MICROBIOLOGICAL SURVEY OF RETAIL FRESH PRODUCE OF IMPORTED, DOMESTIC CONVENTIONAL AND DOMESTIC ORGANIC ORIGIN

FINAL REPORT

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SUMMARY

A microbiological survey of 891 imported conventional (n=226) and domestically grown conventional (n=349) and organic (n=316) fresh fruits and vegetables purchased from a variety of retail outlets in Auckland and Christchurch was conducted over a 15 month period. For each sample, concentrations of faecal coliforms and generic *E. coli*, and the prevalence of shiga-toxin producing *E. coli* (STEC) O157, *Salmonella* spp. and *Campylobacter* spp were determined. Testing was conducted using most probable number (MPN) and enrichment-based standard methods on 250 g samples (excluding melon where one whole fruit was analysed per test). Results were assessed as satisfactory, marginal or unsatisfactory using relevant microbiological reference criteria for salads, sprouted seeds and ready-to-eat foods.

Campylobacter spp. and *E. coli* O157 were not detected in any sample. However, *Salmonella* Typhimurium phage type RDNC-May06 was detected in two domestic organic lettuces from the same grower, both of which were deemed satisfactory/marginal in terms of limits for faecal coliforms and *E. coli*. A site visit identified bird faeces on hail netting located directly above growing produce, which was particularly concentrated in areas where birds were able to land on metal hoops holding the netting up. It is likely that contamination occurred either through direct defecation onto plants below or indirectly via overhead irrigation and/or precipitation.

In terms of microbiological quality, between 95.4% and 96.6% of produce items sampled were satisfactory based on microbiological limits for faecal coliforms and/or *E. coli*. All imported samples (apples, capsicums, grapes, melons and strawberries) were of a satisfactory nature, while at least 54% of marginal and unsatisfactory samples were attributed to domestic conventional and organically grown leafy greens. Strawberries, sprouted seeds and capsicums also contributed to a lesser extent.

A review of current domestic practices for leafy greens is suggested to determine whether microbiological quality and safety can be improved. The dominance of *E. coli* as a proportion of the faecal coliform population on leafy greens suggests that the current Ministry of Health guidelines are scientifically no longer appropriate. A guideline based on *E. coli* rather than faecal coliforms might be more robust. The inclusion of testing for additional pathogens such as non-O157 STECs, *Listeria monocytogenes*, viruses and protozoa would allow future produce surveys to more comprehensively assess the risks associated with the production, processing and consumption of fresh fruits and vegetables.

1 INTRODUCTION

A discussion document commissioned by the New Zealand Food Safety Authority (NZFSA) has recently reviewed the status of pathogens in fruits and vegetables in New Zealand and overseas (McIntyre *et al*., 2008). It identified green leafy vegetables (e.g. spinach, lettuce), melons, tomatoes, raw berry fruits, unpasteurised fruit juices and sprouted seeds as food commodity of increasing concern internationally in terms of consumer safety. These fresh produce items have posed a particular challenge to producers and regulators alike for a number of reasons including (i) the numerous opportunities for contamination to occur throughout the production chain, (ii) their ability in some cases to support pathogen growth and (iii) a general absence of suitable and effective decontamination measures. A number of other produce items, including fresh herbs, have also been implicated in outbreaks.

Despite the obvious potential for produce-related food safety issues to occur, only one confirmed outbreak – Hepatitis A in raw blueberries – has been documented in New Zealand (Calder *et al*., 2003). However, other unconfirmed outbreaks have been identified, including two *Salmonella* outbreaks tentatively linked to carrots (Neuwelt *et al*., 2006) and watermelon (M. Wilson, pers. comm.), and a further outbreak investigation which revealed the presence of shiga-toxin producing *E. coli* (STEC) O157 in stream water being used as a source of farm-level wash water (M. Wilson, pers. comm.). These outbreaks signal failures in good agricultural practices at a number of levels, including poor hygiene and sanitation, inadequate product washing or the use of contaminated wash water.

A review of national microbiological surveys carried out to date identified the absence of *E. coli* O157 and *Salmonella* spp. in 765 samples of hydroponically grown leafy vegetables, sprouted seeds and herbs (Graham & Dawson, 2002) and conventional and organically-grown lettuces (Wong, 2003). *Salmonella* Typhimurium DT12a was however detected in one of 230 batches of organic apples (Wong, 2003). Surveys conducted in the U.S. and elsewhere have reported prevalence values ranging from 0 to 100% for various bacterial groups, which is unsurprising given the variability occurring between studies in relation to testing criteria such as choice of target pathogen(s), methodology and sample size. However, such variability also suggests that this is an area that needs to be addressed in order to ensure appropriate, comparable and robust testing protocols for both survey work and routine testing.

