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Campylobacter and farm dairy effluent irrigation

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Abstract Campylobacter were studied at a mole-tile drained farm over a 12-month period. Farm dairy effluent was applied to the farm in January, October, and November. In January, herd 1 was on the farm and effluent that contained 10^3 campylobacter 100 ml^{-1} was sourced from a storage pond. In October and November herd 2 was on the farm and effluent that contained 10^5 and 10^6 campylobacter 100 ml^{-1} respectively was sourced from the dairy shed. In January and November, when irrigation caused preferential flow, concentrations in the drainage water were similar to those in the applied effluent, creating a potential for water contamination. *C. jejuni* was the predominant campylobacter species recovered. Only one subspecies type (i.e., one Penner serotype and one PFGE type) was observed in the effluent, drainage water, and soil samples collected after the January application. This recovery of a single subtype allowed tracing from effluent into drainage water and soil. In contrast, several subspecies types were observed after the October and November applications, and the study demonstrated that when there is a diversity of subtypes, typing was less effective as a means of tracking campylobacter. Penner serotyping of *C. jejuni* in these effluents revealed serotypes linked to the human illness, campylobacteriosis.

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INTRODUCTION

New Zealand has a particularly high rate of campylobacter infection with a nine-fold increase in incidence, from 14 to 120 cases per 100 000 population, between 1981 and 1990, and a further three-fold increase from 128 to 363 cases from 1991 to 1998 (Friedman et al. 2000). In New Zealand, epidemic type outbreaks have been attributed to both contaminated drinking water, and to consumption of raw milk (Brieseman 1984; Anon. 1991). As in many parts of the world, small unprotected rural water supplies may be a particular source.

Worldwide, campylobacter is the most common bacterial cause of diarrhoeal illness and represented approximately 1% of reported foodborne and waterborne outbreaks in the USA between 1996 and 1998 (Friedman et al. 2000). Campylobacter are highly infective, with *Campylobacter jejuni* accounting for most of reported infectious illness followed by *C. coli* and *C. lari*. Studies have found that ingestion of as few as 500–800 *C. jejuni* has resulted in a high probability of infection and clinical illness (Robinson 1981; Black et al. 1988). Black et al. (1988) administered a range of doses of *C. jejuni* (serotypes 27 and 23,36) to healthy adult volunteers. At a dose rate of 800 cells of serotype 27, 50% of the volunteers became infected and 10% became ill. When the dose was increased to 1×10^6, 79% of the volunteers became infected and 11% became ill. For serotype 23,36, 100% of the volunteers became infected in response to a dose of 10^6 and 46% became ill, demonstrating differences in infectivity for different strains. As for asymptomatic animal carriers, passive human carriers will shed campylobacter with potential to enter the environment via sewage-contaminated water. Unlike many enteric pathogens, there is apparently limited person-to-person spread of campylobacter (Skirrow & Blaser 1992). In the main, campylobacter

infections result from the ingestion of contaminated foods of animal origin (Park 2002).

Campylobacter are found in the intestinal tract of many wild and domesticated animals where they show no ill effect to the animal. These animals can introduce the pathogen into the environment and potentially be a risk to humans. Stanley et al. (1998) reported campylobacter isolation from faeces of healthy dairy cows and beef cattle at a carriage rate of 70–90%. Campylobacter, in particular *C. jejuni*, are known to be very diverse in their genotype and some serotypes are common to both cattle and humans (Jones et al. 1984; Frost 2001).

Campylobacter are resistant to a wide range of antibiotics, a feature that is exploited in the design of the selective media used for laboratory growth, and the optimal growth temperature is 42°C. Campylobacter require a microaerophilic atmosphere (5% O_2 is optimal; Doyle & Jones 1992), and *C. jejuni* does not grow at temperatures less than 30°C. These characteristics restrict the ability of *C. jejuni* to multiply outside an animal/human host.

Although contamination of water with campylobacter has been associated with sewage effluent (Bolton et al. 1987), it has also been linked with agricultural runoff and grazing animals (Jones et al. 1999; Obiri-Danso & Jones 2000). Bolton et al. (1987) found that surface runoff from farmland following heavy rains increased the number of campylobacter in surrounding waterways; *C. jejuni* was the prevalent species.

Studies in New Zealand have demonstrated that campylobacter are frequently present in rural waterways (Hudson 1997; Till et al. 2000), and that the concentration can be high when streams are in flood (Donnison et al. 2001). In a recent Ministry for the Environment publication, McBride et al. (2002) reported that thermotolerant campylobacter and human adenoviruses were the pathogens most likely to cause human waterborne illness to recreational freshwater users in New Zealand. A mechanism for introducing faecal microorganisms such as campylobacter into the farm environment and potentially into rural waterways is irrigation with farm effluents. In some areas, the soils irrigated with farm effluents are not free draining, and mole-tile drainage systems are installed to prevent soil degradation.

