications. Bacterial suspensions were prepared by centrifugation of cultures (6,000 × *g* for 10 min at 4°C). The pellets were suspended in DMEM with 1% FBS at a density of approximately 1×10^8 CFU/ml. An aliquot (100 μl) of this suspension was inoculated into duplicate wells containing semiconfluent monolayers of Vero cells and 150 μl of DMEM. Infected monolayers were incubated (3 h, 5% CO_2, 37°C) to allow bacteria to attach to Vero cells. After that, monolayers were washed five times in 500 μl of DMEM with 1% FBS and incubated for another 3 h under the same conditions to allow bacterial invasion of Vero cells.

To measure intracellular bacteria, 150 μl of medium containing 250 μg of gentamicin (Sigma Aldrich) was added to some of the wells to kill all the extracellular bacteria attached to Vero cells. Medium without antibiotic was added to other wells to measure the total amount of bacteria. After 3 h of incubation, monolayers were washed with 200 μl of Hanks balanced saline solution (Sigma Aldrich) and lysed with 100 μl of 0.1% Triton X-100. The suspensions were diluted, and the viable bacterial population was enumerated.

CDT activity assay. Toxin activity was determined using the method described by Coote and Arain *(4)* with some modifications. To prepare *Campylobacter* filtrates, an aliquot of each strain was inoculated onto MH agar and incubated at 42°C for 48 h. Cells were then suspended and adjusted to 1×10^9 CFU/ml in PBS, and 1-ml aliquots were centrifuged (1,500 × *g* for 20 min at 4°C). Cell pellets were then resuspended in 1 ml of DMEM with 0.15% polymyxin B (Sigma Aldrich) to promote the release of cell-associated material. After incubation (37°C for 30 min), 1 ml of treated cells was centrifuged (2,500 × *g* for 20 min), and the supernatant was filtered (0.45-μm-pore-size filter) and used for the following assays.

To determine CDT activity, the MTT (2-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide) dye reduction assay was

TABLE 1. *Motility and chemotactic activity of* C. jejuni *and* C. coli *native strains*

Species	Strain	Strain origin	Motility diam (mm)[a]	Chemotactic compounds[b]								
				Attractants					Repellents			
				Asp	Cit	Cys	Glu	Ser	Che	Cho	Deo	Tau
C. jejuni	NCDC 5653	Pork	3.1 ± 0.01	0	0	0	0	0	−	−	−	−
	009 B	Pork	<1	0	0	0	0	0	−	−	−	−
	27 PFF	Raw chicken	12.8 ± 0.2	0	0	0	0	0	−	0	−	−
	31 PVB	Raw chicken	2.2 ± 0.2	0	0	0	+	0	−	−	−	−
	034 B	Raw chicken	3.7 ± 0.4	0	0	0	0	0	−	0	−	−
	35 PVB	Raw chicken	10 ± 2.5	0	0	0	+	0	−	−	−	−
	37	Raw chicken	32.2 ± 0.8	0	−	0	0	0	−	−	−	−
	50 sp	Healthy person	<1	0	0	0	+	0	−	0	−	−
	57 sp	Healthy person	<1	0	0	0	0	0	−	0	−	−
	060 B	Raw chicken	<1	0	0	0	0	0	−	0	−	−
	101	Raw chicken	8.3 ± 0.1	0	0	0	0	0	−	−	−	−
	102	Live hen	<1	0	0	0	0	0	−	−	−	−
	173 ip	Diarrheic person	10.6 ± 0.0	0	0	0	0	0	−	−	−	−
	180 ip	Diarrheic person	<1	0	0	0	0	0	−	0	−	−
	193 ip	Diarrheic person	<1	0	0	0	0	0	−	−	−	−
	238 ip	Diarrheic person	5.6 ± 0.5	0	0	0	+	0	−	−	−	−
C. coli	Cc 1	Live chicken	7.4 ± 0.3	0	0	0	+	0	−	−	−	−
	Cc 30 PFB	Raw chicken	<1	0	0	0	0	0	−	0	−	−
	Cc 48	Live chicken	12.4 ± 0.3	0	0	0	+	0	−	−	−	−
	Cc BCO48	Raw chicken	13.7 ± 0.1	0	0	0	+	0	−	0	−	−
	Cc 54B	Raw chicken	<1	0	0	0	0	0	−	−	−	−
	Cc 60	Live pig	12.7 ± 0.1	0	0	0	+	0	−	−	−	−
	Cc 61 PVB	Raw chicken	<1	+	+	0	+	0	−	−	−	−
	Cc 62F	Raw chicken	<1	0	0	0	0	0	−	−	−	−
	Cc 66F	Raw chicken	<1	0	0	0	0	0	−	0	−	−
	Cc 67 PVB	Raw chicken	2 ± 0.6	0	0	0	0	0	−	−	−	−
	Cc 68 PVB	Raw chicken	<1	+	0	0	+	0	−	−	−	−
	Cc 74F	Raw chicken	<1	0	0	0	+	0	−	−	−	−
	Cc 81	Live turkey	13 ± 0.2	0	0	0	0	0	−	−	−	−
	Cc 90	Raw chicken	4.1 ± 0.1	0	0	0	−	0	−	−	−	−
	Cc 100	Raw chicken	2.9 ± 0.1	0	0	0	0	0	−	0	−	−
	Cc M424	Beef sausage	<1	0	0	0	+	0	−	−	−	−

