

## NOTES

### Prevalence of *Campylobacter jejuni* in Chicken Wings

HAILU KINDE, CONSTANTIN A. GENIGEORGIS,\* AND MARGUERITE PAPPAIOANOU

Department of Epidemiology and Preventive Medicine, School of Veterinary Medicine, University of California, Davis, California 95616

Received 20 September 1982/Accepted 22 November 1982

*Campylobacter jejuni* was found in 82.9% of 94 chicken wing packages analyzed on the day of arrival at supermarkets and in 15.5% of 45 packages obtained from the supermarket shelves a few days later. The number of bacterial cells ranged from  $10^2$  to  $10^{3.9}$  per wing. The prevalence of *C. jejuni* in the wings varied with the brand, the day of sampling, and the age of the product.

In recent years, *Campylobacter jejuni* has been established as a major cause of infectious enteritis in humans, with a frequency rivaling that of *Salmonella* species (2, 3, 5, 9, 14, 16, 23). Food-borne transmission has been implicated as one route of infection (1, 9). Outbreaks associated with consumption of undercooked chicken have been reported (1, 4, 18, 22). Recent studies have revealed isolation rates of *C. jejuni* from processed chickens that range from 1.7 to 83% (1, 7, 11, 13, 19, 20, 23), and other studies have found that the prevalence of the organism in the feces of birds ranges from 30 to 100% (1, 11, 12, 20). The objective of this study was to estimate the prevalence and number of *C. jejuni* in chicken wings sold in supermarkets in Davis, Calif.

Chicken wing packages marketed as brands A and B were obtained from four supermarkets. Most of the packages were obtained on the day of arrival (fresh); however, a number of packages, which could have been on the shelves for 2 or 3 days, also were sampled (old). The refrigerated products remained on the supermarket shelves for a maximum of 10 days. A total of 3 wings from each package of 10 were placed in a polyethylene bag containing 30 ml of nutrient broth (polypeptone-yeast extract-NaCl; BBL Microbiology Systems, Cockeysville, Md.) with polymyxin B (5,000 IU/liter), trimethoprim lactate (5 mg/liter), and vancomycin (10 mg/liter) (the antibiotics were added to the broth as presterilized solutions after being autoclaved and cooled). Each bag was massaged for 4 min, and 10 ml of the washing was centrifuged for 5 min at  $3,000 \times g$ . The supernatant was plated on a selective medium made with 52 g of brain heart infusion agar (Difco Laboratories, Detroit, Mich.), 0.5 g of yeast extract (Difco Laboratories), and 1,000 ml of distilled water. The pH

was adjusted to 7.4. After being autoclaved and cooled to between 47 and 50°C, 50 ml of lysed cow erythrocytes and the three antibiotics were added at the previously described concentrations. The inoculated plates were incubated at 42°C for 48 h in an atmosphere of 10% CO<sub>2</sub>-5% O<sub>2</sub>-85% gas. Colonies suspected of being *C. jejuni* were checked microscopically for typical morphology and motility, counted, and then subcultured in differential media for further classification, as described by Ullmann (24).

Based on a 90% expected prevalence of *C. jejuni* (1), the minimum number of chicken wing packages that were required to estimate the prevalence of *C. jejuni* within 5%, at a level of significance of 0.05, was 138 (8). Assuming that brands A and B shared equally in the market for packaged wings in Davis, Calif., a total of 70 brand A and 69 brand B chicken wing packages were analyzed for *C. jejuni* during a 3-month period (14 sampling dates) (Table 1).

The estimated mean prevalence of *C. jejuni* in fresh chicken wings, based on eight samplings, was 82.9% (standard error [SE], 3.9%). The estimated mean prevalence in fresh brand A chicken wings (four samplings) was 98%, and that in brand B chicken wings (four samplings) was 64.3%. This difference in prevalence was significant ( $P < 0.001$ ) (15). The rate of recovery of *C. jejuni* from fresh brand A and B products over the study period ranged from 30 to 100% (Table 2), which is comparable to rates reported previously (1, 7, 13, 19, 20, 23). The mean *C. jejuni* count for the 51 fresh brand A positive samples was  $10^{3.21}$  cells per wing (range,  $10^{2.48}$  to  $10^{3.91}$  cells per wing), and that for the 27 fresh brand B positive samples was  $10^{2.7}$  cells per wing (range,  $10^{2.0}$  to  $10^{3.66}$  cells per wing).

