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Wide geographical distribution of internationally rare *Campylobacter* clones within New Zealand

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SUMMARY

During the southern hemisphere winter of 2006 New Zealand experienced a significant increase in the number of reported cases of *Campylobacter* infection. In total, 112 *Campylobacter* isolates from eight district health boards (DHBs) located across New Zealand were submitted for PFGE, MLST and Penner serotyping analysis. Distinct clusters of *Campylobacter* isolates were identified, several of which were composed of isolates from up to five different DHBs located on both the North and South islands of New Zealand. One sequence type, ST-474, was identified in 32 of the 112 isolates and may represent an endemic sequence type present in New Zealand. The spatial pattern of genotypes, combined with the generalized increase in notifications throughout the country is consistent with a common source epidemic, most likely from a source contaminated with the dominant sequence types ST-474 and ST-190 and may also represent widely distributed stable clones present in New Zealand.

INTRODUCTION

Campylobacter jejuni and *Campylobacter coli* are the most commonly identified bacterial agents of gastroenteritis in the developed world [1]. The majority of *Campylobacter* infections appear to be sporadic with relatively few outbreaks being reported, although this may be due to inadequate ascertainment of related cases [2]. The predominant source of the infectious agent is thought to be food. However, the widespread distribution of *Campylobacter* spp. in the environment suggests that other sources may be important.

New Zealand has one of the highest rates of campylobacteriosis recorded in the developed world [3]. The notification rate in 2006 was 383·5/100 000, which is at least threefold higher than seen in other industrialized countries [4]. The causes of the increased incidence in New Zealand have yet to be identified, although, as found elsewhere in the world, poultry consumption has been identified as a risk factor [4–8]. The seasonality and age distribution of cases are similar to those found elsewhere [3, 9, 10]. To identify potential sources of contamination and transmission routes of *Campylobacter* spp., the distribution of individual strains within the environment and the relationship between strains must be determined. Attempts to do this in New Zealand using pulsed-field gel electrophoresis (PFGE) have suggested possible transmission routes such as animal faeces and water but definitive answers require a more detailed knowledge of the population structure [11].

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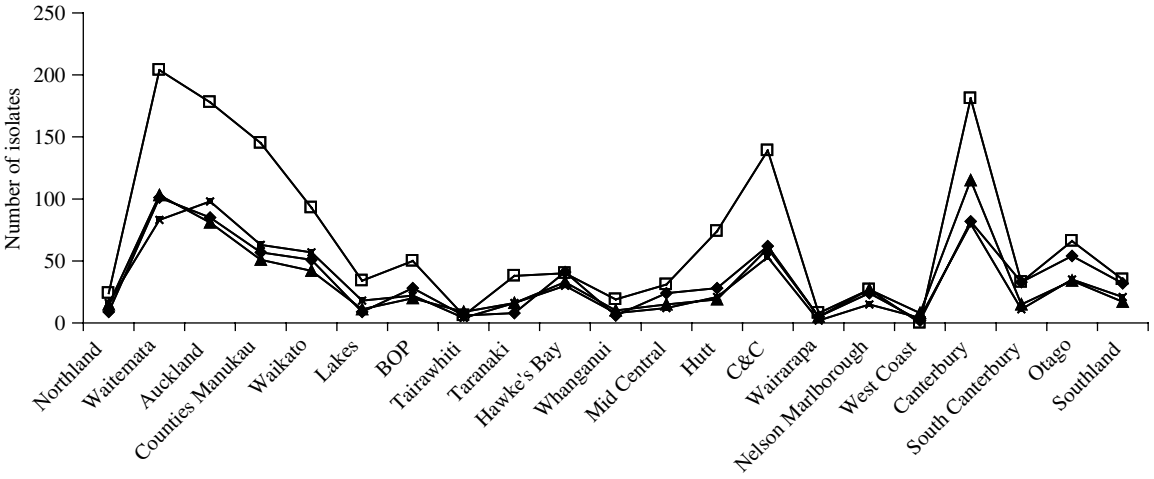

Fig. 1. The number of campylobacteriosis reports in the district health boards of New Zealand, May 2003–2006. C&C, Capital & Coast; BOP, Bay of Plenty; ▲, 2003; ■, 2004; ◆, 2005; □, 2006.