In light of the limited nature of national produce surveys to date, a more comprehensive microbiological survey was commissioned by the NZFSA to investigate the quality and safety of imported, domestic conventional and domestic organic produce available for retail purchase in this country, and to establish a bench mark against which future surveys can be compared. Produce types to be surveyed were selected on the basis of international evidence for their involvement as vehicles of infection, availability (particularly for imported produce) and methods of growth. Pathogens of interest, sample size and methods of analysis were determined in the context of both domestic and international evidence of outbreaks and previously conducted surveys.

2 MATERIALS AND METHODS

2.1 Survey scope

To determine the potential exposure of New Zealand consumers to pathogens in fruits and vegetables, samples were purchased at the retail level to take into consideration all possible routes of contamination (including post-pack house transportation and handling) excluding those related to consumer food handling in the home. Supermarkets, green grocers, specialist organic shops and weekend farmers markets in both Auckland and Christchurch were selected to represent the produce buying habits of a broad cross-section of NZ consumers, and to enable the capture of sufficient samples of domestic conventional, domestic organic and imported origin. The produce items tested are listed in Table 1, with a justification for their selection. To achieve the greatest diversity (and thus minimise bias) in terms of producers, samples were collected at intervals during the survey period. However, it was necessary to sample intensively over a short time period for certain organic and imported commodities with short seasonal availability which may have biased sampling to some extent. Nonetheless, this reflects the New Zealand situation where only a small number of producers exist for certain commodities. This was particularly true for organic strawberries which had to be sourced directly from the only grower (located on the South Island) due to insufficient sample availability at the retail level.

Three pathogen groups – *E. coli* O157, *Salmonella* spp. and *Campylobacter* spp. – were selected as the most relevant pathogenic analytes due primarily to the frequency of international recalls and outbreaks associated with *E. coli* O157 and *Salmonella* (McIntyre *et al*., 2008), their association with animal faecal material (a potential source of contamination either directly or via contaminated water) and the predominance of *Campylobacter* as the most frequently notified cause of bacterial gastrointestinal foodborne disease in New Zealand (ESR, 2008).

2.2 Sample collection

Produce sampling from supermarkets, greengrocers, organic shops and farmers markets commenced in Christchurch on 5th May 2008 and in Auckland on 16th June 2008. Samples were collected by ESR staff in Christchurch and a Food Act Officer in Auckland.

Up to 25 sample units of different produce items, weighing between 750 g and 1000 g were collected weekly, alternating between the two sampling locales. Sampling was pre-determined to some extent by seasonal availability and, where possible, samples were collected from a range of retail outlets. Samples collected in Auckland were packed in chilly bins with gel freezer packs and couriered overnight to ESR's Public Health Laboratory (PHL) at Christchurch Science Centre for next day analysis. Farmers market samples collected at weekends were held under refrigeration at ESR's Auckland site (Mt. Albert Science Centre) prior to overnight courier delivery to PHL. In total, 50 samples of each item were collected with an approximate 50:50 split between sampling locations (see Table 3 for full details).

Table 1: Produce items selected for inclusion in survey with justification

Item	Imported (conventional / organic)	Domestic conventionally grown	Domestic organically grown	Justification for selection
Melons	✓	-	-	Recent outbreak in Australia. Concerns regarding retail cutting and storage practices.
Tomatoes	-	✓	✓	Previous international outbreaks.
Strawberries	✓	✓	✓	Grown in close proximity to ground/soil. More highly handled with minimal washing.
Apples	✓	✓	✓	Has been previously surveyed in NZ. More likely to be contaminated by birds flying overhead.
Table grapes	✓	-	-	Mostly imported. Some local grapes (but majority grown for wine). Thought to be more of a risk due to birds.
Leafy greens*	-	✓	✓	Previous international outbreaks. Grown in close proximity to ground/soil.
Capsicums	✓	✓	✓	Grown in close proximity to ground/soil. All three types sourced in large amounts.
Carrots	-	✓	✓	*Salmonella* outbreak in NZ associated with carrots (not confirmed). Grown in close proximity to ground/soil.
Sprouted seeds	-	✓	-	Previous international outbreaks (incl. Australia). Sprouts not imported into NZ but seed stock imported from Australia. Contaminated seed is believed to be the source of pathogens detected on sprouts.

