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Occurrence of Thermophilic *Campylobacter* spp. Contamination on Vegetable Farms in Malaysia

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The aim of the present study was to examine the prevalence of thermophilic *Campylobacter* spp. (*Campylobacter jejuni* and *Campylobacter coli)* in soil, poultry manure, irrigation water, and freshly harvested vegetables from vegetable farms in Malaysia. *C. jejuni* was detected in 30.4% and 2.7% of the soil samples, 57.1% and 0% of the manure samples, and 18.8% and 3% of the vegetable samples from farm A and farm B, respectively, when using the MPN-PCR method. *Campylobacter* spp. was not found in any of the irrigation water samples tested. Therefore, the present results indicate that the aged manure used by farm A was more contaminated than the composted manure used by farm B. Mostly, the leafy and root vegetables were contaminated. *C. coli* was not detected in any of the samples tested in the current study. Both farms tested in this study were found to be contaminated by campylobacters, thereby posing a potential risk for raw vegetable consumption in Malaysia. The present results also provide baseline data on *Campylobacter* contamination at the farm level.

Keywords: *Campylobacter*, prevalence, pre-harvest, vegetable, farm

Campylobacteriosis is a serious global issue owing to the heavy economic burden caused by the disease. In the United States, as much as US$ 4.3 billion is estimated to be used in fighting against this disease in one year [2].

The epidemiology of *Campylobacter* infections in humans is not well understood, yet campylobacteriosis is known to be sporadic and rarely associated with large outbreaks. Although the vehicle of transmission is not always identified [19], the most common vehicles are poultry, poultry products, raw milk, and contaminated drinking water [19, 27].

Produce as a potential route for transmitting foodborne diseases to humans has recently gained more attention owing to changes in diet [8]. As the overall consumption of fresh produce, especially raw vegetables, has increased as a result of an increase in health consciousness, raw vegetables could serve as a potential vehicle for the transmission of foodborne pathogens to humans. Although *Campylobacter* spp. are not usually detected in produce or other produce-related products [25, 33], the Center for Disease Control [3] has a record of 18 outbreaks of *Campylobacter* enteritis associated with produce worldwide from 1990 to 1999, and the first reported *Campylobacter* outbreak associated with fresh produce occurred in 1993 in the United States and was linked to melons and strawberries [3].

Accordingly, the present study was undertaken to determine the prevalence and concentration of *Campylobacter* spp., especially *C. jejuni* and *C. coli*, in selected environmental samples (soil, poultry manure, and irrigation water) and freshly harvested vegetables from two vegetable farms practicing organic and traditional farming in Malaysia. No comparison of organic and traditional farming approaches was originally intended. Various methods for detecting *Campylobacter* were used, including common culturing methods (direct-plating and enrichment-plating), a direct-PCR, and MPN–PCR. The data obtained in this study can provide useful information for future studies on the risk assessment of *Campylobacter* in Malaysian food.

The sampling was carried out at two vegetable farms, representing an organic vegetable farm (farm A) and traditional vegetable farm (farm B), from January to July 2007, based on five field visits. Both farms were of medium

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size (4,000 to 6,000 m^2) and produced various types of crops from time to time. The samples collected from both farms were soil, poultry manure, irrigation water, and vegetables. Manure application was about twice a month, whereas crop harvesting was dependent on the type of crop. The exact sources of the poultry manure were unknown.

The soil samples (200 g) were collected in a sterile plastic sampling cup from randomly selected planting sites. At farm A, the irrigation water samples were taken from the source of the underground water, main reservoir, and distribution tank, whereas at farm B, the samples were taken from pond water and underground water. The poultry manure (200 g) was randomly collected from a manure aging site at farm A, and composted manure in packages at farm B.

Freshly harvested vegetables were collected on a random basis and the sample collection was dependant on the type of vegetable available during each visit, which included cabbages, tomatoes, and red radishes at farm A, and water spinach, yard-long beans, Indian pennywort, and Vietnamese coriander at farm B.

Immediately after collection, all the samples were transported on ice to the laboratory. The samples were then examined within 24 h of collection.