The characterization of *Campylobacter* populations has undergone significant improvements in recent years with the development of a number of genotypic methods [12]. Many studies have utilized variation in macrorestriction profiles or flagellin gene (*flaA*) sequence to identify possible outbreaks and examine sources of infection [5, 13, 14]. However, the large degree of genetic variation seen in both the PFGE and *flaA* sequence profiles has limited the application of these methods for population studies of *C. jejuni* and *C. coli*. The development of a highly portable multi-locus sequence typing (MLST) scheme for *C. jejuni* and *C. coli* has provided significant information on the population structure of *C. jejuni and C. coli* isolates in human clinical samples, animal hosts and the environment [15–18]. A limited number of clonal complexes (CC) have been detected and host-specific sequence types (ST) and alleles identified. The current *C. jejuni*/*C. coli* MLST database containing strain and sequence information is composed mainly of isolates from a limited number of countries (http://pubmlst.org/campylobacter/).

During May and June of 2006 New Zealand experienced an increase in the number of reported campylobacteriosis cases [19]. This unexpected rise in cases observed by the majority of New Zealand district health boards (DHBs) (Fig. 1) prompted this investigation. In total, 112 human isolates of *Campylobacter* were collected from eight DHBs within a 2-month period and characterized by PFGE, MLST and Penner serotyping.

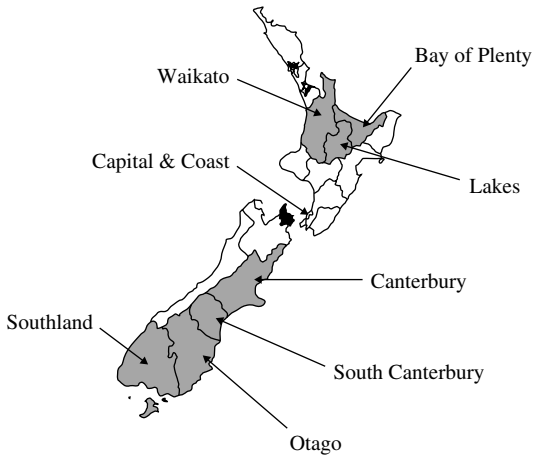

Fig. 2. The location of the eight district health boards in New Zealand that sent *Campylobacter* isolates for typing and subtyping analysis.

MATERIAL AND METHODS

As part of the investigation into the increase in *Campylobacter* reports, isolates were requested from laboratories serving the major New Zealand DHBs and eight responded (Fig. 2). All isolates came from diarrhoeic patients (60 females, 52 males) with ages ranging from 0 to 93 years (Table 1). DHBs were asked to submit consecutive isolates excluding repeat isolates and isolates from families. A total of 112 *Campylobacter* isolates were characterized (five *C. coli* and 107 *C. jejuni*). All isolates were grown on 5% (sheep blood) Columbia Blood agar plates at 42 °C

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for 48 h in microaerophilic conditions and speciated following standard microbiological procedures.

MLST was performed as described previously [16]. Chromosomal DNA was prepared from freshly grown cultures by boiling for 10 min followed by centrifugation of the disrupted cells. The supernatant was decanted to a fresh tube and used for amplification. The amplifications were performed in a 25 μl volume reaction using Applied Biosystems AmpliTaq Gold mastermix (Applied Biosystems, Auckland, New Zealand) and 5 pmol of each primer. Products were sequenced on an ABI 3130XL automated DNA sequencer using ABI BigDye v3.1 (Applied Biosystems) following the manufacturer's instructions. Sequence data was collated and alleles assigned using the *Campylobacter* PubMLST database (http://pubmlst.org/campylobacter/). Novel alleles and sequence types were submitted for allele and sequence-type designation as appropriate.

PFGE analysis was performed as described previously [20]. Isolates were prepared and digested with *Sma*I and run under standard PulseNet conditions. *Salmonella* Braenderup H9812 strain restricted with *Xba*I was run as a size standard. Patterns were clustered using BioNumerics v4.6 (Applied Maths, Ghent, Belgium). A second restriction enzyme, *Kpn*I, was used for further discrimination of isolates with indistinguishable *Sma*I patterns. PFGE clusters were defined using the BioNumerics software at 95% similarity using an optimization of 0·5% and a position tolerance of 1·5%. Patterns were compared with those in the PulseNet Aotearoa New Zealand Campylobacter database containing 1600 *Sma*I PFGE patterns and 505 *Kpn*I PFGE patterns from isolates obtained from diverse sources throughout New Zealand since 2001.