*lettuce, baby (salad) spinach, kale

2.3 Methodology

2.3.1 Sample preparation

One sub-sample of approximately 250 g (or one melon) was prepared for each microbiological analysis in a manner consistent with consumer preparation of the fruit/vegetable (FDA, 2005). Lettuces had their outer lettuce leaves removed. Grapes (and tomatoes as necessary) were carefully removed from stalks. Samples were bagged and rinsed with tap water using gentle agitation for 30 s to remove any visible dirt. With the exception of lettuces which were cored, samples were tested whole to avoid the release of acidic cell exudates which could reduce the pH of the enrichment medium and therefore potentially reduce pathogen recovery (Burnett and Beuchat, 2001). This resulted in sample weights typically greater than (or rarely, less than) 250 g. The sub-sample was assumed to be microbiologically representative of the original 750 – 1000 g analytical unit collected. However, it is acknowledged that individual fruits/vegetables may have been exposed to potentially different levels/types of contamination, and it is therefore conceivable that a sub-sample tested for one pathogen may have been positive for one/both of the other pathogens of interest.

2.3.2 Rinse method for faecal coliforms and generic *E. coli* enumeration

Samples to be tested for coliforms, *E. coli* and *E. coli* O157 were placed into a sterile stomacher bag and a 1:1 weight of buffered peptone water (BPW; Merck KGaA, Darmstadt, Germany) was added. The bag contents were gently shaken to avoid tissue damage at 100 rpm for 5 minutes using a mechanical shaker. A 50 mL volume of sample rinse was then removed and used to set up 3 levels of a 3-tube MPN (from 10 to 0.1 mL) using LT broth (Fort Richard Laboratories Ltd., Auckland, New Zealand). The remaining broth was used to set up enrichments for *E. coli* O157 as described in sections 2.3.3 and 2.3.4. Tubes were incubated at 35°C for up to 48 h followed by sub-culture of positive tube contents into EC-MUG broth (Fort Richard Laboratories Ltd., Auckland, New Zealand) incubated at 44.5°C for 48 h. In the event that results were positive for all 3 levels of MPN, retained rinse held at 4°C was re-tested at higher dilutions (0.01 to 0.0001 mL) to obtain an MPN count. The presence of *E. coli* was confirmed by fluorescence and plating on LEMB agar (Merck KGaA, Darmstadt, Germany) followed by indole testing of positive colonies. Further characterisation of *E. coli* isolates was not conducted.

2.3.3 Enrichment-based pathogen detection methods

For melons, tomatoes, capsicums, strawberries, apples and grapes, a *floating enrichment* method, based on the FDA soak method for *Salmonella* analysis in cantaloupe melon, was devised for all pathogen analyses (FDA, 2005). Each sample was placed into a sterile plastic bag and sufficient enrichment broth was added to allow it to float (typically 1.5 times the weight of sample). The bag was then loosely tied. For melons only, bags were hung from the incubator shelf to ensure sufficient immersion, ensuring that each bag did not come into contact with others. For lettuce, carrots and sprouted seeds, sufficient volumes (at least 250 mL) of enrichment broths were added to adequately immerse samples (*soak enrichment*). The bag was then loosely tied. Media, incubation temperatures and confirmation testing are described below for each specific pathogen.

2.3.3.1 *E. coli* O157 detection

Samples enriched in BPW (Merck KGaA, Darmstadt, Germany) were incubated at 42°C for 24 h. A 1 mL volume of enriched BPW was then removed and immunomagnetic separation was conducted according to the manufacturer's instructions (Invitrogen Dynal AS, Oslo, Norway) using CHROMagar (Fort Richard Laboratories Ltd., Auckland, New Zealand) and CT-SMAC (Becton Dickinson, Sparks, MN, USA) for selective plating. Suspect colonies were plated onto EHEC agar (Fort Richard Laboratories Ltd., Auckland, New Zealand) and identified using Microgen™ GN-IDA and GN-IDB kits (Microgen Bioproducts Ltd., Camberley, UK).

2.3.3.2 *Salmonella* detection

Samples enriched in lactose broth (Merck KGaA, Darmstadt, Germany) were incubated at 35°C for 24 h. Following incubation the pre-enrichment broths were manually mixed and selectively enriched by removing 0.1 mL and dispensing into 10 mL RVS broth (Fort Richard Laboratories Ltd., Auckland, New Zealand) incubated at 42°C for 24 h. A further 1.0 mL volume of pre-enrichment broth was dispensed into 10 mL TET broth (Merck KGaA, Darmstadt, Germany) and incubated at 35°C for 24 h. Selective plating was conducted using XLD agar (Merck KGaA, Darmstadt, Germany), Hektoen agar (Becton Dickinson, Sparks, MN, USA) and Bismuth Sulfite agar (Becton Dickinson, Sparks, MN, USA). Presumptive positive colonies were further characterised by biochemical testing (indole, urease, TSI and LIA), serotyping, phage typing and PFGE.