The aim of this study was to determine the occurrence of campylobacter in farm dairy effluent, and to investigate the use of subspecies typing as a tool to trace the fate of these organisms in the farm environment after irrigation.

METHODS AND MATERIALS

Study site

Two experimental plots 27 × 35 m, were constructed side by side on a dairy farm in West Otago. The soil type was a naturally imperfectly drained Waikoikoi/Athol complex (Fragic perch-gley and Typic perch-gley Pallic soils respectively, (Bruce et al. 2001). One plot was irrigated periodically with farm dairy effluent while the adjacent plot was a non-irrigated control. Each plot was individually drained with isolating drains, plus a plastic sheet to prevent horizontal water movement. The drains from the two plots were separated both from each other and the surrounding pasture. A tipping bucket and siphon system, linked to a datalogger that allowed automatic records and representative samples to be taken of drainage water entering the mole-tile drain under each plot, was connected to the drains. Effluent was applied to a depth of 23, 8, and 9.4 mm for irrigations 1, 2, and 3, respectively.

During the study period, two separate herds of cows grazed the plots. Herd 1, comprising 220 cows, grazed from 5 January to 5 May, after which herd 1 was removed from the farm. Ponded effluent from herd 1 was used for irrigation 1 on 29 January 2001. After 5 May, no cows grazed the farm until late August when herd 2 was introduced. Herd 2 comprised 210 cows that calved on the study plots from late August to early September. Untreated effluent from herd 2 was used for irrigations 2 and 3, in October and November 2001.

For the first application, on irrigation day farm dairy effluent was collected from an effluent storage pond near the cowshed. For subsequent applications, effluent was collected in a sump during milking and sprayed immediately onto the designated plot. After irrigation both plots were grazed according to a typical twice monthly farm rotation. Site measurements included soil temperature at a depth of 10 cm and of daily rainfall (mm).

Campylobacter analysis was carried out for 12 months during which time effluent was applied three times as part of an on going study of irrigation to mole-tile drained soils (Monaghan et al. 2001).

Sample collection

Samples of drainage water and soil were collected from the irrigated plot on irrigation day on each of three irrigations. Drainage water samples were collected from the tipping bucket system both immediately after irrigation and after heavy rainfall events. Soil samples were collected from time to time

throughout the study as 10 separate cores each 2 × 5 cm deep. All 10 cores from each plot, including pasture (<5 cm length) and the underlying soil were composited prior to analysis.

Irrigation 1

Irrigation 1 took place on 29 January 2001. Subsequent samples of drainage water and soil were collected on 28 May, 12 June, and 27 June. The last grazing of herd 1 was 5 May.

Irrigation 2

Irrigation 2 took place on 25 October 2001. For this irrigation there was no drainage available to sample and only soil samples were collected from the plots.

Irrigation 3

Irrigation 3 took place on 4 November 2001. On this occasion a soil sample was collected from the irrigation plot immediately prior to irrigation. After irrigation, soil samples were collected on 12, 19, and 26 November and on 4 December. Samples of drainage water were collected on the 21 November.

Samples of effluent were collected periodically during the course of each irrigation and subsamples composited prior to analysis.

Sampling dates are included in Table 1. After collection, samples were packed in an insulated container with frozen ice packs and transported to the laboratory where they were analysed within 24 h.

Sample analysis

Campylobacter

Samples were analysed for thermotolerant campylobacter (hereafter referred to as campylobacter) using a three-tube three-dilution Most Probable Number technique (le Roux et al. 2001) except for the soil samples for irrigation 1 in which the presence/absence technique was used and the presence of campylobacter determined in three 1-g aliquots from each plot.

For each sample, a colony that had been confirmed as thermotolerant campylobacter, was subjected to biochemical testing (le Roux et al. 2001) to identify campylobacter species. Isolates identified as *C. jejuni* were stored at –80°C for subspecies typing.

Soil moisture

The soil moisture in these samples (top 5 cm) was obtained by drying a subsample (approximately 30 g) overnight at 70°C.

Subspecies typing of *C. jejuni*

Eighteen *C. jejuni* isolates were typed by pulse field gel electrophoresis (PFGE) using a method supplied by Massey University (M. Leyland pers. comm.). Fourteen of the *C. jejuni* isolates were also subspecies typed by Penner serotyping (ESR, Kenepuru Science Centre).