The sequence types identified by MLST were assigned to clonal complexes using the eBURST3 programme [21, 22]. Isolates were defined as belonging to a clonal complex if they shared four or more alleles with the central or founder sequence type. Penner serotyping was performed using a panel of 43 *C. jejuni* antisera produced in-house according to the method of Penner & Hennessy [23].

RESULTS

The 107 *C. jejuni* and five *C. coli* isolates received were characterized using PFGE, MLST and Penner serotyping. MLST analysis identified 25 sequence types including four that had not previously been recognized and another three that had been identified in New Zealand isolates only. One new *aspA* allele was identified in three *C. coli* isolates (ST-2397) (Table 1). Over one quarter of the isolates were ST-474 (32/112 isolates), 18 were ST-190 and nine were ST-354. ST-190 isolates were identified in all DHBs and ST-474 isolates in seven of the eight DHBs (Table 2). Over half the isolates (59%) belonged to two clonal complexes, CC ST-21 (32 isolates) and CC ST-48 (34 isolates).

The PFGE typing data identified isolates from throughout New Zealand with indistinguishable PFGE patterns (Table 1). Within the 112 isolates, 17 PFGE groups of two isolates or more (81 isolates) were identified, and of these 17 groups, 15 had isolates from two or more DHBs (74 isolates). PFGE analysis showed that 23 of the 32 ST-474 isolates belonged to three clusters (cluster J 7 isolates, cluster K 9 isolates and cluster L 7 isolates) with isolates from multiple DHBs. Digestion of the isolates with a second enzyme *Kpn*I indicated that isolates within these clusters were indistinguishable. Similarly, among the 18 ST-190 isolates, three clusters of 10, 3 and 3 isolates were identified (Table 1). Again, digestion with the second enzyme showed that isolates within each cluster were indistinguishable. Comparison with the PulseNet Aotearoa New Zealand Campylobacter database showed that six of the 17 *Sma*I clusters (B, K, L, M, P and S; Table 1, Fig. 3) had patterns not previously seen in New Zealand. Comparison of the *Sma*I PFGE patterns from each of the clusters showed that the CC ST-48 patterns were more closely related to each other than to other cluster patterns, as were the CC ST-21 patterns (Fig. 3).

Penner serotyping identified 15 different serotypes with the four-complex accounting for 32% of the isolates. All ST-190 isolates except one (untypable) belonged to serogroup 2 and all ST-474 isolates belonged to the four-complex serogroup. Isolates with identical sequence types and indistinguishable PFGE patterns using both restriction enzymes had identical serogroups (Table 1) except for one of the 10 isolates from ST190 cluster F.

DISCUSSION

Outbreaks of campylobacteriosis are rarely identified in relation to the number of cases reported. In 2005, 13 839 cases of campylobacteriosis were reported in New Zealand, yet only 47 *Campylobacter*-associated outbreaks were reported involving 252 cases [3]. This

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Table 1. *Subtyping data from New Zealand isolates* (*isolates are ordered by sequence type*)