2.3.3.3 *Campylobacter* detection

Samples enriched in Exeter broth (Oxoid Ltd., Basingstoke, UK) were pre-incubated for 4 h at 37°C followed by 42°C incubation for a further 44 ± 2 h using a 10% CO_2 incubator. After incubation, enrichment broths were manually mixed and streaked onto mCCDA plates (Fort Richard Laboratories Ltd., Auckland, New Zealand) which were incubated for a further 48 ± 2 h prior to determination of colony morphology and further characterisation as necessary.

Positive and negative controls were set up for all analyses.

2.4 Data analysis

Results were interpreted using the New Zealand Ministry of Health (MoH) microbiological reference criteria (Ministry of Health, 1995) and Food Standards Australia New Zealand (FSANZ) guidelines for the microbiological examination of ready-to-eat (RTE) foods (FSANZ, 2001), both of which are currently applicable to fruits and vegetables. Samples were deemed satisfactory, marginal or unsatisfactory based on the microbiological limits summarised in Table 2.

Table 2: Microbiological limits used for interpretation of survey data

Food	Test	Satisfactory	Marginal	Unsatisfactory	Potentially hazardous	Reference
Fruits and vegetables	Faecal coliforms (/g)	$<10^2$	$\geq 10^2 - <10^3$	$\geq 10^3$		5.25; MoH, 1995
Fruits and vegetables	*Salmonella*	Not detected in 25 g				5.25; MoH, 1995
Sprouted seeds	*E. coli*	Not detected in 1 g				5.5; MoH, 1995
Sprouted seeds	*Salmonella*	Not detected in 25 g				5.5; MoH, 1995
Ready-to-eat foods	*E. coli* (/g)	<3	3 – <100	≥100	*	FSANZ, 2001
Ready-to-eat foods	*Campylobacter*	Not detected in 25 g			Detected	FSANZ, 2001
Ready-to-eat foods	*Salmonella*	Not detected in 25 g			Detected	FSANZ, 2001

* Pathogenic strains of *E. coli* should be absent.

3 RESULTS AND DISCUSSION

3.1 Samples and Premises

A summary of sample purchases and premises types is presented in Table 3. Over the 15 month survey period, 891 samples comprising imported (n=226), domestic conventional (n=349) and domestic organic (n=316) produce were collected from farmers markets (n=40), greengrocers (n=247), specialist organic shops (n=249) and supermarkets (n=355) in Auckland (n=424) and Christchurch (n=467). Overall, the frequency of sampling in each premises type was similar for both locations, although approximately 10% more samples were purchased from supermarkets in Christchurch. In terms of produce type, most organic produce items (75%) were sourced from specialist organic shops, while imported produce were sampled most frequently from supermarkets (62%). However, domestic conventionally grown items were sampled more often from supermarkets (63%) than greengrocers (26%) in Christchurch, and vice versa for Auckland (35% v 54%, respectively). In Auckland, seven domestic conventional and four imported items were procured from specialist organic shops.

3.2 Microbiological analysis results

Data interrogated using relevant microbiological limits (summarised in Table 2) are presented in Table 4. Overall, 96.6% (812/841) and 95.4% (850/891) of tested produce was satisfactory based on microbiological limits for salads (excluding sprouted seeds) and RTE foods (including sprouted seeds), respectively. Combining the salads and sprouts data and applying both Ministry of Health microbiological limits gave a result of 95.6% (852/891), virtually identical to that obtained using the RTE Foods guidelines.

A further 3.4% (29/841) of salads (excluding sprouted seeds) and 4.6% (41/891) of RTE foods (including sprouted seeds) were considered either marginal or unsatisfactory. All were of domestic origin. Regardless of the limits applied, leafy greens (lettuce, spinach and kale) were responsible for between 71% (29/41) and 76% (22/29) of the marginal and unsatisfactory results obtained, with counts ranging from 1.5×10^2 to 1.1×10^4 MPN/g for faecal coliforms and 4.8×10^0 to 1.1×10^4 MPN/g for *E. coli* (Table 5).

Application of the MoH faecal coliforms reference criterion resulted in 20/29 marginal samples and 9/29 unsatisfactory. However, of these same 29 samples, 24 (83%) samples had *E. coli* counts of the same magnitude as faecal coliforms. This finding has potential implications regarding the use of bacterial indicator organisms to assess the quality of fresh produce, particularly when the MoH reference criteria permit a 10-fold higher level of faecal coliforms than the FSANZ RTE foods limits based on *E. coli*. In terms of interpreting these data, Kornacki and Johnson (2001) have stated that "since the proportion of *E. coli* within the fecal coliform population varies between samples, there is little reason to stop at the fecal coliform test when *E. coli* is really the object of interest". In this case *E. coli* appears to dominate the faecal coliform population, particularly for leafy greens, suggesting that the use of the MoH faecal coliforms reference criterion is potentially inappropriate when evaluating the microbiological status of these (and possibly other) fresh produce items. Further consideration of the most appropriate quality indicator appears to be warranted based on these findings.

