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Research

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Detection of *Listeria* Species in Fresh Produce Samples from Different Retail Shops in Canterbury, New Zealand

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ABSTRACT

Aim: This study investigates the prevalence of *Listeria* spp. in fresh produce sold in the Canterbury region of New Zealand.
Background: *L. monocytogenes* is a common pathogenic bacterium that can be present and grow on fresh produce. Therefore, systematic risk assessment is needed to avoid a food safety scare.
Results: Microbiological analysis to detect *Listeria* spp. in fresh produce samples showed that lettuce from the retail shops had the highest level (4.2 log cfu/g) >cucumber (3.2 log cfu/g) >cabbage (2.5 log cfu/g) >carrot (nil). In terms of *Listeria* prevalence, carrot samples had the lowest *Listeria* spp. load (<1.05 log cfu/g) and lettuce samples had the highest (>4 log cfu/g). None of the samples showed a positive result for *L. monocytogenes* presence.
Conclusion: Although the *L. monocytogenes* was not detected in this study, the potential hazard for contaminating fresh produce by this organism still exist due to high prevalence of *Listeria* spp.

KEYWORDS: *Listeria monocytogenes*; Foodborne pathogens; Retail shops; Fresh produce; Lettuce.

INTRODUCION

Fresh produce is transported from farm to distribution centres and then to retail shops. It is a general practice that fresh vegetables are washed in water before they are supplied to retailers. After washing, fresh produce commodities are packed and transferred to different shops by different food chains. At any of these steps a careless omission would give *Listeria monocytogenes* a chance to contaminate fresh vegetables, such as during storage conditions (including temperature, moisture, and oxygen content) and/or sanitation conditions during packing processes and transport. At the end of 2014, a listeriosis outbreak related to caramel apples occurred, which led to 35 people being infected by *L. monocytogenes*.[1] After testing, *L. monocytogenes* was detected in the apple-packing factory.

Most fresh produce is pre-treated, i.e. by ultraviolet (UV),[2] washing[3] or chlorine disinfection[4] before being sold. However, *Listeria* spp. and *L. monocytogenes* were found in fresh produce from retail shops; for example, in pre-packaged mixed vegetable salads,[5] parsley[6] and lettuces.[7]

Fresh vegetables are consumed by almost all families. According to a local report,[8] in 2013, local consumers spent NZ$ 41.8 million on fresh lettuces and NZ$ 30 million on fresh carrots. New Zealand produced nearly 1.4 million tonnes of vegetables, while 500,000 tonnes were exported and over 900,000 tonnes were consumed locally. Therefore, to understand the safety of fresh produce in retail shops is at least as significant as that in farms. The research described in this article investigated the prevalence of *Listeria* spp. in selective salad vegetables from different retail shops in the Canterbury region of South Island, New Zealand.

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MATERIAL AND METHODS

Sample Collection

Four different retail shops were selected in this study: (1) an open market (Market A); (2) a supermarket (Market B); (3) an Asian grocery store (Market C); and (4) a fruit and vegetable market (Market D). A total of 96 samples were collected from 4 local markets. Lettuce, cabbage, cucumber and carrot samples were collected from these markets over a period of 6 weeks.

Sample Preparation

Fresh produce samples were packaged separately and stored at 5 °C before the analysis. To reduce the influence of objective factors, samples were washed in the same tap water and cut with sterile tools (including knives and cutting boards). All samples were tested for the presence of *L. monocytogenes* following the procedure recommended by the International Organisation for Standardisation (ISO) procedure.

Listeria spp. Detection and Enumeration

Twenty-five grams of a representative portion from each sample was introduced aseptically into a sterile stomacher bag containing 225 ml of peptone water. *Listeria* selective agar (CM0856, OXOID) and Difco™ plate count agar (for aerobic plate counts) were plated the after making suitable dilutions. Following incubation at 35 °C for 24-48 h, the colonies were counted and results were reported in cfu/g. Gram staining and carbohydrate utilization profiles were used to further characterize the *Listeria* colonies.

