Differential behaviour of *Escherichia coli* and *Campylobacter* spp. in a stream draining dairy pasture

Rebecca Stott, Robert Davies-Colley, John Nagels, Andrea Donnison, Colleen Ross and Richard Muirhead

ABSTRACT

The faecal indicator bacterium *Escherichia coli* and thermotolerant *Campylobacter* spp., which are potentially pathogenic, were investigated in the Toenepi Stream draining a pastoral catchment dominated by dairying. Bacteria concentrations were monitored routinely at fortnightly intervals over 12 months and intensively during storm events to compare the transport dynamics of bacterial indicator and pathogen under varying hydro-meteorological conditions. Routine monitoring indicated median concentrations of 345 *E. coli* MPN 100 ml^{-1} and relatively low concentrations of 2.3 *Campylobacter* MPN 100 ml^{-1}. The bacterial flux was three orders of magnitude greater under elevated stream flow compared with base-flow. *E. coli* peak concentrations occurred very close to the turbidity peak and consistently ahead of the *Campylobacter* spp. peak (which was close to the hydrograph peak). We postulate that, under flood conditions, the *E. coli* peak reflects the entrainment and mobilisation of in-stream stores on the flood wave front. In contrast, *Campylobacter* spp. are derived from wash-in from land stores upstream and have travelled at the mean water velocity which is slower than the speed of the flood wave. Our findings of different dynamics for *E. coli* and *Campylobacter* spp. suggest that mitigation to reduce faecal microbial impacts from farms will need to take account of these differences.

Key words | *Campylobacter* spp., dairying, *E. coli*, faecal contamination, floods, stream

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INTRODUCTION

In a number of countries including England (DEFRA 2003) and New Zealand (MfE 2007) microbial water quality is poor in rivers and streams impacted by pastoral farming. For example, Larned *et al.* (2004) assessed low-elevation rivers in New Zealand and reported that the adverse impacts of pastoral farming included *Escherichia coli* concentrations exceeding guidelines recommended for contact recreation. To address such issues the dairy industry, in partnership with policy and regulatory agencies, has introduced a range of initiatives under the *Clean Streams Accord* including fencing and bridging streams to prevent direct contact of animals with water. A suite of studies summarised by Collins *et al.* (2007) identified a number of best management practices (BMPs) to reduce microbial pollution from pastoral farming, including fencing. The dairy industry in New Zealand has initiated a programme in which representative regional 'focus' catchments are being assessed for soil and water quality, and the effectiveness of BMPs determined. In the Waikato Region the representative dairy focus catchment is the Toenepi where water quality has been monitored for more than 10 years (Wilcock *et al.* 2006).

The only natural habitat of *E. coli* is the intestines of warm-blooded animals and this bacterium is consistently present in high numbers in their faeces. *E. coli* is widely

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used as an 'indicator' of faecal contamination, and is the preferred indicator for freshwaters used for recreation (MfE & MoH 2003; EW 2006). To assess water quality in rivers and streams it is usual practice to implement an ongoing routine monitoring programme whereby samples are collected at regular intervals (e.g. monthly) for a range of variables. Such a sampling protocol, although not random, is expected to give the same results (i.e. unbiased estimates of population statistics) as truly random sampling and is sometimes referred to as 'pseudo-random' (Gilbert 1987). This approach provides general information on the status of a stream or river and identifies trends over time, but is not well designed to capture the variability produced by transient events such as floods. For example, in the course of natural flood events, *E. coli* concentrations in the water column can increase by more than two orders of magnitude (from about 10^2 to 10^4 100 ml^{-1}; Nagels *et al.* 2002). *E. coli* are typically well-correlated with turbidity, with maximum values of these two variables occurring ahead of peak water flow (Nagels *et al.* 2002). Muirhead (2001) estimated that on the first day of a single large flood event, more *E. coli* were delivered by the Topehaehae Stream (adjoining the Toenepi) than the total annual yield estimated from monthly monitoring data. Artificial floods were generated by Muirhead and colleagues (Muirhead *et al.* 2004) in the Topehaehae Stream over several days during dry weather, and the findings suggested that the initial high concentrations in floods were mainly attributable to re-suspension of in-stream stores rather than 'wash-in' from land stores. *E. coli* concentrations were comparably high with those in natural floods, but the concentrations declined markedly through a series of identical artificial floods owing to depletion of in-stream stores (Muirhead *et al.* 2004).