ERL no.	Sex	Age (yr)	Submitting DHB	ST	CC	Serotype	*Sma*I	*Kpn*I	Species
ERL06-2307	M	52	C&C (N)	5	ST-353	4c			*C. jejuni*
ERL06-2420	M	12	Waikato (N)	21	ST-21	2	E		*C. jejuni*
ERL06-2261	M	71	BOP (N)	42	ST-42	23,36	N		*C. jejuni*
ERL06-2304	M	22	C&C (N)	42	ST-42	23,36	N		*C. jejuni*
ERL06-2332	M	8	Otago (S)	42	ST-42	23,36			*C. jejuni*
ERL06-2340	M	12	Otago (S)	42	ST-42	23,36			*C. jejuni*
ERL06-2357	M	35	Canterbury (S)	45	ST-45	42	M		*C. jejuni*
ERL06-2423	F	0	Waikato (N)	45	ST-45	UT	M		*C. jejuni*
ERL06-2268	F	44	Waikato (N)	45	ST-45	42	P		*C. jejuni*
ERL06-2377	M	24	Otago (S)	45	ST-45	57			*C. jejuni*
ERL06-2295	M	22	C&C (N)	48	ST-48	4c			*C. jejuni*
ERL06-2329	F	48	BOP (N)	50	ST-21	1c	A	10	*C. jejuni*
ERL06-2305	F	6	C&C (N)	50	ST-21	1c	A	10	*C. jejuni*
ERL06-2359	F	62	Canterbury (S)	50	ST-21	1c	A	10	*C. jejuni*
ERL06-2330	M	26	Otago (S)	50	ST-21	1c	A	10	*C. jejuni*
ERL06-2372	M	51	Otago (S)	50	ST-21	1c	A	10	*C. jejuni*
ERL06-2378	F	71	Southland (S)	50	ST-21	1c	A	10	*C. jejuni*
ERL06-2339	M	1	Otago (S)	50	ST-21	1c	A	10	*C. jejuni*
ERL06-2351	F	73	Canterbury (S)	50	ST-21	1c			*C. jejuni*
ERL06-2257	F	18	C&C (N)	52	ST-52	5	B	11	*C. jejuni*
ERL06-2298	F	32	C&C (N)	52	ST-52	5	B	11	*C. jejuni*
ERL06-2341	M	61	Otago (S)	52	ST-52	5	B	11	*C. jejuni*
ERL06-2272	F	52	Waikato (N)	52	ST-52	5	B	11	*C. jejuni*
ERL06-2343	M	19	Canterbury (S)	53	ST-21	2	C	12	*C. jejuni*
ERL06-2345	F	28	Canterbury (S)	53	ST-21	2	C	12	*C. jejuni*
ERL06-2347	F	77	Canterbury (S)	53	ST-21	2	C	12	*C. jejuni*
ERL06-2358	F	3	Canterbury (S)	53	ST-21	2	C	12	*C. jejuni*
ERL06-2355	M	11	Canterbury (S)	53	ST-21	2			*C. jejuni*
ERL06-2267	F	77	Waikato (N)	61	ST-61	4c			*C. jejuni*
ERL06-2309	M	8	C&C (N)	190	ST-21	2	D	1	*C. jejuni*
ERL06-2327	F	33	Lakes (N)	190	ST-21	2	D	1	*C. jejuni*
ERL06-2336	M	48	Otago (S)	190	ST-21	2	D	1	*C. jejuni*
ERL06-2258	F	18	BOP (N)	190	ST-21	2	E	2	*C. jejuni*
ERL06-2291	F	32	C&C (N)	190	ST-21	2	E	2	*C. jejuni*
ERL06-2275	M	15	Waikato (N)	190	ST-21	2	E	2	*C. jejuni*
ERL06-2364	M	3	Canterbury (S)	190	ST-21	2	F	3	*C. jejuni*
ERL06-2367	M	4	Canterbury (S)	190	ST-21	2	F	3	*C. jejuni*
ERL06-2369	M	15	Canterbury (S)	190	ST-21	2	F	3	*C. jejuni*
ERL06-2331	M	22	Otago (S)	190	ST-21	2	F	3	*C. jejuni*
ERL06-2342	F	86	Otago (S)	190	ST-21	2	F	3	*C. jejuni*
ERL06-2374	F	23	Otago (S)	190	ST-21	2	F	3	*C. jejuni*
ERL06-2376	F	20	Otago (S)	190	ST-21	2	F	3	*C. jejuni*
ERL06-2356	M	2	South Canterbury (S)	190	ST-21	2	F	3	*C. jejuni*
ERL06-2334	F	2	Southland (S)	190	ST-21	UT	F	3	*C. jejuni*
ERL06-2337	F	30	Southland (S)	190	ST-21	2	F	3	*C. jejuni*
ERL06-2249	F	17	Lakes (N)	190	ST-21	2			*C. jejuni*
ERL06-2274	M	1	Waikato (N)	190	ST-21	2			*C. jejuni*
ERL06-2260	F	44	BOP (N)	257	ST-257	11	G	4	*C. jejuni*
ERL06-2289	M	76	BOP (N)	257	ST-257	11	G	4	*C. jejuni*
ERL06-2571	F	62	BOP (N)	257	ST-257	11	G	4	*C. jejuni*
ERL06-2354	F	39	Canterbury (S)	257	ST-257	11	G	4	*C. jejuni*
ERL06-2365	F	28	Canterbury (S)	257	ST-257	11	G	4	*C. jejuni*
ERL06-2328	M	52	BOP (N)	354	ST-354	UT	H	5	*C. jejuni*
ERL06-2255	M	71	C&C (N)	354	ST-354	UT	H	5	*C. jejuni*
ERL06-2290	M	57	C&C (N)	354	ST-354	UT	H	5	*C. jejuni*
ERL06-2297	M	60	C&C (N)	354	ST-354	UT	H	5	*C. jejuni*
ERL06-2270	F	49	Waikato (N)	354	ST-354	UT	H	5	*C. jejuni*
ERL06-2418	F	76	Waikato (N)	354	ST-354	UT	H	5	*C. jejuni*