Observed differences in prevalence and

TABLE 1. Mean prevalence of *C. jejuni* in 14 samples of brand A and brand B chicken wings obtained from four supermarkets in Davis, Calif. from 29 March 1981 to 23 June 1981

Brand	Fresh samples			Old samples			Both types of samples (total)	
	No. sampled	No. (%) <sup>a</sup> positive	SE	No. sampled	No. (%) <sup>a</sup> positive	SE	% positive <sup>a</sup>	SE
A	52	51 (98.0)	1.9	18	4 (22.0)	9.8	78.6	4.9
B	42	27 (64.3)	7.4	27	3 (11.0)	6.5	43.5	6.0

<sup>a</sup> Based on a pool of washings of three chicken wings for each sample and a detection level of >100 cells per wing. The mean positive prevalence of *C. jejuni* in fresh samples was 82.9% (SE, 3.9). The mean positive prevalence of *C. jejuni* in old samples was 15.5% (SE, 5.5). The total mean positive prevalence of *C. jejuni* was 61.1% (SE, 4.1).

counts for the two brands may have been caused by variations in transportation times from the slaughterhouse to the supermarkets and possible differences in handling. The brand A operation is located within California; therefore, the distance between the slaughterhouse and the supermarkets is short. The brand B operation is located outside of California and, hence, the product must be transported a longer distance, resulting in more refrigeration time. A study on the effect of cold storage on the prevalence and number of *C. jejuni* in 16 chicken wing packages over a period of 6 days supported this speculation. At day 0, the mean *C. jejuni* count was  $10^{3.3}$  cells per wing, at day 3 it was  $10^{2.6}$  cells per wing, and at day 6 it was reduced to  $10^{2.0}$  cells per wing. The large difference in the prevalence of *C. jejuni* between fresh (82.9%) and old (15.5%) samples (Table 1) also reflects the detrimental

effect of aging in cold storage. *C. jejuni* possibly is killed because of an effective competition from the increasing numbers of psychrotrophic spoilage microorganisms on the meat. Masking of *C. jejuni* by the competing flora on the selective agar was not observed.

Overall, the prevalence of *C. jejuni* showed greater variability from sampling to sampling in brand B chicken wings (SE, 7.4) than in brand A chicken wings (SE, 1.9). In addition, the prevalence of *C. jejuni* in brand A chicken wings remained close to 100% during the entire sampling period, whereas that in brand B chicken wings decreased (Table 2). The reasons for this difference can only be speculated. Brand A is a vertically integrated operation, with the farms and the slaughterhouse owned by the same company. This may minimize variability in overall management of bird-raising and processing operations. Similar information was not available for brand B. The reasons for the decreasing *C. jejuni* prevalence in fresh brand B samples during the sampling period also are unknown. Information on the parameters affecting the carrier state in birds is lacking. Similarly, the effects of transportation and slaughterhouse practices on the prevalence of *C. jejuni* in poultry meat have not been critically evaluated but are presently being studied.

Even though *C. jejuni* is prevalent in poultry products, these products have been rarely implicated as a cause of campylobacteriosis. There are possible explanations for this apparent paradox. *C. jejuni* may be a heterogeneous group, and many of the poultry strains may not be pathogenic for humans. Antigenic heterogeneity already has been demonstrated (5, 21). An additional reason might be the special growth requirements of the organism. Contrary to the other enteric organisms, *C. jejuni* does not grow below 30°C (9). Also, recent thermal death studies (6, 10) have indicated that the organism is heat sensitive, with minimal survival potential in chicken meat that is subjected to normal cooking.

TABLE 2. Rate of recovery of *C. jejuni* from 14 samples of brand A and brand B chicken wings obtained from four supermarkets in Davis, Calif., from 29 March 1981 to 23 June 1981

Brand	Sampling date (mo/day) <sup>a</sup>	No. of samples	% Positive <sup>b</sup>	
			Fresh	Old
A	3/29	2	— <sup>c</sup>	100
	3/31	4	100	—
	4/12	7	—	0
	4/16	9	—	22
	4/24	16	93	—
	5/7	16	100	—
	6/20	16	100	—
B	3/31	3	—	67
	4/5	8	—	18
	4/12	9	100	—
	4/16	7	71	—
	5/6	16	62	—
	5/7	16	—	0
	6/23	10	30	—

<sup>a</sup> Sampling was done in 1981.

<sup>b</sup> Based on a detection level of >100 cells per wing.

<sup>c</sup> —, No sampling.

Ingestion of 500 organisms by a human volunteer caused illness after 4 days (17). With such a low infective dose, avoiding cross contamination of cooked food with raw poultry or utensils exposed to raw poultry is significant in the prevention of campylobacteriosis.

This work was supported in part by funds provided by the Dean's Office as part of California's allocation to the School of Veterinary Medicine for fiscal year 1980 to 1981.