Thermotolerant *Campylobacter* species are zoonoses (i.e. microorganisms that are infectious for both humans and warm-blooded animals), but although they are a known cause of disease in humans they do not appear to affect the health of animals (Blackmore & Humble 1987). Most cases of campylobacteriosis are sporadic, and, unusually for an enteric pathogen, there is apparently limited person-to-person transfer. Transfer among animal species appears to occur readily as recovery of *Campylobacter jejuni* with a very similar genetic composition (indicated by the pulsed-field gel electrophoresis pattern) was reported from the faeces of cows, sparrows, rodents and from flies on a dairy farm in the Manawatu (Adhikari *et al.* 2004). *Campylobacter* spp. are highly infectious (Hunt *et al.* 1998) with the species *C. jejuni* accounting for about 90% of reported illness followed by *Campylobacter coli* and *Campylobacter lari*. The serotypes commonly found in human infection are frequently isolated from animals and environmental sources (Jones *et al.* 1984; Devane *et al.* 2005).

Campylobacter spp. are widespread in the environment (Jones 2001), but the main reservoir is animals, and human infection is usually acquired by contact with infected individuals (Hopkins & Scott 1983) or consumption of food or water contaminated by their faeces (Harris *et al.* 1986). Poultry are a major vector (Jacobs-Reitsma 2000), but cattle and sheep farms are important reservoirs of potentially infectious *Campylobacter* spp. (Stanley & Jones 2003). Increases in *Campylobacter* spp. have been observed in rural streams and rivers following land application of cattle manure or slurries (Jones 2001) and runoff from farms after rainfall (Jones & Hobbs 1996; Bates & Phillips 2005). As there are no reports that *Campylobacter* spp. can multiply outside of host animals, and their environmental survival is much shorter than that of bacteria such as *E. coli* (Sinton *et al.* 2007a), the presence of *Campylobacter* spp. in surface water implies recent faecal contamination (Bolton *et al.* 1987).

New Zealand has a higher reported incidence of campylobacteriosis than other developed countries (Till & McBride 2004) including in the Waikato region (New Zealand Public Health Observatory 2006; WDHB 2005). In an investigation of sources and transmission routes, Devane *et al.* (2005) found the same sub-species types of *C. jejuni* in human clinical cases, faeces of dairy cows, beef cattle, sheep and ducks and also on chicken carcasses. *Campylobacter* spp. have been isolated from rivers impacted by pastoral farming and by point-source discharges of sewage and meat processing wastewaters (Donnison & Ross 1999) and there was a 60% recovery rate of thermotolerant campylobacters over two years at 25 freshwater beaches (Savill *et al.* 2001). Epidemiological quantitative microbial risk assessment suggests that about 5% of New Zealand's cases of campylobacteriosis can be attributed to contact recreation in freshwater (Till *et al.* 2008). A similar recovery rate (57%) was reported for recreational water in Northern Ireland; 83% of the campylobacters were identified as *C. jejuni* or *C. coli* and the remainder as

urease-positive thermotolerant campylobacter (UPTC) (Moore *et al*. 2001). As there are very few reports of recovery from human clinical specimens, UPTC are not considered a common human pathogen (Matsuda & Moore 2004).

This paper describes studies on the faecal microbial status of the Toenepi Stream, a tributary of the Piako River and one of five dairy 'focus' catchments in New Zealand (Wilcock *et al*. 2006). We compare monitoring data for thermotolerant *Campylobacter* spp. with that for *E. coli*, and for storm events as well as base-flow. This comparison reveals contrasting dynamics that provides insight into different sources and environmental behaviour for these two bacteria.

METHODS

The stream investigated in this study, the Toenepi Stream, is a tributary of the Piako River in the Waikato Region of New Zealand. The substrate of this stream is 100% silt-sand (Duggan *et al*. 2002). The stream drains a catchment dominated by dairy farming. Although the main channel is fenced, other channels in the Toenepi stream network are only partially fenced, allowing direct access to water by livestock. The characteristics of the Toenepi catchment are summarised in Table 1.