Ten g or 10 ml of the test samples were placed in a sterile stomacher bag containing 90 ml of a Bolton enrichment broth (Merck, Darmstadt, Germany) supplemented with 5% lysed horse blood, and homogenized for 1 min using a stomacher (Interscience, Saint Nom La Bretèche, France). The mixture was then incubated at room temperature for 30 min and subjected to examination using culturing and MPN-PCR methods.

For the direct-plating method, serial dilutions (from 10^{-1} to 10^{-2}) of the homogenized samples were made using a Bolton broth, and 0.1 ml of each dilution was spread onto mCCDA (Merck, Darmstadt, Germany) plates. For the enrichment-plating method, the homogenized samples were incubated under microaerobic conditions at 37°C for 24 h and 0.1 ml of the enriched cultures was spread onto mCCDA plates in duplicate. The plates were then incubated microaerobically at 42°C for 36 to 48 h, where the microaerobic conditions (85% N_2, 5% O_2, 5% H_2, 5% CO_2) were created in an anaerobic jar using an Anaerocult C Gaspak system (Merck). Finally, the incubated mCCDA plates were examined for presumptive *Campylobacter* colonies, which were identified according to the procedure described by the ISO [10].

The direct-PCR detection method involved direct isolation of DNA from the samples and identification of *Campylobacter* using a PCR technique. The direct-PCR detection method was not applied to the vegetable samples. The protocol used for direct DNA extraction from the soil and poultry manure samples was a modification of the method of Studer *et al.* [31]. Briefly, sterile distilled water (sdH_2O) was added to 10-g samples to make a final volume of 40 ml. The solutions were then incubated in a shaker incubator at 45°C for 1 h, followed by centrifugation at 600 ×*g* for 15 min. Thereafter, the supernatant was filtered through a sterile cheese cloth and centrifuged at 10,000 ×*g* for 10 min. The pellet was suspended in 400 µl of sdH_2O, boiled for 10 min, frozen for 5 min, and centrifuged at 12,000 ×*g* for 10 min. The supernatant was then mixed with two volumes of cold 95% ethanol and centrifuged at 12,000 ×*g* for 10 min. Finally, the pellet was washed with 70% ethanol, dried under a laminar air flow, dissolved in 200 µl of sdH_2O, and examined using PCR methods, as described by Chai *et al.* [5]. The 16S rRNA gene [16] (primers: C412F 5'-GGA TGA CAC TTT TCG GAG C-3'; C1288R 5'-CAT TGT AGC ACG TGT GTC-3') specific to *Campylobacter* spp., *hip* gene [15] (primers: HIP400F 5'-GAA GAG GGT TTG GGT GGT G-3'; HIP1134R 5'-AGC TAG CTT CGC ATA ATA ACT TG-3') specific to *C. jejuni*, and *ceu*E gene [7] (primer: F 5'-ATG AAA AAA TAT TTA GTT TTT GCA-3'; R 5'-ATT TTA TTA TTT GTA GCA GCG-3') specific to *C. coli* were used in the PCR identification of the pathogens found in the samples.

The DNA extraction from water was modified from the method of Studer *et al.* [31]. Briefly, 50 ml of water was centrifuged at 600 ×*g* for 15 min to spin down any sand or coarse particles present in the water sample. The supernatant was then transferred to a new 50-ml centrifugation tube and centrifuged at 12,000 ×*g* for 30 min. Thereafter, the pellet was suspended in 400 µl of sdH_2O, boiled, treated with 97% ethanol, dissolved in 200 µl of sdH_2O, and subjected to a PCR examination [5].

The MPN-PCR detection method was carried out following the protocol described in our previous publication [5].

To test the detection limits of the four detection methods used in this study, *C. jejuni* provided by WHO was grown in a Bolton broth and the culture concentration determined using the standard direct plating method. The culture was then serially diluted in a Bolton broth and detected using the direct plating method, enrichment plating method, direct-PCR method, and MPN-PCR method. These same methods of detection were also used to test the presence of the test strain in the above broth culture when serially diluted in sterile distilled water.