Table 3: Summary of sampling location and retail premises used during survey

		Auckland					Christchurch					
Food item	**Type**	**Farmers Markets**	**Green Grocers**	**Specialist Organic Shops**	**Super-markets**	**Total**	**Farmers Markets**	**Green Grocers**	**Specialist Organic Shops**	**Super-markets**	**Total**	**Grand Total**
Apples	Conventional	4	21			25	1	6		18	25	50
	Imported[1]				25	25		10		15	25	50
	Organic		2	19	4	25	3		20	3	26	51
Capsicums	Conventional		17		8	25	6	10		12	28	53
	Imported							22		4	26	26
	Organic			25		25		10	14	1	25	50
Carrots	Conventional	1	13	3	10	27	5	5		15	25	52
	Organic		1	19	5	25	4	1	15	5	25	50
Grapes	Imported		17	2	6	25		9		16	25	50
Leafy greens	Conventional	7	8		10	25	4	9		12	25	50
	Organic		1	21	3	25	1		21	11	33	58
Melons	Imported		10	2	13	25		7		18	25	50
Sprouted seeds	Conventional		3		16	19		3		22	25	44
	Organic				6	6						6
Strawberries	Conventional		12	4	9	25		9		16	25	50
	Imported		2		23	25		5		20	25	50
	Organic			21	1	22			28		28	50
Tomatoes	Conventional		19		6	25	3	5		17	25	50
	Organic			22	3	25	1	10	13	2	26	51
Grand Total		12	126	138	148	424	28	121	111	207	467	891
%		2.8%	29.7%	32.5%	34.9%		6.0%	25.9%	23.8%	44.3%		

[1] Imported produce assumed to be conventionally grown unless otherwise stated

Table 4: Interpretation of faecal coliforms[a] and *E. coli*[b] results (/g) using Ministry of Health and FSANZ microbiological limits

		5.25 Salads (MoH, 1995)[a]			5.5 Sprouts (MoH, 1995)[b]		RTE Foods (FSANZ, 2001)[b]		
Food	**Source**	**Satisfactory $<10^2$**	**Marginal $\geq10^2$ - $<10^3$**	**Unsatisfactory $\geq10^3$**	**Satisfactory *E. coli* -ve**	**Unsatisfactory *E. coli* +ve**	**Satisfactory <3**	**Marginal 3 - <100**	**Unsatisfactory ≥100**
Apples	Conventional	50					50		
	Imported	50					50		
	Organic	51					51		
Capsicums	Conventional	52	1				52	1	
	Imported	26					26		
	Organic	49		1			48	1	1
Carrots	Conventional	52					52		
	Organic	50					50		
Grapes	Imported	50					50		
Leafy greens	Conventional	46	3	1			42	5	3
	Organic	40	13	5			37	4	17
Melons	Imported	50					50		
Sprouted seeds	Conventional				35	9	43	1	
	Organic				5	1	6		
Strawberries	Conventional	49		1			48	1	1
	Imported	50					50		
	Organic	46	3	1			44	2	4
Tomatoes	Conventional	50					50		
	Organic	51					51		
Total		812	20	9	40	10	850	15	26
Percentage		96.6%	2.4%	1.0%	80.0%	20.0%	95.4%	1.7%	2.9%

Table 5: Summary of marginal and unsatisfactory results based on faecal coliforms and *E. coli* counts