The following formula was used to calculate cfu/g:

$$\text{cfu/g of sample} = \frac{A \times 10^{n}}{V1} \times \frac{V2}{m}$$

where A - number of colonies (average of the plates);
10^{n} - level of dilution at which the counting was carried out;
V1 - volume of inoculum;
V2 - total volume of peptone water;
m - total sample weight

Statistical Analysis

Statistical analysis was conducted using Minitab Statistical Software version 17 (Minitab Pty Ltd., NSW, Australia). The software functions were used to analyze the experimental data. The Tukey test was used to compare the means. When values differed at $p<0.05$ level, the results were considered to be as a significantly different.

RESULTS AND DISCUSSION

Comparsion of Microbial Levels by Different Retail Markets

Market A was an open market that operated each Sunday. Four types of vegetable samples were collected over a 6-week period to compare the total microbial counts and the presence of *Listeria* spp. Data was statistically analysed using the Tukey test. The total microbial populations in the cabbage, carrot and lettuce were not significantly different and the mean counts were around 7 log cfu/g. The cucumber samples had the lowest microbial count (4.5 log cfu/g). The *Listeria* spp. detected in 4 different types of fresh produce samples were significantly different ($p<0.05$). The highest population of *Listeria* spp. was detected in lettuce (4.5 log cfu/g), followed by cucumber (about 3 log cfu/g; mean). The *Listeria* spp. count was the 2nd lowest load in cabbage (1.5 log cfu/g). The *Listeria* spp. contamination was lowest in carrot with less than 1 log cfu/g (Figure 1).

Market B was an outlet from a supermarket chain, which had constant suppliers providing fresh vegetables. Four types of vegetables were sampled over the 6-week collection period. Therefore, it was not suitable to compare the microbial levels and *Listeria* spp. levels for these 4 types of vegetable samples from Market B. The results were similar to as observed

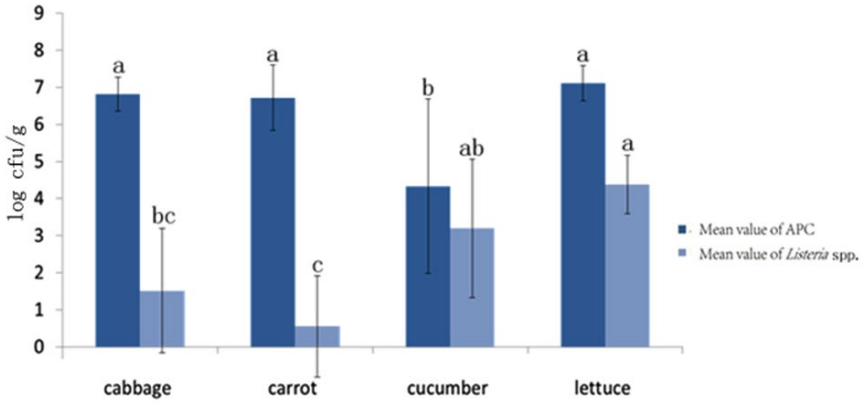

Figure 1: Comparative microbial analysis of fresh produce samples from Market A. Error bars represent standard deviations of the means. Different letters on each bar show that the results have significant differences using the Tukey test, $p<0.05$.

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for Market A, where cabbage, carrot and lettuce belonging to the same group were contaminated and a range between 6 to 7 log cfu/g was recorded. In contrast, the APC value of cucumbers was lower (3 log cfu/g). The level of *Listeria* spp. in lettuce was the highest mean (4 log cfu/g) among the 4 types of vegetables from Market B. The second highest mean population of *Listeria* spp. was in cabbage (2.5 log cfu/g) followed by cucumber (2 log cfu/g). The population of *Listeria* spp. in carrot was the lowest (1 log cfu/g) (Figure 2).

Market C was an ethnic retail shop that sold a variety of commodities, including fresh produce. Four types of vegetable samples were collected over six weeks to test for total microbial counts and the presence of *Listeria* spp. There were no significant differences (p>0.05) in the total plate counts of the fresh produce samples sold in Market C (Figure 3). The mean APC values of the fresh produce samples in Market C ranged from 5.5 to 7 log cfu/g. Leafy vegetables (cabbage and lettuce) in Market C had similar *Listeria* spp. contamination levels when analyzed by the Tukey test. However, the *Listeria* spp. levels in lettuce (4 log cfu/g) were higher compared to those in cabbage (3 log cfu/g), followed by those in cucumber (3 log cfu/g). Carrot contained the lowest of *Listeria* spp. population (less than 1 log cfu/g) (Figure 3).