The resulting data were subjected to a Chi-square test using MINITAB Release 14 statistical software to determine any significant differences ($p \leq 0.05$) between the vegetable farms.

A total of 172 samples collected from farm A (n=73) and farm B (n=99) were examined. All the samples that tested positive for *Campylobacter* spp. were also positive for *C. jejuni*. *C. coli* was not detected in any of the samples tested. A higher prevalence of *Campylobacter* spp. and *C. jejuni* (hereinafter abbreviated as campylobacters) were

Table 1. Prevalence of *Campylobacter* spp. and *C. jejuni* in various samples collected from farms A and B[a].

Sample		Farm A				Farm B			
		DP[b]	EP[b]	d-PCR[b]	MPN-PCR[b]	DP	EP	d-PCR	MPN-PCR
Soil		4.3 (1/23)[c]	0.0 (0/23)	13.0 (3/23)	30.4 (7/23)	2.7 (1/37)	0.0 (0/37)	2.7 (1/37)	2.7 (1/37)
Irrigation water	Source	0.0 (0/9)	0.0 (0/9)	0.0 (0/9)	0.0 (0/9)	NA[d]	NA	NA	NA
	Underground water (distribution)	0.0 (0/9)	0.0 (0/9)	0.0 (0/9)	0.0 (0/9)	NA	NA	NA	NA
	Underground water (reservoir)	0.0 (0/9)	0.0 (0/9)	0.0 (0/9)	0.0 (0/9)	0.0 (0/9)	0.0 (0/9)	0.0 (0/9)	0.0 (0/9)
	Pond water	NA	NA	NA	NA	0.0 (0/9)	0.0 (0/9)	0.0 (0/9)	0.0 (0/9)
Poultry manure		14.3 (1/7)	28.6 (2/7)	42.9 (3/7)	57.1 (4/7)	0.0 (0/11)	0.0 (0/11)	0.0 (0/11)	0.0 (0/11)
Vegetables		6.25 (1/16)	6.25 (1/16)	NA	18.8 (3/16)	3.0 (1/33)	3.0 (1/33)	NA	3.0 (1/33)

[a]All samples that tested positive for *Campylobacter* spp. were also positive for *C. jejuni*.
[b]DP, direct plating; EP, enrichment plating; d-PCR, direct-PCR; MPN-PCR, most-probable-number-PCR.
[c]Expressed in percentage (number of positive samples/number of tested samples).
[d]Not available.

detected at farm A than at farm B with all the detection methods used in this study ($p<0.05$). For farm A, campylobacters were detected in the soil samples and poultry manure samples with a prevalence of 30.4% and 57.1%, respectively (MPN-PCR method). However, campylobacters were only detected in 1 out of 37 soil samples (2.7%, MPN-PCR method) and none of the poultry manure samples from farm B (Table 1). All four detection methods used in this study failed to detect campylobacters in any of the irrigation water samples from either farm (Table 1). Whereas 3 of 16 vegetables (18.8%) from farm A were found to be contaminated with campylobacters, only 1 of 33 vegetables (3.0%) from farm B tested positive for campylobacters (Table 1). The vegetables that tested positive for campylobacters included cabbage, radish (farm A), and Vietnamese coriander (farm B) (Table 2).

The prevalence data shown in Table 1 indicates that, among the four detection methods employed, the MPN-PCR method was the most sensitive method for detecting campylobacters, and the direct-plating method was the least sensitive method. The direct-plating method, enrichment-plating method, direct PCR method, and MPN-PCR method were able to detect the presence of the test strain in samples containing 10^2 CFU/ml, 10 CFU/ml, 10^3 CFU/ml, and 10 CFU/ml, respectively. However, the detection of *C. jejuni* in water when using the four detection methods was lower at 10^6 CFU/ml, 10^6 CFU/ml, 10^4 CFU/ml, and 10^2 CFU/ml, respectively.