Table 1 Recovery of campylobacter from effluent, drainage water, and soil. cmp = campylobacter; gDW = gram dry weight.

				Irrigated		Non-irrigated	
Irrigation	Herd	Date sampled	Effluent (cmp/100 ml)	Drainage (cmp/100 ml)	Soil (cmp/gDW)	Drainage (cmp/100 ml)	Soil (cmp/gDW)
1	1	29 Jan	10^3	10^3	*		0
		28 May		0	0.13	0	0.13
		12 Jun		0	15	0	15
		27 Jun		0	0	0	0
2	2	25 Oct	10^6	†	36.2		3.5
		4 Nov			1.8		
3	2	4 Nov	10^5	10^4	10^3		0
		12 Nov			0		5.5
		19 Nov			0		0
		21 Nov		4		50	
		26 Nov			0		0
		4 Dec			0		0

*3 (of 3) samples positive, but not enumerated.
†Nil drainage.

RESULTS

Recovery of campylobacter

The recovery of campylobacter from effluent, drainage, and soil is summarised in Table 1. *C. jejuni* was identified in all samples with one exception. *C. coli* was identified in the sample tested from non-irrigated plot drainage collected on 21 November, 17 days after irrigation 3.

Irrigation 1

For irrigation 1, campylobacter were recovered in the farm dairy effluent and in the drainage water. The concentration in the drainage water was the same as that in the effluent, 10^3 per 100 ml. No campylobacter were recovered from the non-irrigated plot soil, but they were recovered (not enumerated) from all three soil samples from the irrigated plot.

There was insufficient rain to produce drainage water for the next 4 months. After this period no campylobacter were detected in the drainage water from either the irrigated or the non-irrigated plot, but they were recovered from samples of both irrigated and non-irrigated soil (0.13 per gram dry weight (DW)). The moisture content in the top 5 cm of soil from the irrigated plot was 33% and the non-irrigated plot 32%. The average daily temperature, from when herd 1 was last grazed on 3–5 May until the samples were collected, was 7°C.

Further rainfall 2 weeks later produced more drainage. Campylobacter were not detected in this drainage water but were found in the soil from both the irrigated and non-irrigated plots (about 15 per gram DW). The average daily soil temperature decreased to 3.4°C in the 2 weeks between samplings. The farmer reported that a large flock of seagulls camped on the experimental plots for most of May.

Samples were collected again 2 weeks later after further rain, but no campylobacter were found in either the drainage water or the soil of either the irrigated or non-irrigated plots.

Irrigation 2

The farm dairy effluent used for irrigation had a campylobacter concentration of 10^6 per 100 ml. No drainage water was available for collection after this irrigation event because the soil moisture deficit was greater than the volume of effluent applied (C. Smith, AgResearch, pers. comm.). Campylobacter were recovered from non-irrigated soil (3.5 per gram

Table 2 Subspecies typing of *C. jejuni* strains isolated from irrigation effluent, mole-tile drainage, and soil samples collected on a dairy farm. Eleven different pulse field patterns were found in 18 isolates and seven different Penner serotypes in 14 isolates. kb = kilobases (size of DNA fragment) and each combination of numbers represents a PFGE pattern.

Date isolated (2001)	Sample type	DNA in kb	Penner serotype
29 Jan	Irrigated soil	525, 368, 327, 150, 124	Not done
29 Jan	Irrigated soil	525, 368, 327, 150, 124	Not done
29 Jan	Irrigated soil	525, 368, 327, 150, 124	42
29 Jan	Effluent	525, 368, 327, 150, 124	42
29 Jan	Preferential drainage	525, 368, 327, 150, 124	42
28 May	Non-irrigated soil	368, 327, 199, 166, 150, 124, 56, 37	Not done
28 May	Irrigated soil	525, 368, 327, 199, 166, 150, 124, 56, 37	5
12 Jun	Non-irrigated soil	525, 368, 327, 199, 166, 150, 124, 56, 37	Not done
12 Jun	Irrigated soil	525, 368, 327, 199, 166, 150, 124, 56, 37	5
25 Oct	Effluent	433, 368, 327, 306, 199, 177, 166, 150, 124 56, 42, 37	23,36
25 Oct	Non-irrigated soil	525, 433, 306, 199, 150, 124, 42	23,36
25 Oct	Irrigated soil	433, 327, 306, 199, 150, 124, 42	23,36
4 Nov	Pre-irrigation soil	368, 306, 150, 124, 56, 37	4
4 Nov	Irrigated soil	433, 368, 327, 306, 272, 199, 177, 163, 150 124, 104, 42	untypable
4 Nov	Effluent	433, 368, 327, 306, 272, 199, 177, 166, 150 124, 104, 42	23,36
4 Nov	Preferential drainage	433, 327, 199, 166, 150, 124, 56, 37	10
12 Nov	Non-irrigated soil	368, 327, 199, 166, 150, 124, 56, 37	2
21 Nov	Irrigated plot drainage	525, 464, 422, 367, 305, 186, 162, 141, 120 61, 39	1