Market D was a fruit and vegetable retail shop outlet where fresh produce was the major commodity sold. Four different types of vegetable samples were collected to evaluate the microbiological quality and the presence of *Listeria* spp. contamination during 6-weeks of sampling. Statistically significant differences were recorded for total plate counts using the Tukey test. The microbial populations were highest in the cabbage and lettuce samples (7 log cfu/g). The population of total microbes in carrots (6.5 log cfu/g) was slightly lower and significantly different from cabbage and lettuce (7 log cfu/g) (p=0.05). The APC in cucumber were lowest in the samples from Market D (fewer than 5 log cfu/g). In contrast, the levels of *Listeria* spp. in the cabbage and lettuce were higher than in other types of vegetables. No *Listeria* spp. were detected in carrot samples from Market D (Figure 4).

Comparsion of Microbial Levels by Fresh Produce Type

The microbial quality (*Listeria* spp. and APC) of fresh produce was compared between four retail shops. Statistical analysis (Tukey test) of the microbial analyses showed that, the fresh produce samples were not significantly different (p>0.05) among the retail shops selected (Figure 5). The mean *Listeria* spp. levels in the cabbage samples ranged from 1.5 to 3 log cfu/g (whereas APC values were about 7 log cfu/g). *Listeria* spp. levels in the

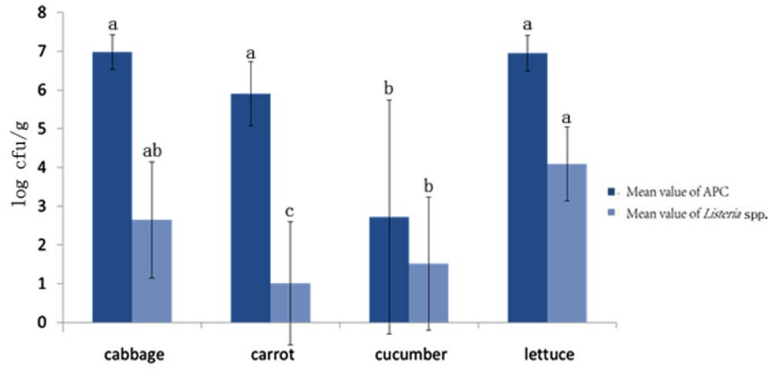

Figure 2: Comparative microbial analysis of fresh produce samples from Market B. Error bars represent standard deviations of the means. Different letters on each bar show that the results have significant differences using the Tukey test, p<0.05.

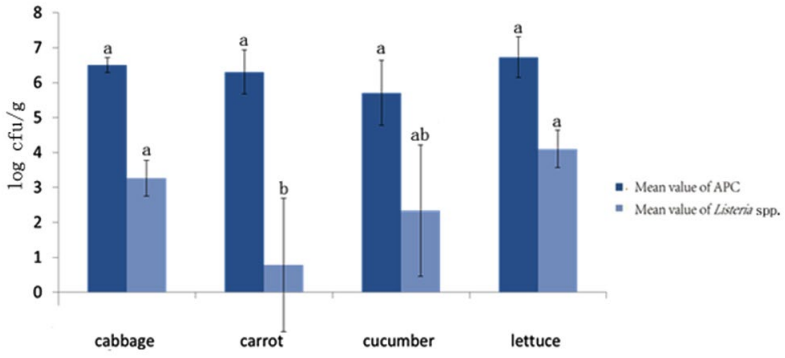

Figure 3: Comparative microbial analysis of fresh produce samples from Market C. Error bars represent standard deviations of the means. Different letters on each bar show that results have significant differences using the Tukey test, p<0.05.