The mean MPN count of campylobacters harbored in the soil and poultry manure samples from both farms ranged from 6.10 to 6.85 MPN/g (*Campylobacter* spp.) and 3.57 to 6.10 MPN/g (*C. jejuni*) (Table 3). The highest mean MPN count was observed in the freshly harvested vegetables. The mean MPN for the vegetables from farm A was 370.0 MPN/g (*Campylobacter* spp.) and 53.0 MPN/g (*C. jejuni*), whereas that for the vegetables from farm B was 11.0 MPN/g (*Campylobacter* spp.) and 9.1 MPN/g (*C. jejuni*). Mostly, the leafy and root vegetables were contaminated.

Cases of gastroenteritis occur frequently among children in Malaysia (23.6 episodes per 100 children per year) [14]. A number of reports from Malaysia have already recorded a very high prevalence of *Campylobacter* spp., especially *C. jejuni,* at poultry farms, in poultry and poultry products from retail outlets, and in house crows [6, 26, 29]. The

Table 2. Prevalence (MPN-PCR) of *Campylobacter* spp. and *C. jejuni* in vegetables collected from farms A and B[a].

Source	Type of vegetable	Scientific name	Prevalence	
Farm A	Radish	*Raphanus L.*	(1/6)[b]	16.7%
	Tomato	*Lycopersicon esculentum*	(0/6)	0.0%
	Cabbage	*Brassica oleacea*	(2/4)	50.0%
Farm B	Water spinach	*Ipomoea aquatic*	(0/12)	0.0%
	Yard-long bean	*Vigna unguiculata*	(0/9)	0.0%
	Indian Pennywort	*Centella asiatica*	(0/4)	0.0%
	Vietnamese coriander	*Poligonum minus*	(1/8)	12.5%

[a]All samples that tested positive for *Campylobacter* spp. were also positive for *C. jejuni*.
[b](Number of positive samples/number of tested samples).

Table 3. Most probable number (MPN/g) of *Campylobacter* spp. and *C. jejuni* in soil, manure, and vegetable samples from farms A and B.

Sample	Vegetable farm	*Campylobacter* spp.		*C. jejuni*	
		Mean	Range	Mean	Range
Soil	Farm A	6.16	3.0–9.1	3.57	3.0–6.1
	Farm B	6.10	0	6.10	0
Poultry manure	Farm A	6.85	3.0–11.0	4.05	3.0–7.2
	Farm B	-	-	-	-
Vegetables	Farm A	370.00	3.0–1,100	53.00	3.0–150
	Farm B	11.0	0	9.10	0

current authors also recently reported an extremely high prevalence of thermophilic *Campylobacter* spp. in fresh salad vegetables from retail outlets in Malaysia [5]. At the same time, a high multi-antibiotic resistance has also been found in the *C. jejuni* isolated from vegetables [4]. Therefore, these findings prompted the current investigation of how vegetables are contaminated with campylobacters.

Data on *Campylobacter* contamination in fresh produce are limited [5, 13, 21, 23] and no studies have yet evaluated *Campylobacter* contamination in fresh vegetables at the pre-harvest stage. As such, this study revealed the entrance of *Campylobacter* spp., especially *C. jejuni*, into vegetables at the pre-harvest stage on vegetable farms. Among the environmental samples collected, the soil and poultry manure samples were found to carry *Campylobacter* spp., especially *C. jejuni*. As *Campylobacter* spp. are fecal organisms, their presence was expected in the poultry manure used on both farms. Hutchison *et al.* [9] previously reported high concentrations ($2.6–5.9\times10^2$ CFU/g) and a prevalence (7.7–19.4%) of *Campylobacter* spp. in poultry manure. However, in the present study, *Campylobacter* spp. was only detected in the poultry manure collected from farm A (57.1%). The soil samples from farm A (30.4%) also showed a higher prevalence than the soil samples from farm B (2.7%). Thus, it is tempting to speculate that the different farming practices of the farms played a role in this phenomenon. Whereas farm B practices bed burning before seeding to produce porous soil and only uses composted poultry manure, farm A does not practice bed burning and uses aged poultry manure to fertilize the crops. According to previous studies, *Campylobacter* spp. can survive up to three months in manure and one month in soil, yet are very sensitive to drying and heat (D_{55C}=1 min) [19, 20, 28], thereby providing some explanations for the absence of *Campylobacter* spp. in the poultry manure and soil from farm B. However, further studies are needed to determine whether this speculation is correct.