## LITERATURE CITED

- Blaser, M. J. 1982. *Campylobacter jejuni* and food. *Food Technol.* 36:89-92.
- Blaser, M. J., F. M. La Force, N. A. Wilson, and W. L. Wang. 1980. Reservoirs for human campylobacteriosis. *J. Infect. Dis.* 141:665-669.
- Blaser, M. J., and L. B. Reller. 1981. *Campylobacter* enteritis. *N. Engl. J. Med.* 305:1444-1452.
- Brouwer, R., M. J. A. Mertens, T. H. Siem, and J. Katchaki. 1979. An explosive outbreak of *Campylobacter* enteritis in soldiers. *Antonie van Leeuwenhoek. J. Microbiol. Serol.* 45:517-519.
- Butzler, J. P., and M. B. Skirrow. 1979. *Campylobacter* enteritis. *Clin. Gastroenterol.* 8:737-765.
- Christopher, F. M., G. C. Smith, and C. Vanderzant. 1982. Effect of temperature and pH on the survival of *Campylobacter fetus*. *J. Food Prot.* 45:253-259.
- Christopher, F. M., G. C. Smith, and C. Vanderzant. 1982. Examination of poultry giblets, raw milk, and meat for *Campylobacter fetus* subsp. *jejuni*. *J. Food Prot.* 45:260-262.
- Cochran, W. G. 1977. *Sampling techniques*, 3rd ed. John Wiley & Sons, Inc., New York.
- Doyle, M. P. 1981. *Campylobacter fetus* subsp. *jejuni*: an old pathogen of new concern. *J. Food Prot.* 44:480-488.
- Doyle, M. P., and J. Roman. 1981. Growth and survival of *Campylobacter fetus* subsp. *jejuni* as a function of temperature and pH. *J. Food Prot.* 44:596-601.
- Grant, I. H., N. J. Richardson, and V. D. Bokkenheuser. 1980. Broiler chickens as potential source of *Campylobacter* infections in humans. *J. Clin. Microbiol.* 11:508-510.
- Leuchtefeld, N. W., M. J. Blaser, L. B. Reller, and W.-L. Wang. 1980. Isolation of *Campylobacter fetus* subsp. *jejuni* from migratory waterfowl. *J. Clin. Microbiol.* 12:406-408.
- Park, C. E., Z. K. Stankiewicz, J. Lovett, and J. Hunt. 1981. Incidence of *Campylobacter jejuni* in fresh eviscerated whole market chickens. *Can. J. Microbiol.* 27:841-842.
- Public Health Laboratory Service, United Kingdom. 1981. *Campylobacter* infections, 1977-80. *Br. Med. J.* 282:1484.
- Remington, R. D., and M. A. Shork. 1970. *Statistics with applications to the biological and health sciences*. Prentice-Hall, Inc., Englewood Cliffs, N.J.
- Rettig, P. J. 1979. *Campylobacter* infections in human beings. *J. Pediatr.* 94:855-864.
- Robinson, D. A. 1981. Infective dose of *Campylobacter jejuni* in milk. *Br. Med. J.* 282:1584.
- Schaefer, J. R., E. V. Conklin, D. F. M. Bunce, R. D. Storck, F. K. Arnold, J. P. Viner, F. B. Merritt, D. Krish, A. J. Roth, R. W. Currier, L. A. Wintermeyer, and J. P. Davis. 1979. *Campylobacter* enteritis—Iowa. *Morbid. Mortal. Weekly Rep.* 28:565-566.
- Shanker, S., J. A. Rosenfield, G. R. Davey, and T. C. Sorrell. 1982. *Campylobacter jejuni*: incidence in processed broilers and biotype distribution in human and broiler isolates. *Appl. Environ. Microbiol.* 43:1219-1220.
- Simmons, N. A., and F. J. Gibbs. 1979. *Campylobacter* spp. in oven-ready poultry. *J. Infect.* 1:159-162.
- Skirrow, M. B., and J. Benjamin. 1980. 1001 *Campylobacter*: cultural characteristics of intestinal campylobacters from man and animals. *J. Hyg.* 85:427-442.
- Skirrow, M. B., R. G. Fidoe, and D. M. Jones. 1981. An outbreak of presumptive food-borne *Campylobacter* enteritis. *J. Infect.* 3:234-236.
- Svedhem, A., B. Kaijer, and E. Sjögren. 1981. The occurrence of *Campylobacter jejuni* in fresh food and survival under different conditions. *J. Hyg.* 87:421-425.
- Ullmann, U. 1979. *Methods in Campylobacter*, p. 435-452. In T. Bergan and J. R. Morris (ed.), *Methods in microbiology*, vol. XIII. Academic Press, Inc., New York.