Table 1 (*cont.*)

ERL no.	Sex	Age (yr)	Submitting DHB	ST	CC	Serotype	*Sma*I	*Kpn*I	Species
		58			ST-354	UT	H	5	*C. jejuni*
ERL06-2421	F		Waikato (N)	354					
ERL06-2363	F	19	Canterbury (S)	354	ST-354	UT			*C. jejuni*
ERL06-2417	M	30	Waikato (N)	354	ST-354	UT			*C. jejuni*
ERL06-2366	F	1	Canterbury (S)	436	UA	UT			*C. jejuni*
ERL06-2375	F	40	Southland (S)	436	UA	4c			*C. jejuni*
ERL06-2422	M	1	Waikato (N)	436	UA	UT			*C. jejuni*
ERL06-2288	F	49	BOP (N)	474	ST-48	4c	J	6	*C. jejuni*
ERL06-2253	F	23	C&C (N)	474	ST-48	4c	J	6	*C. jejuni*
ERL06-2250	F	18	Lakes (N)	474	ST-48	4c	J	6	*C. jejuni*
ERL06-2265	F	39	Waikato (N)	474	ST-48	4c	J	6	*C. jejuni*
ERL06-2271	F	51	Waikato (N)	474	ST-48	4c	J	6	*C. jejuni*
ERL06-2416	M	39	Waikato (N)	474	ST-48	4c	J	6	*C. jejuni*
ERL06-2424	M	57	Waikato (N)	474	ST-48	4c	J	6	*C. jejuni*
ERL06-2256	F	22	C&C (N)	474	ST-48	4c	K	7	*C. jejuni*
ERL06-2292	M	54	C&C (N)	474	ST-48	4c	K	7	*C. jejuni*
ERL06-2293	M	25	C&C (N)	474	ST-48	4c	K	7	*C. jejuni*
ERL06-2296	M	28	C&C (N)	474	ST-48	4c	K	7	*C. jejuni*
ERL06-2287	F	30	Lakes (N)	474	ST-48	4c	K	7	*C. jejuni*
ERL06-2326	M	52	Lakes (N)	474	ST-48	4c	K	7	*C. jejuni*
ERL06-2551	M	21	Lakes (N)	474	ST-48	4c	K	7	*C. jejuni*
ERL06-2373	F	12	Otago (S)	474	ST-48	4c	K	7	*C. jejuni*
ERL06-2266	F	24	Waikato (N)	474	ST-48	4c	K	7	*C. jejuni*
ERL06-2308	F	36	C&C (N)	474	ST-48	4c	L	8	*C. jejuni*
ERL06-2313	F	46	C&C (N)	474	ST-48	4c	L	8	*C. jejuni*
ERL06-2368	F	78	Canterbury (S)	474	ST-48	4c	L	8	*C. jejuni*
ERL06-2370	F	22	Canterbury (S)	474	ST-48	4c	L	8	*C. jejuni*
ERL06-2338	M	26	Otago (S)	474	ST-48	4c	L	8	*C. jejuni*
ERL06-2415	F	85	Waikato (N)	474	ST-48	4c	L	8	*C. jejuni*
ERL06-2419	M	0	Waikato (N)	474	ST-48	4c	L	8	*C. jejuni*
ERL06-2361	M	61	Canterbury (S)	474	ST-48	4c	Q	13	*C. jejuni*
ERL06-2352	M	0	South Canterbury (S)	474	ST-48	4c	Q	13	*C. jejuni*
ERL06-2412	F	19	BOP (N)	474	ST-48	4c	S	14	*C. jejuni*
ERL06-2306	F	24	C&C (N)	474	ST-48	4c	S	14	*C. jejuni*
ERL06-2310	M	50	C&C (N)	474	ST-48	4c			*C. jejuni*
ERL06-2312	F	93	C&C (N)	474	ST-48	4c			*C. jejuni*
ERL06-2325	M	1	Lakes (N)	474	ST-48	4c			*C. jejuni*
ERL06-2549	M	15	Lakes (N)	474	ST-48	4c			*C. jejuni*
ERL06-2262	F	2	Waikato (N)	474	ST-48	4c			*C. jejuni*
ERL06-2259	F	36	BOP (N)	530	UA	23,36			*C. jejuni*
ERL06-2286	F	24	Lakes (N)	583	ST-45	6			*C. jejuni*
ERL06-2353	F	70	Canterbury (S)	1581	UA	UT			*C. coli*
ERL06-2344	F	73	South Canterbury (S)	2026	ST-403	35			*C. jejuni*
ERL06-2371	F	24	Southland (S)	2343	ST-48	UT			*C. jejuni*
ERL06-2413	F	29	BOP (N)	2345	ST-206	1c			*C. jejuni*
ERL06-2335	F	43	Otago (S)	2345	ST-206	1c			*C. jejuni*
ERL06-2362	M	18	Canterbury (S)	2347	UA	8,17			*C. jejuni*
ERL06-2252	F	52	C&C (N)	2397	ST-828	37	R	9	*C. coli*
ERL06-2254	M	46	C&C (N)	2397	ST-828	37	R	9	*C. coli*
ERL06-2269	F	45	Waikato (N)	2397	ST-828	37	R	9	*C. coli*
ERL06-2311	M	58	C&C (N)	2398	UA	42			*C. jejuni*
ERL06-2360	M	11	Canterbury (S)	2534	UA	UT			*C. coli*
ERL06-2263	M	73	Waikato (N)	2535	UA	UT	M		*C. jejuni*
ERL06-2273	M	53	Waikato (N)	2535	UA	41	P		*C. jejuni*
ERL06-2294	M	27	C&C (N)	2535	UA	UT			*C. jejuni*