[a] Values are mean ± standard deviation.

[b] Chemotactic compounds: Asp, L-aspartic acid; Cit, sodium citrate; Cys, L-cysteine; Glu, L-glutamate; Ser, L-serine; Che, chenodeoxycholic acid; Cho, cholic acid; Deo, deoxycholic acid; Tau, taurocholic acid. +, chemotaxis positive (attractant); −, chemotaxis negative (repellent); 0, no chemotaxis observed.

used *(4)*. Vero cell monolayers were detached by trypsinization. Cells were harvested and washed in fresh medium and seeded into 96-well tissue culture trays at 2×10^5 cells per well and incubated (4 h, 5% CO_2, 37°C) to allow the cells to adhere. Then, 150 μl of various dilutions of bacterial cell filtrates (twofold serial dilutions from undiluted to 1:128 in DMEM) were added to the wells and incubated for 24 h. After incubation, 20 μl of 0.5% (wt/vol) MTT (Sigma Aldrich) diluted in PBS was added, and the system was incubated for an additional 4 h.

The overlying medium was removed, and the formazan product (from cleavage of MTT by cellular mitochondria) was solubilized by addition of 100 ml of 0.04 N HCl in dimethylsulfoxide (Sigma Aldrich). Absorbance was measured at 620 nm (microplate reader 550, Bio-Rad, Hercules, CA). *Escherichia coli* verotoxin (Sigma Aldrich) was used as a positive control, and DMEM was used as a negative control. Results were expressed as the percentage of cell death, using the following formula:

$$\text{Percentage of cell death} = 1 - (\text{OD}_{620\,\text{nm}} \text{ of test well} / \text{OD}_{620\,\text{nm}} \text{ of negative control well}) \times 100$$

Statistical analysis. All experiments were conducted in at least triplicate with two repetitions each. Results were analyzed using SPSS 15.0 (SPSS Inc., Chicago, IL) and NCSS 07.1.10 software (NCSS, Kaysville, UT).

RESULTS

Motility. Among the 31 wild-type *Campylobacter* strains, 9 of 15 *C. jejuni* and 8 of 16 *C. coli* strains exhibited motility (Table 1). The extent of motility differed greatly among strains, but most produced a motility zone of 2 to 13 mm. Analysis revealed significant differences ($P < 0.01$) in motility among strains. For

example, *C. jejuni* strain 37 exhibited greater motility (32 mm) than did the other *C. jejuni* strains. For *C. coli,* strains Cc 81 and Cc BCO48 had the greatest motility (13 mm) compared with other strains.

Chemotaxis. Most compounds (aspartic acid, cysteine, serine, and sodium citrate) reported to be attractants for *Campylobacter* did not have a positive attractant effect on the native strains tested (Table 1). However, L-glutamate had an attractant effect in 12 of the 31 strains tested. Taurocholic acid, deoxycholic acid, and chenodeoxycholic acid, reported to be repellents for *C. jejuni (12),* exerted a repellent effect on all native strains of both species. However, cholic acid acted as a repellent in 22 strains of both species and had no effect on the others.

HLA. All strains of *C. jejuni* and *C. coli* analyzed produced the red-pink zone in the assay for α-HLA and the transparent zone in the assay for β-HLA, indicating that they were all positive for hemolytic activity.