DW) and in a higher concentration from the irrigated plot (36.2 per gram DW) immediately after irrigation. The average daily soil temperature between irrigations 2 and 3 was 11.7°C and there was 25.9 mm of rainfall.

Irrigation 3

Irrigation 3 occurred 10 days after irrigation event 2. The irrigation effluent contained campylobacter at a concentration of 10^5 per 100 ml, and 10^4 per 100 ml were recovered from the drainage water immediately after irrigation. Campylobacter were recovered from the irrigated soil plot prior to irrigation at a concentration of 1.8 per gram DW, and after irrigation at a concentration of 10^3 per gram DW; none were recovered from the non-irrigated plot. Soil samples were collected from both the irrigated and non-irrigated plots for 4 consecutive weeks after the third irrigation event. Campylobacter were not recovered from the irrigated plot over this time, but were recovered from the non-irrigated plot on one occasion (5.5 per gram DW). The cows were grazing on both study plots at the time the samples were collected. The average daily soil temperature over this sampling period was 12.6°C.

Seventeen days after irrigation 3 (during which time there was 40 mm of rain) drainage water was collected from both irrigated and non-irrigated plots. Campylobacter were found to be present in both irrigated and non-irrigated drainage samples at a concentration of 4 per 100 ml in the irrigated plot drainage and 50 per 100 ml in the non-irrigated plot drainage. *C. jejuni* was isolated from the irrigated drainage water and *C. coli* from the non-irrigated drainage water.

Subspecies typing of *C. jejuni*

When campylobacter were recovered, an isolate was subjected to species identification. *C. jejuni* was the predominant species but *C. coli* was present in one sample. Results of the subspecies typing of *C. jejuni* by both PFGE and Penner serotyping are given in Table 2. Eleven different PFGE patterns and seven different Penner serotypes were observed over the course of the study.

DISCUSSION

The aim of this study was to investigate the occurrence and fate of thermotolerant campylobacter after irrigation with farm dairy effluent and its potential to contaminate water.

Occurrence of campylobacter in effluent and transfer to drainage water

All three effluents contained campylobacter. Irrigation 1 effluent was sourced from a storage pond and had a lower concentration of campylobacter than that in the untreated effluents used in irrigations 2 and 3 (10^3 per 100 ml compared with 10^5 to 10^6 per 100 ml). All three effluents contained *Campylobacter jejuni*, one of the two species (*C. jejuni* and *C. coli*) that are responsible for most of the disease campylobacteriosis (Savill et al. 2002).

During irrigations 1 and 3, when preferential flow occurred, the concentration of campylobacter was similar in effluent and drainage water. This demonstrated that at least a proportion of the effluent was channelled directly into the drainage system.

Survival of campylobacter in soil

Campylobacter was recovered from soil of both irrigated and non-irrigated plots. Soil concentrations were high on irrigation day and when seagulls and not cows were present on the plots. On one occasion campylobacter were recovered from the plot that was grazed but not irrigated.

Soil campylobacter had declined substantially 10 days after irrigation (Table 1). Survival of enteric bacteria in soils depends on the species of microorganism and its physiological state and the nature of the soil. Survival is enhanced in cool, wet, alkaline, highly nutritive fine soils, especially clay that retains moisture (Abu-Ashour et al. 1993). The water content in the top 5 cm was 30–40%, the average daily temperature in the 10 days between irrigations 2 and 3 was 11.7 and 12.4°C for the month following irrigation 3. Theoretically, campylobacter would be expected to survive under these conditions over this period.

There is little published information on the survival of campylobacter in soil, but several studies have reported that campylobacter can survive outside the host for some time. Rollins & Colwell (1986) reported survival in streams at 4°C for > 4 months, and Easton (1996) reported survival in cattle slurry for 24 days at 8°C. Work in our laboratory has demonstrated that both *C. jejuni* and *C. coli* survive in soil stored at 10°C for 14 days (C. Ross, AgResearch, unpubl. data).