DHB, District health board; ST, Sequence type; CC, clonal complex; N, North Island; S, South Island; C&C (N), Capital & Coast DHB; BOP (N), Bay of Plenty DHB; UA, unassigned; UT, untypable.

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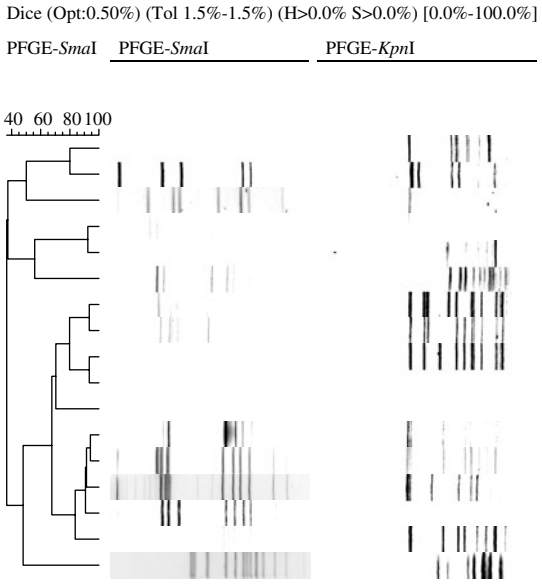
Table 2. *Sequence types* (*ST*) *identified in individual district health boards* (*DHBs*)

ST	BOP	C&C	Canterbury	Lakes	Otago	S. Canterbury	Southland	Waikato	Total
5		1							1
21								1	1
42	1	1			2				4
45			1		1			2	4
48		1							1
50	1	1	2		3		1		8
52		2			1			1	4
53			5						5
61								1	1
190	1	2	3	2	5	1	2	2	18
257	3		2						5
354	1	3	1					4	9
436			1				1	1	3
474	2	10	3	6	2	1		8	32
530	1								1
583				1					1
1581			1						1
2026						1			1
2343							1		1
2345	1				1				2
2347			1						1
2397		2						1	3
2398		1							1
2534			1						1
2535		1						2	3
Total	11	25	21	9	15	3	5	23	112

BOP, Bay of Plenty DHB; C&C, Capital & Coast DHB.