Adherence and invasion. The numbers of attached and invasive bacterial cells were determined in the same assay. Results indicated that 0.12 to 6.3% of *C. jejuni* cells (mean, 1.69%), and 0.19 to 3.8% of *C. coli* cells (mean, 0.836%) attached to Vero cells. Invasion by strains of *C. jejuni* and *C. coli* occurred at a mean of 0.006 and 0.0098% of cells, respectively. No significant difference ($P > 0.05$) in adherence and invasion capability was noted between *C. jejuni* and *C. coli* (Table 2).

CDT activity. Cytotoxic effects were observed when Vero cells were exposed to various dilutions of *C. coli* and *C. jejuni* cell filtrates. Bacterial extracts diluted 1:2 and 1:4 produced high mortality of Vero cells; however, at the 1:8 dilution, some extracts caused no detectable mortality of Vero cells (Table 2).

DISCUSSION

Phenotypes of members of the genus *Campylobacter* include both directional swimming and tumbling motility, and these behaviors have been linked to pathogenesis *(6).* Both motility and the presence of an intact flagellum contribute to the ability of *Campylobacter* to colonize the intestinal epithelium in vivo and to invade an epithelial cell monolayer in vitro *(9).* In the present study, 17 of the 31 wild-type *Campylobacter* strains exhibited motility. However, motility of this bacterium is known to be phase variable, although the mechanism of such variation remains unknown *(15).* Reversible expression of flagella may occur in response to the environment. The formation of motile or nonmotile cells may confer flexibility upon bacteria for adapting to changing environmental conditions when flagellar expression and motility are desirable or undesirable *(15).*

Attraction to intestinal mucosal tissue has been demonstrated for *Vibrio cholerae, Salmonella* Typhimurium, and *E. coli,* and in these studies the nonchemotactic mutants were not attracted to mucus, suggesting that bacterial chemotaxis may be an important factor in the colonization of the intestinal tract *(12).* In our study, all the strains tested had similar chemotactic properties. In most cases, compounds reported to be attractants (L-glutamate, L-serine, L-aspartic acid, sodium citrate, and L-cysteine) did not have a significant attractant effect on native strains, whereas most compounds reported to be repellents (taurocholic acid, deoxycholic acid, chenodeoxycholic acid, and cholic acid) produced a repulsive effect in most strains. The HAP technique was used for the chemotactic assay, and although bacteria are variable in their responses to some chemotactic substances *(12),* results in this work were highly reproducible.

Results of clinical and experimental studies have revealed that invasion of *Campylobacter* plays a role in the disease process *(29).* At present, the least understood aspect of *Campylobacter* virulence is the interaction of this organism with the intestinal cells. After the bacteria have contacted a target cell, *C. jejuni* exhibits two potentially pathogenic characteristics: invasiveness and cytotoxin production. Invasiveness is associated with bloody diarrhea, often with endoscopic evidence of colitis and bacteremia in some infected individuals. Intestinal infection produces inflammatory lesions in the bowel of animal models such as rabbits, hamsters, and mice *(27). Campylobacter* invades the cells of the intestinal tract via a mechanism that starts with the efficient movement of the pathogen into the subcellular space (subvasion). Subvasion is followed by an invasion at the basal cell side *(27).* This bacterium is able to trigger signal transduction events to induce host cytoskeletal rearrangements and bacterial uptake *(11).*

Cell culture has been used as a model to examine the invasiveness of many bacteria *(11).* Several cell lines have been used to study and detect CDT activity in *Campylobacter*: CHO, INT-407 *(20),* Vero *(11, 16, 20),* HeLa *(18),* and Caco-2 *(21, 25).* In the present study, Vero cells were used as a model to assay adhesion, invasion, and CDT activity, and all bacterial strains analyzed produced variable levels of CDT activity, adhesion, and invasion; however, no significant differences ($P > 0.05$) among the strains were detected. Recent studies of the distribution of separate *cdtA*, *cdtB,* and *cdtC* genes or the *cdt* cluster in *C. jejuni* and *C. coli* have revealed a high prevalence of these genes in isolates that can exceed 90%; however, levels of CDT production differed between strains *(21–23).* In a previous study, human isolates were more invasive and cytotoxic to Vero cells than were poultry isolates *(20).* In the present study, the isolates from different sources did not differ in toxicity.