Produce	Micro limit used	Result	Count range (MPN/g)	Mean count (MPN/g)	No. marginal / unsatisfactory samples	Sample type
Leafy greens	Faecal coliforms	Marginal	$1.5 \times 10^2 – 9.2 \times 10^2$	3.8×10^2	16	3 conv (spinach), 13 org (10 kale, 2 spinach, 1 lettuce)
Leafy greens	Faecal coliforms	Unsatisfactory	$1.5 \times 10^3 – 1.1 \times 10^4$	5.6×10^3	6	1 conv (spinach), 5 org (kale)
Leafy greens	*E. coli*	Marginal	$4.8 \times 10^0 – 9.2 \times 10^1$	4.6×10^1	9	5 conv (1 lettuce, 4 spinach), 4 org (lettuce)
Leafy greens	*E. coli*	Unsatisfactory	$1.5 \times 10^2 – 1.1 \times 10^4$	1.9×10^3	20	3 conv (spinach), 17 org (15 kale, 2 spinach)
Capsicum	Faecal coliforms	Marginal	2.9×10^2	-	1	1 conv
Capsicum	Faecal coliforms	Unsatisfactory	1.1×10^4	-	1	1 org
Capsicum	*E. coli*	Marginal	$1.1 \times 10^1 – 7.5 \times 10^1$	4.3×10^1	2	1 conv, 1 org
Capsicum	*E. coli*	Unsatisfactory	1.1×10^4	-	1	1 org
Sprouted seeds	*E. coli*	Unsatisfactory	$1.0 \times 10^{-1} – 1.1 \times 10^1$	6.8×10^0	10	9 conv, 1 org
Sprouted seeds	*E. coli*	Marginal	1.1×10^1	-	1	1 conv
Strawberries	Faecal coliforms	Marginal	$4.2 \times 10^2 – 9.2 \times 10^2$	7.4×10^2	3	3 org
Strawberries	Faecal coliforms	Unsatisfactory	$1.1 \times 10^3 – 1.1 \times 10^4$	6.0×10^3	2	1 conv, 1 org
Strawberries	*E. coli*	Marginal	$4.6 \times 10^0 – 9.2 \times 10^1$	3.6×10^1	3	1 conv, 2 org
Strawberries	*E. coli*	Unsatisfactory	$4.2 \times 10^2 – 1.1 \times 10^4$	2.7×10^3	5	1 conv, 4 org

Between 72% (21/29) and 82% (18/22) of the marginal and unsatisfactory results obtained for leafy greens were due to organically grown spinach, lettuce and kale. However, it was noted that 15 samples of organic kale, of uniformly poor microbiological quality, were purchased from the same premises on a single sampling day which increased the number of marginal/unsatisfactory samples obtained overall. Excluding these results reduced the percentage of marginal and unsatisfactory results attributed to organic leafy greens to 43%, but leafy greens as a whole were still responsible for the highest proportion (54%; 14/26) of marginal and unsatisfactory results obtained overall. The next highest category was strawberries with 31% (8/26).

All imported samples (apples, capsicums, grapes, melons and strawberries; n=226) were of a satisfactory nature, with domestic conventional and domestic organic products ranging from 96.6% to 98.0% and 90.8% to 92.6%, respectively, depending on the microbiological limits applied (Table 6).

Table 6: Comparison of satisfactory results obtained for imported, domestic conventional and domestic organic produce

	5.25 Salads (MoH, 1995)	5.5 Sprouts (MoH, 1995)	RTE Foods (FSANZ, 2001)
Imported	100% (226/226)	-	100% (226/226)
Domestic conventional	98.0% (299/305)	80% (40/50)	96.6% (337/349)
Domestic organic	92.6% (287/310)	100% (6/6)	90.8% (287/316)

While direct comparisons between the different types of produce are difficult, as not all items were sampled in parallel, a direct comparison of imported and domestic conventional apples, capsicums and strawberries (Table 7) revealed little difference in microbiological quality between the two groups.

Table 7: Comparison of satisfactory results obtained for imported and domestic conventional fruits and vegetables

	5.25 Salads (MoH, 1995)[a]		RTE Foods (FSANZ, 2001)[b]	
	Imported	Domestic conventional	Imported	Domestic conventional
Apples	100% (50/50)	100% (50/50)	100% (50/50)	100% (50/50)
Capsicums	100% (26/26)	98.1% (52/53)	100% (26/26)	98.1% (52/53)
Strawberries	100% (50/50)	98.0% (49/50)	100% (50/50)	96% (48/50)

Employing both microbiological limits applicable to sprouted seeds created profound differences in terms of the interpretation of results. Using Ministry of Health reference criteria which do not tolerate the presence of *E. coli* per gram, 20% (10/50) of samples were categorised as unsatisfactory despite negative pathogen results. In contrast, 98% (49/50) of samples were deemed satisfactory using FSANZ limits which permit an *E. coli* count of <3/g. A number of samples (25) had faecal coliform counts greater than 10 MPN/g, of which two were > 10^4 MPN/g. However, in contrast to leafy greens, only one sample had an *E. coli* count equivalent to the level of faecal coliforms (11 MPN/g) and was considered marginal.

There is ample evidence to support food safety concerns regarding sprouted seeds, and the stricter approach adopted by the Ministry of Health in 1995 may be more of a reflection of the level of concern existing at that particular time point. This is, however, a potentially moot point as it could also be argued that the application of these limits is somewhat inappropriate given the larger 250 g sample sizes tested in this survey as opposed to smaller sample sizes (1 – 25 g) upon which these limits were likely established.