As infection by campylobacters can be waterborne, and these bacteria are frequently detected in surface water and drinking water [18, 32], the irrigation water used during the pre-harvest stage was considered as a potential source of *Campylobacter* contamination in the pre-harvest vegetables. However, no *Campylobacter* spp. was detected in any of the irrigation water samples collected from either farm in this study. Nonetheless, the possibility of water as a potential source of contamination in the current study can not be totally excluded, as the lowest detection limit among the four methods applied in this study was 10^2 cells/ml with the MPN-PCR method. As such, the methods used in this study were unable to detect water samples with less than 100 *Campylobacter* cells/ml. Thus, the detection of *Campylobacter* spp. in irrigation water requires methods with a higher sensitivity to bacteria concentrations.

Overall, the vegetables from farm A (18.8%) were more frequently contaminated with campylobacters than the vegetables from farm B (3.0%) (Table 1). However, an interesting finding in this study was that all the *Campylobacter*-positive vegetables were harvested from a contaminated planting site (soil sample tested positive for *Campylobacter* spp.). Therefore, this result and its association with the distribution of campylobacters in the soil samples (explained above) reiterate the speculation of *Campylobacter* contamination of the vegetables by poultry manure through soil contamination. Freshly harvested vegetables available during our visits were sampled from both farms. The vegetable samples that tested positive for campylobacters included cabbage and Vietnamese coriander (leafy vegetables) and radish (root vegetables). Meanwhile, none of the tomato samples tested positive for campylobacters (Table 2), suggesting that vegetables in close contact with soil may have a higher possibility of contamination by campylobacters. Other researchers have also detected *Campylobacter* spp. in leafy vegetables (lettuce and spinach) and root vegetables (potatoes, green onion, and radish) [22].

Although many previous studies have reported a high prevalence of *C. jejuni* and *C. coli* in poultry [12, 17, 27, 30], *C. coli* was not detected in any of the samples from either farm in the present study. However, *C. jejuni* has been proven to survive under various conditions and is approximately 13-times more resistant to mesophilic anaerobic digestion in animal manure than *Salmonella typhimurium* [11]. Thus, even though *C. jejuni* may not be able to grow, it can persist well in environmental water [28]. The maximal survival of *C. jejuni* has been identified at low temperatures, where survival times of up to 4 months have been identified [24]. Yet, information on the survivability

of *C. coli* in the environment is extremely scarce. Hence, a comparison of the survivability of *C. coli* and *C. jejuni* in the environment would seem to be important to discover the reason for the zero detection of *C. coli* in this study.

The present study found a marked difference in the prevalence of campylobacters among the different detection methods. The MPN-PCR method used in this study was evidently more effective for detecting campylobacters than the other methods used, as it combines an enrichment culture step and the PCR method. Nonetheless, the four methods used in this study were able to detect *C. jejuni* grown in a Bolton broth and diluted in distilled water at concentrations of 10 CFU/ml – 10^6 CFU/ml, with the MPN-PCR method being the most sensitive quantitative method. However, most previous studies of campylobacters in raw vegetables did not employ an MPN-PCR-based method, and reported either a nil or low occurrence of campylobacters [13, 21, 22]. As such, the low occurrence of campylobacters in vegetables reported by these studies may be partly due to the lack of appropriate methods for detecting campylobacters in produce samples.

In conclusion, the current study demonstrated a potential risk posed by campylobacters through consumption of raw vegetables and raised the speculation that organic farming (farm A) resulted in more contamination than traditional farm using composted manure (farm B). However, a further study with a larger sample size is needed to confirm this speculation. National level surveillance is also needed to better comprehend the situation to set a basis for future risk assessment work and establish food safety standards in Malaysia and associated countries where fresh vegetables are traded.