This is the first reported study of the virulence factors of native *C. jejuni* and *C. coli* isolates from foods and animals in Mexico. Although a small number of isolates were studied, the *Campylobacter* strains analyzed had virulence factors that gave them the potential to cause disease. Study of a larger number of isolates will give more information on the prevalence and virulence of the strains from different sources in Mexico. The results of this work indicate that public health officials should be aware of the *Campylobacter* threat and should evaluate regulations needed to prevent the contamination of foods and the infection of humans and animals.

TABLE 2. *Adherence, invasion, and CDT activity of native* C. jejuni *and* C. coli *strains*[a]

Species	Strain	Strain origin	Adhesion CFU (%)	Invasion CFU (%)	% mortality of Vero cells from effect of CDT at bacterial supernatant dilution of:		
					1:2	1:4	1:8
C. jejuni	NCDC 5653	Pork	$4.3 \times 10^3 \pm 2.2 \times 10^2$ (0.43)	$4.2 \times 10^1 \pm 3.0 \times 10^1$ (0.0042)	85 ± 10	38 ± 2	24 ± 5
	009 B	Pork	$1.2 \times 10^4 \pm 8.4 \times 10^1$ (0.12)	$3.0 \times 10^1 \pm 3$ (0.003)	90 ± 4	6 ± 4	0
	27 PFF	Raw chicken	$3.3 \times 10^3 \pm 1.2 \times 10^3$ (0.33)	$2.6 \times 10^2 \pm 1.8 \times 10^2$ (0.026)	93 ± 3	25 ± 15	0
	31 PVB	Raw chicken	$5.4 \times 10^4 \pm 1.3 \times 10^4$ (5.4)	$1.2 \times 10^1 \pm 2$ (0.0012)	94 ± 3	25 ± 8	0
	034 B	Raw chicken	$2.9 \times 10^3 \pm 2.0 \times 10^2$ (0.29)	$5.0 \times 10^1 \pm 2.3 \times 10^1$ (0.005)	95 ± 2	54 ± 25	29 ± 7
	35 PVB	Raw chicken	$6.6 \times 10^3 \pm 4.9 \times 10^3$ (0.66)	7 ± 4 (0.0007)	90 ± 4	40 ± 23	26 ± 14
	37	Raw chicken	$4.0 \times 10^4 \pm 1.2 \times 10^4$ (4)	$1.0 \times 10^1 \pm 1$ (0.001)	94 ± 1	19 ± 10	0
	50 sp	Healthy person	$6.3 \times 10^4 \pm 1.2 \times 10^4$ (6.3)	7 ± 5 (0.0007)	84 ± 2	17 ± 5	0
	57 sp	Healthy person	$4.3 \times 10^3 \pm 2.2 \times 10^2$ (0.43)	$4.2 \times 10^1 \pm 3.0 \times 10^1$ (0.0042)	57 ± 2	39 ± 14	0
	060 B	Raw chicken	$7.9 \times 10^3 \pm 5.0 \times 10^2$ (0.79)	2 ± 1 (0.0002)	87 ± 3	29 ± 19	0
	101	Raw chicken	$1.2 \times 10^3 \pm 2.8 \times 10^2$ (0.12)	3 ± 1 (0.0003)	78 ± 14	19 ± 10	0
	102	Live hen	$2.3 \times 10^4 \pm 7.7 \times 10^2$ (2.3)	$5.5 \times 10^1 \pm 3.5 \times 10^1$ (0.0055)	96 ± 4	51 ± 12	29 ± 3
	173 ip	Diarrheic person	$1.9 \times 10^4 \pm 1.2 \times 10^3$ (1.9)	$1.4 \times 10^2 \pm 2.1 \times 10^1$ (0.014)	95 ± 2	32 ± 20	0
	180 ip	Diarrheic person	$3.3 \times 10^3 \pm 2.9 \times 10^2$ (0.33)	$1.8 \times 10^1 \pm 1.0 \times 10^1$ (0.0018)	91 ± 4	20 ± 10	0