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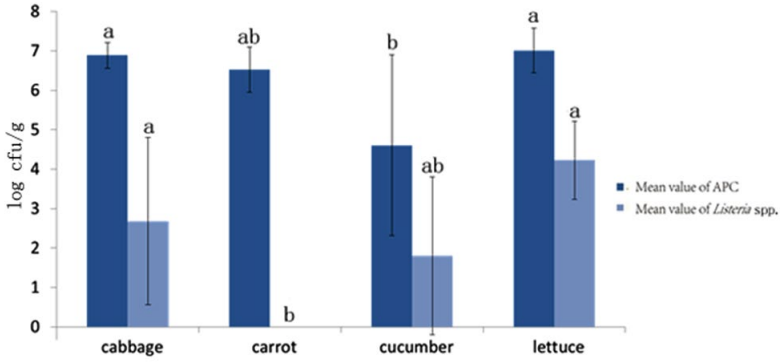

Figure 4: Comparative microbial analysis of fresh produce samples from Market D. Error bars represent standard deviations of the means. Different letters on each bar show that results have significant differences using the Tukey test, $p<0.05$.

carrot samples ranged from 0 to 1 log cfu/g and APC values ranged from 6 to 7 log cfu/g. *Listeria* spp. levels in the cucumber samples ranged from 1.5 to 3.5 log cfu/g (APC levels ranged from 2.5 to 6 log cfu/g). For lettuce samples, *Listeria* spp. levels were about 4 log cfu/g (and APC levels were about 7 log cfu/g).

Overview of *Listeria* spp. in Fresh Produce Samples from Retail Shops

In general, the average APC values were similar for cabbage, carrot and lettuce samples collected from the four retail shops (Figure 6A). Similarly the samples from the farms, the APC values were between 6 to 7 log cfu/g. The mean APC of the carrot samples from Market B had the lowest value, which was fewer than 6 log cfu/g. On average, lettuce samples from Market A and Market D had comparatively higher APC (>7 log cfu/g). The cucumber samples had the lowest microbial load, ranging from 2.72 to 5.68 log cfu/g. However, a research report on the microbiological analysis of salad vegetables in markets in India[9] reported an average APC of 9 log cfu/g. The *Listeria* spp. counts for fresh produce from retail shops are presented in Figure 6B. The majority of APC values in cabbage and cucumber were distinguished in the same group using the Tukey test. Carrot samples had the lowest *Listeria* spp. load (<1.05 log cfu/g) and lettuce samples had the highest *Listeria* spp. load (4 log cfu/g). There were no significant differences in the same type of fresh produce among the four retail shops. Other research from Dhaka, which evaluated the prevalence of bacteria in salad veg-

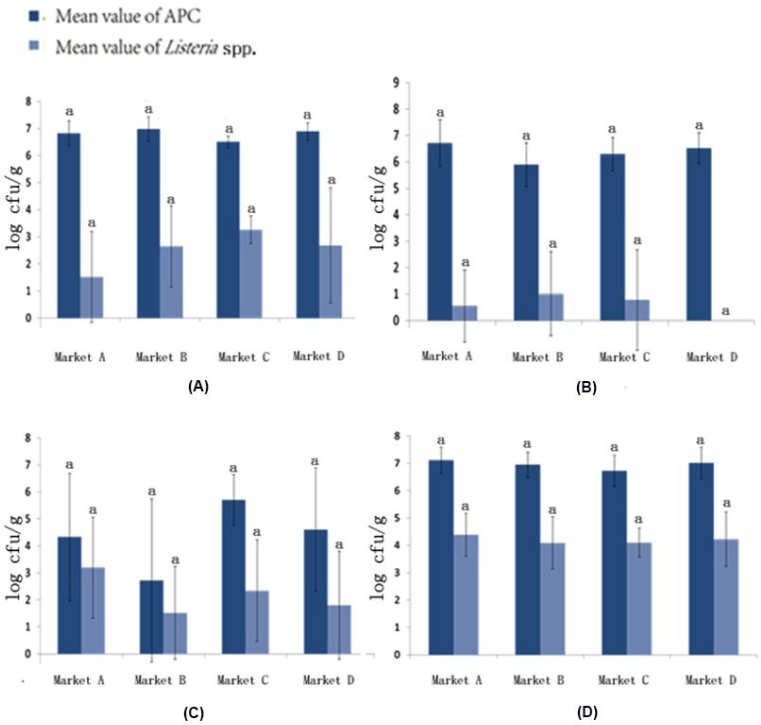

Figure 5: Comparative microbial analysis of fresh produce samples from Market A to D. (A) cabbage (B) carrot (C) cucumber (D) lettuce. Error bars represent standard deviations of the means. Different letters on each bar show that results have significant differences using the Tukey test, $p>0.05$.