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REFERENCES

1. Brackett, R. E. 1999. Incidence, contributing factors, and control of bacterial pathogens in produce. *Postharvest Biol. Technol.* **15:** 305–311.
2. Buzby, J. C. and T. Roberts. 1997. Economic costs and trade impacts of microbial foodborne illness. *World Health Stat. Q.* **50:** 57–66.
3. CDC. 2000. Centers for Disease Control and Prevention, U. S. Foodborne Disease Outbreak Line Listing, 1990–1999. Available from http://www.cdc.gov/ncidod/dbmd/outbreak/us_outb.htm.
4. Chai, L. C., A. B. Fatimah, F. M. Ghazali, H. Y. Lee, R. Tunung, A. T. Shamsinar, *et al.* 2008. Biosafety of *Campylobacter jejuni* from raw vegetables consumed as ulam with reference to their resistance to antibiotics. *Int. Food Res. J.* **15:** 33–43.
5. Chai, L. C., T. Robin, U. M. Ragavan, J. W. Gunsalam, F. A. Bakar, F. M. Ghazali, S. Radu, and M. P. Kumar. 2007. Thermophilic *Campylobacter* spp. in salad vegetables in Malaysia. *Int. J. Food Microbiol.* **117:** 106–111.
6. Ganapathy, K., A. A. Saleha, M. Jaganathan, C. G. Tan, C. T. Chong, S. C. Tang, A. Ideris, C. M. Dare, and J. M. Bradbury. 2007. Survey of *Campylobacter*, *Salmonella* and mycoplasmas in house crows (*Corvus splendens*) in Malaysia. *Vet. Rec.* **160:** 622–624.
7. Gonzalez, I., K. A. Grant, P. T. Richardson, S. F. Park, and M. D. Collins. 1997. Specific identification of the entheropathogens *Campylobacter jejuni* and *Campylobacter coli* by using a PCR test based on the *ceuE* gene encoding a putative virulence determinant. *J. Clin. Microbiol.* **35:** 759–763.
8. Hedberg, C. W., K. L. MacDonald, and M. T. Osterholm. 1994. Changing epidemiology of food-borne disease: A Minnesota perspective. *Clin. Infect. Dis.* **18:** 671–682.
9. Hutchison, M. L., L. D. Walters, S. M. Avery, F. Munro, and A. Moore. 2005. Analyses of livestock production, waste storage, and pathogen levels and prevalences in farm manures. *Appl. Environ. Microbiol.* **71:** 1231–1236.
10. ISO. 1995. Microbiology of food and animal feeding stuffs – Horizontal method for the detection of thermotolerant *Campylobacter*. ISO 10272: 1995 (E) International Standards Organization, Geneva.
11. Kearney, T. E., M. J. Larkin, J. P. Frost, and P. N. Levett. 1993. Survival of pathogenic bacteria during mesophilic anaerobic digestion of animal waste. *J. Appl. Bacteriol.* **75:** 215–219.
12. Klatka, L., M. Hawkins, M. Pass, F. Angulo, D. Rohn, J. Morris, and the EIP FoodNet Working Group. 2002. Risk factors for sporadic *Campylobacter* infections in Maryland. 3rd International Conference on Emerging Infectious Diseases. Atlanta, GA, March 2002. Available from http://www.cdc.gov/foodnet/pub/iceid/2002/klatka_1.htm
13. Kumar, A., R. K. Argawal, K. N. Bhilekaonkar, B. R. Shome, and B. N. Bachhil. 2001. Occurrence of *Campylobacter jejuni* in vegetables. *Int. J. Food Microbiol.* **67:** 153–155.
14. Lim, V. K. E. 2007. Infectious diarrhoea. *Med. J. Malaysia 62:* Editorial. Available from http://www.mma.org.my/Portals/0/MED20%J20%MALAYSIA20%VOL20%6220%NO20%320%AUGUST2007.pdf
15. Linton, D., A. J. Lawson, R. J. Owen, and J. Stanley. 1996. Rapid identification by PCR of the genus *Campylobacter* and of five *Campylobacter* species enteropathogenic for man and animals. *Res. Microbiol.* **147:** 707–718.
16. Linton, D., A. J. Lawson, R. J. Owen, and J. Stanley. 1997. PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* from diarrheic samples. *J. Clin. Microbiol.* **35:** 2568–2572.
17. Mateo, E., J. Carcamo, M. Urquijo, I. Perales, and A. Fernandez-Astorga. 2005. Evaluation of a PCR assay for the detection and identification of *Campylobacter jejuni* and *Campylobacter coli* in retail poultry products. *Res. Microbiol.* **156:** 568–579.
18. Merritt, A., R. Miles, and J. Bates. 1999. An outbreak of *Campylobacter* enteritis on an island resort, North Queensland. *Commun. Dis. Intell.* **23:** 215–219.