	193 ip	Diarrheic person	$3.5 \times 10^3 \pm 2.2 \times 10^2$ (0.35)	$1.7 \times 10^2 \pm 9.8 \times 10^1$ (0.017)	86 ± 4	71 ± 9	12 ± 6
	238 ip	Diarrheic person	$2.1 \times 10^3 \pm 2.1 \times 10^2$ (0.21)	$1.4 \times 10^2 \pm 1.4 \times 10^1$ (0.014)	94 ± 1	27 ± 13	16 ± 3
C. coli	Cc 1	Live chicken	$1.2 \times 10^4 \pm 2.1 \times 10^3$ (1.2)	7 ± 5 (0.0007)	79 ± 10	22 ± 3	0
	Cc 30 PFB	Raw chicken	$3.3 \times 10^3 \pm 1.3 \times 10^3$ (0.33)	2 ± 1 (0.0002)	92 ± 3	21 ± 4	12 ± 10
	Cc 48	Live chicken	$4.1 \times 10^3 \pm 9.9 \times 10^2$ (0.41)	$1.3 \times 10^1 \pm 1.0 \times 10^1$ (0.0013)	88 ± 4	55 ± 17	18 ± 13
	Cc BCO48	Raw chicken	$4.4 \times 10^3 \pm 1.2 \times 10^3$ (0.44)	$2.3 \times 10^2 \pm 2.3 \times 10^1$ (0.023)	94 ± 2	23 ± 18	0
	Cc 54B	Raw chicken	$3.8 \times 10^4 \pm 1.3 \times 10^4$ (3.8)	3 ± 1 (0.0003)	80 ± 8	16 ± 4	0
	Cc 60	Live pig	$1.0 \times 10^4 \pm 1.0 \times 10^3$ (1.0)	$1.6 \times 10^2 \pm 2.1 \times 10^1$ (0.016)	84 ± 7	12 ± 2	0
	Cc 61 PVB	Raw chicken	$1.0 \times 10^4 \pm 1.8 \times 10^3$ (1.0)	$1.2 \times 10^1 \pm 1.1 \times 10^1$ (0.0012)	87 ± 3	25 ± 10	0
	Cc 62F	Raw chicken	$5.8 \times 10^3 \pm 2.0 \times 10^2$ (0.58)	$2.8 \times 10^2 \pm 8.4 \times 10^1$ (0.028)	76 ± 10	39 ± 9	0
	Cc 66F	Raw chicken	$3.9 \times 10^3 \pm 3.6 \times 10^3$ (0.39)	$1.0 \times 10^1 \pm 1$ (0.001)	78 ± 10	24 ± 17	2 ± 0.5
	Cc 67 PVB	Raw chicken	$3.6 \times 10^3 \pm 1.3 \times 10^3$ (0.36)	2 ± 1 (0.0002)	89 ± 3	29 ± 19	0
	Cc 68 PVB	Raw chicken	$4.5 \times 10^3 \pm 2.3 \times 10^3$ (0.45)	$5.0 \times 10^1 \pm 5$ (0.0005)	92 ± 2	22 ± 9	0
	Cc 74F	Raw chicken	$5.4 \times 10^3 \pm 1.9 \times 10^3$ (0.54)	$2.7 \times 10^1 \pm 1.8 \times 10^1$ (0.0027)	90 ± 2	22 ± 14	0
	Cc 81	Live turkey	$1.9 \times 10^3 \pm 9.6 \times 10^2$ (0.19)	$1.4 \times 10^1 \pm 1.2 \times 10^1$ (0.0014)	74 ± 11	22 ± 16	0
	Cc 90	Raw chicken	$2.2 \times 10^3 \pm 2.5 \times 10^2$ (0.22)	$6.2 \times 10^1 \pm 7$ (0.0062)	80 ± 16	23 ± 8	0
	Cc 100	Raw chicken	$5.0 \times 10^3 \pm 2.5 \times 10^3$ (0.5)	$1.7 \times 10^2 \pm 5.0 \times 10^1$ (0.017)	76 ± 7	8 ± 5	0
	Cc M424	Beef sausage	$1.8 \times 10^4 \pm 1.2 \times 10^4$ (1.8)	$5.7 \times 10^2 \pm 7.0 \times 10^1$ (0.057)	89 ± 4	21 ± 5	0

[a] Values are mean ± standard deviation.

ACKNOWLEDGMENTS

This research was supported by the Consejo Nacional de Ciencia y Tecnología de México (CONACYT) and the Programa de Apoyo para la Investigación Científica y Tecnológica, Universidad Autónoma de Nuevo León. We are grateful to CONACYT for the scholarship granted to I. Malagón. The authors also thank Dr. Francisco Contreras for his excellent assistance with some experiments.

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