Strain	Source	Serotype	Cluster	ST	CC
ERL06-2273	Waikato	41	P	2535	UA
ERL06-2357	Canterbury	42	M	45	ST-45
ERL06-2304	Capital & Coast	23,36	N	42	ST-42
ERL06-2297	Capital & Coast	UT	H	354	ST-354
ERL06-2365	Canterbury	11	G	257	ST-257
ERL06-2341	Otago	5	B	52	ST-52
ERL06-2293	Capital & Coast	4,13	K	474	ST-48
ERL06-2338	Otago	4	L	474	ST-48
ERL06-2288	Bay of Plenty	4,13	J	474	ST-48
ERL06-2412	Bay of Plenty	4	S	474	ST-48
ERL06-2352	South Canterbury	4	Q	474	ST-48
ERL06-2291	Capital & Coast	2	E	190	ST-21
ERL06-2327	Lakes	2	D	190	ST-21
ERL06-2329	Bay of Plenty	1,44	A	50	ST-21
ERL06-2358	Canterbury	2	C	53	ST-21
ERL06-2364	Canterbury	2	F	190	ST-21
ERL06-2269	Waikato	37	R	2397	ST-828

Fig. 3. The PFGE patterns of the 17 clusters identified using *Sma*I. The *Kpn*I patterns are also shown. ST, Sequence type; CC, clonal complex; UA, unassigned.

suggests either a significant under-reporting of outbreaks or a very large number of sporadic cases. Unfortunately the volume of *Campylobacter* isolates normally precludes routine subtyping for identification of related cases and may hide the true extent of outbreak-related cases. Recent studies using MLST

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and PFGE subtyping methods have shown that within human *Campylobacter* strains there is a clear temporal distribution of isolates [2, 24, 25]. In New Zealand, PFGE subtyping of all isolates received by a single clinical laboratory over two short time periods identified a significant number of clusters [2]. Distinct clusters defined using both *Sma*I and *Kpn*I PFGE patterns were identified in different isolation periods suggesting outbreaks may be more common than previously thought. Similarly, studies characterizing isolates by MLST, one from urban and rural communities in the United Kingdom [25] and one from New South Wales, Australia [24], also identified a temporal distribution of clonal complexes. The lower discriminatory power of MLST precludes identification of potential outbreaks *per se* but clearly indicates clusters of related isolates present within a collection. In this study we have identified distinct clusters within a discrete time-frame but from isolates distributed across the length of New Zealand.

Two restriction enzymes (*Sma*I and *Kpn*I) were used for the confirmation of individual clusters and MLST was included as an alternative confirmatory technique providing unequivocal data on the relationship of the isolates. It has previously been shown that isolates from a number of confirmed outbreaks were indistinguishable by four subtyping methods including MLST, PFGE and Penner serotyping [26]. Isolates in all the major clusters (Table 1) were indistinguishable by PFGE and MLST and Penner serotyping, although one isolate in ST-190 cluster F had a different serotype. As found previously ST-257 was only identified with serotype 11 and ST-42 with serotype 23,36 [27].