19. Nachamkin, I., A. B. Mishu, and T. Ho. 1998. *Campylobacter* species and Guillain–Barre syndrome. *Clin. Microbiol. Rev.* **11:** 555–567.
20. Nicholson, F. A., S. J. Groves, and B. J. Chambers. 2005. Pathogen survival during livestock manure storage and following land application. *Bioresour. Technol.* **96:** 135–143.
21. Odumeru, J. A., S. J. Mitchell, D. M. Alves, J. A. Lynch, A. J. Yee, S. L. Wang, S. Styliadis, and J. M. Farber. 1997. Assessment of microbiological quality of ready-to-use vegetables for health-care food services. *J. Food Prot.* **60:** 954–960.
22. Park, C. E. and G. W. Sanders. 1992. Occurrence of thermotolerant campylobacters in fresh vegetables sold at farmers outdoor markets and supermarkets. *Can. J. Microbiol.* **38:** 313–316.
23. Phillips, C. A. 1998. The isolation of *Campylobacter* spp. from modified atmosphere packaged foods. *Int. J. Environ. Health Res.* **8:** 215–221.
24. Rollins, D. M. and R. R. Colwell. 1986. Viable but non-culturable stage of *Camp. jejuni* and its role in survival in the aquatic environment. *Appl. Environ. Microbiol.* **52:** 531–538.
25. Sagoo, S. K., C. L. Little, L. Ward, I. A. Gillepsie, and R. T. Mitchell. 2003. Microbiological study of ready-to-eat salad vegetables from retail establishments uncovers a national outbreak of salmonellosis. *J. Food Prot.* **66:** 403–409.
26. Saleha, A. A. 2002. Isolation and characterization of *Campylobacter jejuni* from broiler chickens in Malaysia. *Int. J. Poultry Sci.* **1:** 94–97.
27. Schorr, D., H. Schmid, H. L. Rieder, A. Baumgertner, H. Vorkauf, and A. Burnens. 1994. Risk factors for *Campylobacter* enteritis in Switzerland. *Zentralbl. Hyg. Umweltmed.* **196:** 327–337.
28. Solomon, E. B. and D. G. Hoover. 1999. *Campylobacter jejuni*: A bacterial paradox. *J. Food Safety* **19:** 121–136.
29. Son, R., M. I. A. Karim, G. Rusul, and K. Yusoff. 1996. Plasmids and antimicrobial resistance among *Campylobacter jejuni* isolated from retail fresh poultry. *Asia Pac. J. Mol. Biol.* **4:** 106–111.
30. Studahl, A. and Y. Andersson. 2000. Risk factors for indigenous *Campylobacter* infection: A Swedish case-control study. *Epidemiol. Infect.* **125:** 269–275.
31. Studer, E., M. Domke, B. Wegmuller, J. Luthy, S. Schmid, and U. Candrian. 1998. RFLP and sequence analysis of *Campylobacter jejuni* and *Campylobacter coli* PCR products amplified directly from environmental samples. *Lebensm. Wiss. Technol.* **31:** 537–545.
32. Tauxe, R. V., N. Hargerett-Bean, C. M. Patton, and I. K. Wachsmuth. 1988. *Campylobacter* isolates in the United States, 1982–1986. *Morb. Mort. Wkly. Rep. Surveill. Summ.* **37:** 1–13.
33. Thunberg, R. L., T. T. Tran, R. W. Bennett, R. N. Matthews, and N. Belay. 2002. Microbial evaluation of selected fresh produce obtained at retail markets. *J. Food Prot.* **65:** 677–682.