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etables,[10] reported much higher *Listeria* spp. load (6 to 8 log cfu/g) than the results of this study.

DISCUSSION

The results of this study mainly compared the microbial populations and presence of *Listeria* spp. in selected vegetables from four retail shops in the Canterbury region. Each type of vegetable purchased from the selected shops did not show significant differences in microbial quality (APC). However, slight differences inwere observed (Figures 5 and 6). For cabbage samples, the highest *Listeria* spp. Loads were in samples from the Market C, with a mean of 3.26 log cfu/g, while the lowest population of *Listeria* spp. were detected in the samples from Market A, with a mean of 1.51 log cfu/g. Carrot samples had the lowest *Listeria* spp. load. Among four types of fresh produce from 4 retail shops, the highest loads were in Market B (1.02 log cfu/g) and all samples from Market D showed negative results. The population of *Listeria* spp. in cucumber was similar to that in cabbage, with the highest value of *Listeria* spp. load in Market A (3.2 log cfu/g) and the lowest value of *Listeria* spp. load in Market B (1.52 log cfu/g). The lettuce samples had the highest bacterial load among the 4 types of vegetables with the highest APC values and *Listeria* spp. levels. Lettuce samples from Market A (4.39 log cfu/g) had the highest population of *Listeria* spp. compared to the lowest *Listeria* spp. in the samples from Market B (4.09 log cfu/g).

In the assessment of *L. monocytogenes* associated with lettuce, *L .monocytogenes* was positive in 5 RTE lettuce samples.[7] All colonies isolated belonged to serotype 1/2a. In another research on a risk assessment for *L. monocytogenes* from the farm to the table in Korea,[11] the mean final *L. monocytogenes* contamination levels reported were -1.50 log cfu/g and -0.146 log cfu/g at restaurants and in homes, respectively. In the bacteriological assessment of fresh produce in Norway,[12] just one in 200 lettuce samples was positive for *L. monocytogenes*. In the study on the detection of *Listeria* spp. in RTE products in Malysia,[13] *Listera* spp. were found in 20% of salad and vegetable samples, including 2 lettuce samples, 2 cucumber samples, 1 tomato sample and 3 cabbage samples. *L. monocytogenes* was positive in 1 lettuce sample, 2 cucumber samples and 2 cabbage samples. In the microbial evaluation for minimally-processed vegetables, just one in 112 sprouts samples was positive for *L.*