The sudden and unexpected increase in *Campylobacter* isolates during the winter of 2006 [19] provided an opportunity to examine the relationship and distribution of *Campylobacter* subtypes across New Zealand. This increase was specific to New Zealand and not identified in Australia. The 112 isolates were obtained from eight DHBs, four on the North Island and four on the South Island (Fig. 2). The surprising result was the identification of indistinguishable isolates from upwards of five different DHBs located on both islands (Table 1). This could be attributed to a generalized increase in exposure to multiple sources associated with a common risk factor, or a common source outbreak arising from a single source that was widely distributed throughout New Zealand. The relative frequency and spatial pattern of the genotypes, especially the MLST types, are more consistent with the latter scenario: such a marked increase in notification, accompanied by the predominance of indistinguishable strains with a wide spatial distribution, is consistent with a common source epidemic. Strains most likely associated with a common source are ST-474 and ST-190; these were the most prevalent sequence types, with ST-190 identified in all regions and ST-474 in seven of the eight regions, and the only sequence types isolated in the Lakes region (Table 2). Unfortunately the small numbers associated with each cluster meant that no significant information on possible sources of infection could be identified from epidemiological information gathered and the rural or urban nature of the sample was not recorded.

Comparison of the PFGE patterns from the two major sequence-type groups with those in the PulseNet Aotearoa Campylobacter database showed that the PFGE patterns of two clusters had not been seen previously (ST-474 cluster K and cluster L), one had been seen once (ST-190 cluster D), one four times (ST-474 cluster J), one 23 times (ST-190, cluster F) and one 44 times (ST-190 cluster E). It is clear from the PFGE and MLST data (P. Carter and S. McTavish, unpublished data) that particular *Campylobacter* strains are very stable over a number of years and continue to cause human infections. This stability obfuscates the relationship of these strains in outbreak scenarios and further work surrounding their epidemiology is required. Identification of stable strains within *Campylobacter* populations using MLST, PFGE, *flaA* RFLP typing and AFLP has been reported previously among human and poultry isolates [5, 28, 29].

One unique strain, ST-474 cluster K, was identified in four different DHBs and the PFGE patterns had not previously been seen. The widespread occurrence of this strain argues against a simple local point source normally associated with *Campylobacter* outbreaks but may reflect the need to look at other possible reasons such as food distribution within New Zealand. This scenario is comparable to the widespread dissemination of *E. coli* O157:H7 through large-scale food distribution networks in the United States and highlights the importance of subtyping and surveillance (e.g. by PulseNet USA) in identifying such outbreaks.

The MLST data identified a number of commonly described sequence types that have previously been associated with human infection. CC ST-21 has been

identified in isolates from a wide range of sources accounting for up to a third of human isolates (20–33%) [18, 25, 27, 30] which was also the case in this study. The other major clonal complex in this study, CC ST-48, also accounted for approximately one third of human isolates, comparable with recent data from Australia [24], although significantly more than reported previously in other studies (5–10%) [18, 25, 27, 30]. The majority of the CC ST-48 isolates in New Zealand were ST-474, which is not a commonly identified sequence type internationally. Only one isolate with this sequence type, a Czech isolate from chicken, is logged in the MLST database. Interestingly, the Czech Republic is also reported to have a very high rate of *Campylobacter* infection [31]. It has not been reported in other MLST studies of *Campylobacter* isolates. It is present, however, in significant numbers (about 10% of samples characterized) in human and poultry isolates in New Zealand and has been isolated from sheep and cows (N. French and P. Carter, unpublished results). It may be that this particular sequence type is endemic in New Zealand but not prevalent elsewhere in the world. Potentially endemic strains have also been identified in Australia [24] and Curacao [32]. There were also four isolates with sequence types that had been previously identified in New Zealand as novel sequence types, associated with chicken meat (ST-2343, one isolate and ST-2345, two isolates) and river water (ST-2347, one isolate). The *Sma*I PFGE pattern of the ST-2343 and ST-2347 isolates were indistinguishable from those seen previously. The two ST-2345 isolates in this study, however, gave different patterns to those seen previously. These sequence types may represent other endemic strains of *Campylobacter*.

The data presented here regarding the unusual increase in campylobacteriosis in New Zealand over the winter of 2006 are consistent with a common source epidemic associated with endemic strains of *Campylobacter*. Clones of *Campylobacter* identified by PFGE patterns, MLST and Penner serotyping are widely distributed throughout New Zealand, some of which have been identified before and represent stable clones. The PFGE patterns associated with individual clonal complexes are closely related, consistent with the distribution of epidemic strains via an unknown source. The identification of the New Zealand endemic strain, ST-474, and its association with food sources and serious human illness warrants further investigation.

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DECLARATION OF INTEREST

None.

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