The results of a previous survey of sprouted seeds (Graham & Dawson, 2002) identified 12.8% (15/117) of marginal or unsatisfactory samples based on the Ministry of Health reference criteria. The apparent decline in sprout quality suggested by our results is however more likely to be a function of the larger sample size employed for microbiological analysis in this survey (as mentioned above) and the use of different testing methods.

Campylobacter spp. and *E. coli* O157 were not detected in this survey but the presence of non-O157 *E. coli* was not evaluated. In future surveys, this would be useful to include to further characterise the *E. coli* isolates obtained by enumeration. The presence of *Salmonella* Typhimurium phage type RDNC-May 06 was confirmed on two samples of domestic organic lettuce purchased in Christchurch. PFGE results (Appendix 1) revealed similar profiles. Interestingly both samples were deemed satisfactory in terms of faecal coliforms, while only one sample was considered marginal based on *E. coli* concentrations. A similar lack of association between *E. coli* (presence/concentration) and the presence of *Salmonella* contamination has also been reported in other surveys (Abadias *et al*., 2008; Arthur *et al*., 2007; Mukherjee *et al*., 2004).

A visit to the organic lettuce grower, including observations of growing and harvesting practices, identified bird faeces on hail netting located directly above growing produce, which was particularly concentrated in areas where birds were able to land on metal hoops holding the netting up. It is likely that contamination occurred either through direct defecation onto plants below or indirectly via overhead irrigation and/or precipitation. Testing of facecal material to confirm the presence of the same *Salmonella* phage type isolated from lettuces was not, however, carried out. Regardless, it is important to acknowledge that this type of sporadic contamination event, which could potentially contribute to spikes in illness in consumers from time to time, would usually be very difficult to identify in routine sampling and survey work.

The pathogen detection results obtained in this survey are in strong agreement with the very low prevalence reported from a number of national and international surveys investigating the presence of *E. coli* O157, *Salmonella* and *Campylobacter* in domestic and imported produce grown using conventional and/or organic methods (Table 8). Pathogen prevalence in produce grown in developing countries can be much higher (Viswanathan & Kaur, 2001; Nguz *et* al., 2005; Saroj *et al*., 2006) but it was not considered appropriate to include this information, due to potential differences in growing methods, hygiene and sanitation practices, and regulatory frameworks.

All pathogen detections reported in Table 8, including this survey, have been exclusively *Salmonella* spp. This may be attributable to a number of factors including better environmental persistence, a greater variety of potential sources of the pathogen, the possibility of contamination occurring later in the food chain and differences in detection methods. It is also apparent that organic produce, particularly leafy greens, make up a

Table 8: Summary of pathogen detection results from international produce surveys

Country	Produce types tested	*E. coli* O157	*Campylobacter*	*Salmonella*	Produce positive for pathogens	Reference
Australia	Imported & domestic	0/491	0/479	0/491		Dept. Health, Government of Western Australia, 2005
England & Wales	Imported & domestic (incl. organic)	0/11958	0/10153	5/11958	Conventional and organic lettuce	Little & Gillespie, 2008
Canada	Domestic (incl. organic)	0/1183	N.T.	2/1183	Roma tomato, organic lettuce	Arthur *et al*., 2007
Ireland	Not specified	0/161	N.T.	1/556	Spinach	FSAI, 2002
New Zealand	Domestic (organic & conventional)	0/765	N.T.	1/230	Organic apples	Graham & Dawson, 2002; Wong, 2003
New Zealand	Imported & domestic (incl. organic)	0/850	0/850	2/850	Organic lettuce	This survey
Northern Ireland	Domestic (organic)	0/86	0/86	0/86		McMahon & Wilson, 2001
Norway	Domestic	0/1190	N.T.	0/1190		Robertson *et al*., 2002; Johannessen *et al*., 2002
Spain	Not specified	0/300	0/300	4/300	Corn salad, lettuce, spinach, mixed salad	Abadias *et al*., 2008
USA	Domestic (organic, semiorganic & conventional)	0/2634	N.T.	2/2634	Organic lettuce, organic capsicum	Mukherjee *et al*., 2004; Mukherjee *et al*., 2006
USA	Domestic	0/398	N.T.	3/398	Cantaloupe melon	Johnston *et al*., 2005
USA	Domestic & imported	0/466	N.T.	0/466		Johnston *et al*., 2006
Total		0/20,342	0/11,868	**20/20,342**		

N.T.: Not tested

significant proportion of the pathogen-positive samples detected in these surveys which would be anticipated based on methods of production and opportunities for cross-contamination (McIntyre et al., 2008) and is in agreement with our findings from this survey.