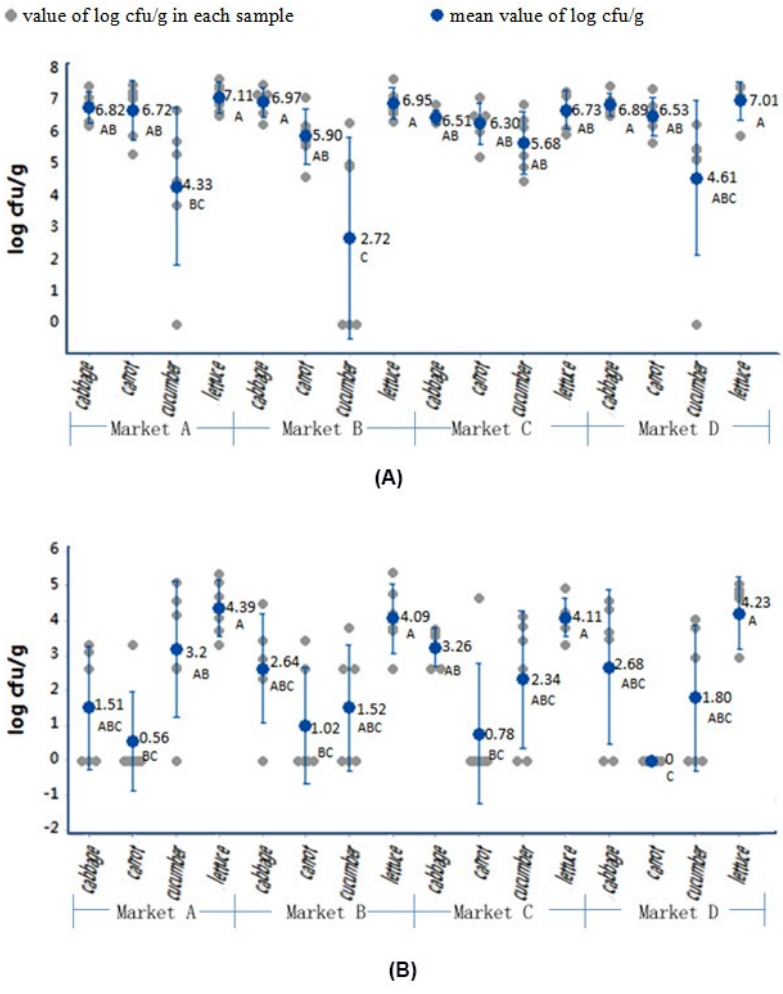

Figure 6: Comparative microbial analysis of samples from four retail shops. A, APC and B, *Listeria* spp. Error bars represent standard deviations of the means. Different letters on each bar show that results have significant differences using the Tukey test, $p<0.05$.

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monocytogenes, and lettuce and other fruit and vegetable samples were negative for this bacterium.[14] In another microbial assessment of fresh produce, *L. monocytgenes* was found in 3.4% of lettuce samples (≤2 log cfu/g).[15] The prevalence of pathogens in fresh produce reported by Rahman and Noor,[10] the population of *Listeria* spp. on fresh produce (cucumber, carrot, lettuce) ranged from 6 log cfu/g to 8 log cfu/g, which was much higher than results reported here. In a previous study, *Listeria* spp. were commonly detected in cabbage and lettuce samples.[16]

Sample collection from retail shops did not seem to be influenced by seasonal changes. Therefore, all fresh produce from retail shops were collected over 6-weeks. Microbial quality may be influenced by storage conditions, including temperature, relative humidity (RH), O_2 and CO_2 contents, storage time and surfaces touched.

A group of scientists completed research on the effect of storage temperature and RH on the growth of *L. monocytogenes*.[17] They examined lettuce leaves, parsley leaves and cucumber epidermis samples at 3 temperatures and at two RH values. *L. monocytogenes* was detected in the cucumber epidermis samples. The results showed that *L. monocytogenes* preferred higher temperature and RH. In addition, *L. monocytogenes* had difficultly surviving on intact vegetable surfaces.[17]

Francis et al[18] studied the effect of atmosphere and acid adaptation on growth of *L. monocytogenes* using intact lettuce leaves to prepare lettuce agar to monitor the growth of *L. monocytogenes*. Lettuce agar plates with *L. monocytogenes* were stored separately under 3 conditions (a. air; b. 10% CO_2, 5% O_2, 85% N_2; and c. 25% CO_2, 75% N_2) at 8 °C. Microbial levels were tested over the storage period. The results showed that the vitality of the *Listeria* spp. was improved when samples were stored under 25% CO_2 conditions, and the most optimal condition for vegetable packing was in a 5 to 10% CO_2 content atmosphere.[18]

In addition, in a research report on storage temperatures, *L. monocytogenes* growth in fresh produce at 7 °C and 15 °C for 6 days was compared.[19] The result showed that the population of *L. monocyogenes* increased more at 15 °C over the storage period. Similar findings have been reported on the effect of storage conditions on *L. monocytogenes* growth at 3 different temperatures (4 °C, 12 °C and 22 °C) for comparison.[20] *L. monocytogenes* survived better at higher temperatures with an increase in cell count of 0.3 log cfu/g in the first 17 h of storage.

CONCLUSIONS

The results of this study showed that cabbage and lettuce had similar levels of total microbial loads. However, the contamination of *Listeria* spp. in lettuce was higher than in cabbage. The total microbial load in carrot samples was higher in the cucumber samples while the prevalence of *Listeria* spp. was higher in the cucumber samples. Overall, all 4 different types of vegetable samples had similar microbiological quality regardless of the market source. However, some variations were observed in the samples when analyzed on a weekly basis. *L. monocytogenes* was not detected in the fresh produce samples tested. However, ongoing risk assessment activities are required to ensure fresh produce safety.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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