Motile microorganisms can migrate through soil to reach conditions that suit their requirements (Abu-Ashour et al. 1993). Campylobacter were not detected in the top 5 cm of soil, and as these bacteria are highly motile, it is possible that they moved

deeper into the soil column. For campylobacter, the favoured conditions include low oxygen tension, cool temperatures and absence of sunlight, conditions that may not occur near the surface.

Campylobacter jejuni subspecies typing

Penner serotyping of *C. jejuni* has been used for several years. However, many isolates do not conform to a recognised serotype (Frost 2001) and therefore are not classifiable by this method (Table 2). In some cases accurate identification of relationships between animal strains and human infection (Hagelton & Bernston 1996; Fitzgerald et al. 2001; Frost 2001) has been possible using information from serotyping in conjunction with a molecular subtyping technique such as PFGE (Lind et al. 1996).

In this study both Penner serotyping and PFGE were used to categorise *C. jejuni*. Eighteen *C. jejuni* isolates were categorised by PFGE and 14 of these were also categorised by Penner serotyping. Eleven different PFGE patterns and seven different serotypes were identified. When herd 1 was on the farm, only one serotype, 42, with one PFGE pattern, was identified in the effluent, drainage, and soil. Serotype 42 has been reported fairly frequently from many sources including humans, chickens, cattle, dogs, goats, and sheep (C. Nicol, ESR, pers. comm.).

Four months after irrigation 1 and 24 and 38 days after herd 1 had left the farm, *C. jejuni* was recovered from the soil on both irrigated and non-irrigated plots. Subtyping demonstrated only one serotype, 5, and two PFGE patterns that were virtually identical, demonstrating a common source. As the cows had been removed for over 3 weeks, this source is not likely to have been cow faeces. During this time a flock of seagulls camped on the plots. Birds have a core temperature of 42°C, the optimal growth temperature of *C. jejuni*, and are widely believed to be the natural hosts of these bacteria (Park 2002; Stanley & Jones 2003). We therefore speculate that seagull faeces rather than the cow faeces were the source. This serotype occurs infrequently but is known from water and human faeces (C. Nicol, ESR, pers. comm.).

In contrast, following irrigation 2, several serotypes and very diverse PGFE patterns were found in effluents, soil, and drainage. Gent et al. (1999) also reported more than one strain can occur in a single source. The five serotypes: 23,36; 4; 10; 2 and 1; all differed from those isolated prior to irrigation 2. Serotype 23,36 occurred in irrigation 2 and 3 effluents and the soil from both irrigated and non-irrigated plots. Both plots were grazed the day prior to irrigation 2. For the *C. jejuni* isolates recovered after irrigation 2 and 3, the discriminatory power of PFGE patterns revealed a diversity that was too great to be useful. Of the five serotypes associated with herd 2, all but serotype 1 have been reported in New Zealand dairy cows and human faeces (Savill et al. 2002). Furthermore, serotype 23,36 has a well established association with disease (Black et al. 1988).

In overseas studies, Jones et al. (1984) compared the serotypes of *C. jejuni* from human and environmental sources and found types 1,44; 2 and 4 to be the commonest types in faeces of patients with enteritis and samples of non-human origin. In this study, two of these types, 2 and 4, were recovered from soils.

The finding of serotypes of known infectivity in farm dairy effluent, drainage, and soils impacted by irrigation or grazing suggests that dairy farms can be an important source of infectious campylobacter subtypes in New Zealand. A similar conclusion was reached for dairy farms in the United Kingdom (Stanley & Jones 2003). Type 5 was recovered when seagulls were present in large numbers on the plots. It has been suggested that migratory birds can introduce new types of *C. jejuni* into herds (Stanley & Jones 2003).

CONCLUSIONS

The recovery of high concentrations of campylobacter in the three effluents, from two herds and at different times of the year, demonstrated a consistent presence in farm dairy effluent. The preferential flow following irrigation with effluent from herd 1 could be clearly traced using subspecies typing by both serotyping and PFGE. This combination of methods also gave credence to the speculation that the source of serotype 5 was not from the cows and could possibly be from the seagulls. For herd 2, which contained a diverse population of subspecies types, the Penner serotyping information was more useful than PFGE and demonstrated that both the grazing cows and the effluent application introduced campylobacter to the soil. Among the serotypes of *C. jejuni* identified in this study were a number that have been commonly reported from humans with campylobacteriosis.

The concentration of campylobacter in farm dairy effluent, together with the presence of potentially harmful serotypes of *C. jejuni*, suggests that farm

workers need to adopt good hygiene practices after contact with faeces and farm dairy effluent.

Due to the high concentration of campylobacter in mole-tile drainage after irrigation it is very important that irrigation is properly managed and preferential flow avoided.

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