4 CONCLUSIONS

This survey of imported and domestically grown conventional and organic produce has revealed very low prevalence of selected pathogens at the retail level in New Zealand, with the majority of produce determined to be of satisfactory quality based on indicator organism levels. This is in strong agreement with international surveys conducted in a number of developed countries. Based on their contribution to marginal and unsatisfactory results, and the detection of *Salmonella* Typhimurium in two organic lettuce samples, leafy greens have been identified as the produce type most likely to be of concern. This is again in keeping with international findings, and signals a need to investigate current practices, both for conventional and organic production, to determine whether microbiological quality and safety can be improved. The dominance of *E. coli* as a proportion of the faecal coliform population on leafy greens suggests that the current Ministry of Health guidelines are scientifically no longer appropriate. A guideline based on *E. coli* rather than faecal coliforms might be more robust.

Overall, despite the low risk associated with fresh produce as indicated by the results of this survey, it is important to acknowledge that sporadic contamination events may occur from time to time which could potentially contribute to spikes in illness in consumers. The inclusion of additional pathogens such as non-O157 STECs, *Listeria monocytogenes*, viruses and protozoa would allow future produce surveys to more comprehensively assess the risks associated with the production, processing and consumption of fresh fruits and vegetables.

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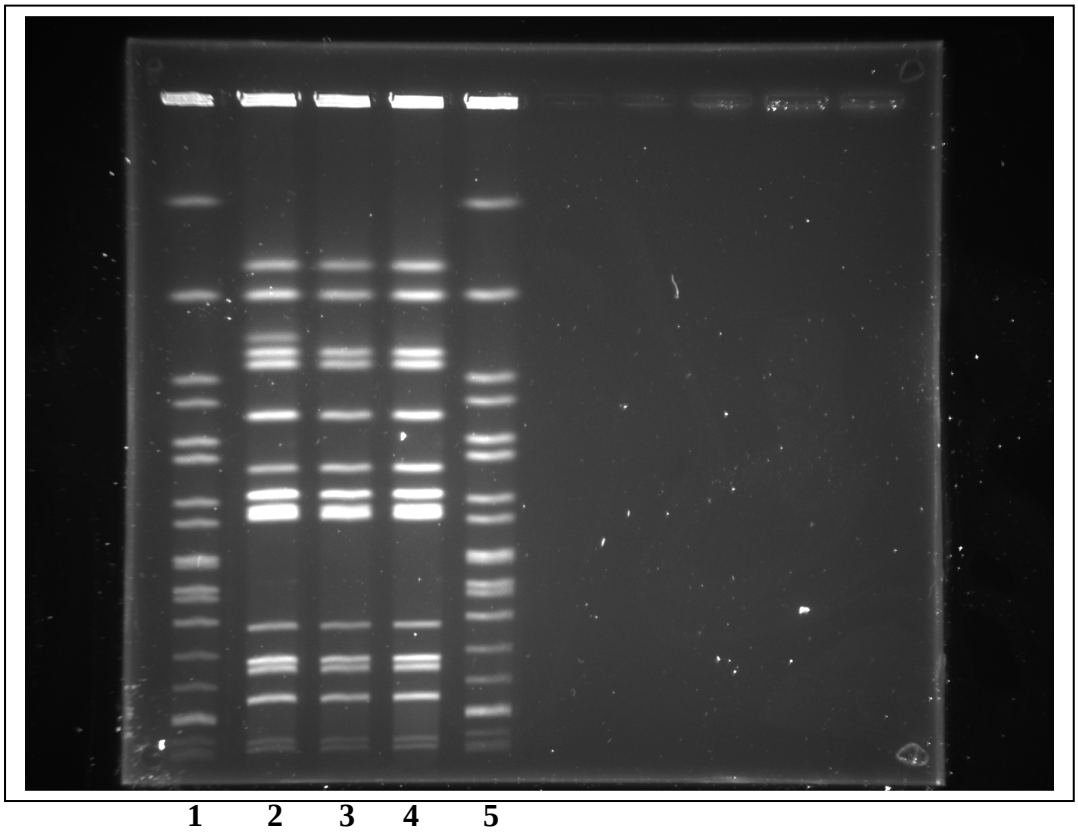
APPENDIX 1: PFGE PROFILES FOR *SALMONELLA* ISOLATES



Lanes 1 & 5: molecular weight markers
Lane 2: Sample CPH0815112 (ERL08-4043)
Lane 3: Sample CPH0815688 (ERL08-4322)
Lane 4: Sample CPH0815688 (ERL08-4323)