Over the period of the study a series of investigations were carried out as detailed below. Each time a sample was collected from the Toenepi Stream the water level was noted at the hydrometric station using an established stage-discharge rating relationship following ISO (1983) protocols.

Routine monitoring

One-litre samples were collected fortnightly between February 2001 and January 2002 from mid-stream below the surface of the water (to avoid any surface debris or microlayers). Samples were stored in the dark (in insulated containers) and returned to the laboratory for analysis of *E. coli* and thermotolerant *Campylobacter* spp. within 4 h of collection. In total, 21 samples were collected either during base-flow (when flow was falling slowly in an exponential pattern, i.e. linear flow rate on a log scale) or during elevated flow (i.e. storm-flow; Figure 1(a)).

Storm-flow monitoring

On four occasions between May and December 2005 (Figure 1(b)) samples were collected during storm-flows, using an automatic sampler (Manning Portable Vacuum Sampler, Model VST; Manning Environmental Inc., Texas, USA). The sampler collected a 0.5 litre sample every 2 h for 48 h, and auto-samples were taken to the laboratory within 48 h and analysed for *E. coli,* turbidity and *Campylobacter* spp. Additionally a continuous turbidity sensor (Model TS100; Greenspan Technology, Coffs Harbour, NSW, Australia) was run from March 2005 with the output recorded

Table 1 | Characteristics of the Toenepi catchment

Characteristic	Toenepi description
Hydrometric site	At Tahuroa Road
(mouth of catchment)	(37° 42' S, 175° 33' E)
Catchment area	15.1 km^2
Elevation	30–139 m above sea level
Slope	89% flat (0–7°), 10% rolling (8–15°), 1% steep (>15°) mainly along boundaries
Soils	Silt (poorly draining: <14% of soils in catchment)
	Yellow brown and allophanic (good soil physical properties; 46% of soils)
	Clay (medium porosity, moderate permeability; 40% of soils)
Land use	100% pasture: 26 farms (18 dairy farms)
Rainfall	1,132 mm yr^{-1}
Median stream flow (authors' data)	0.122 $m^3 s^{-1}$

Source: Davies-Colley & Nagels (2002); Wilcock *et al*. (1999, 2006); Stenger *et al*. (2005)

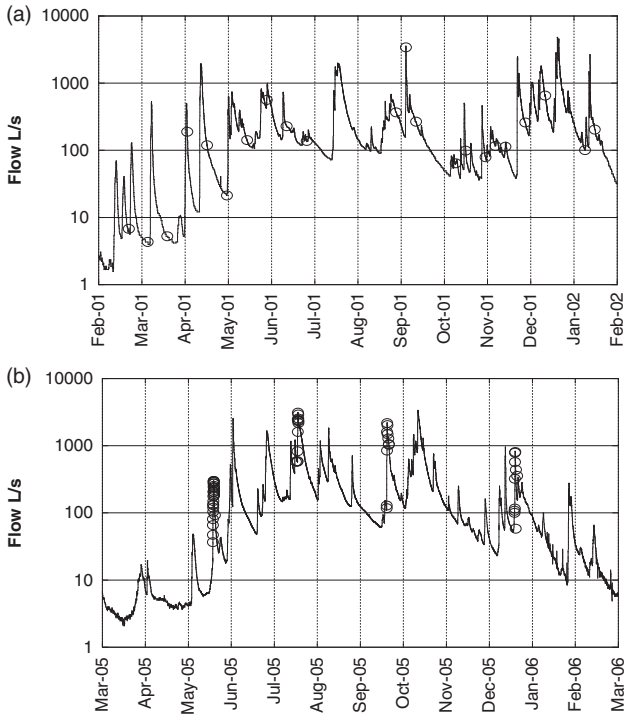

Figure 1 | Toenepi Stream hydrograph and sampling events for *E. coli* and *Campylobacter* spp. (a) Routine monitoring; (b) storm-flow monitoring (*Campylobacter* spp. sampling shown). Hydrograph shown as solid line, sampling times shown as open circles.

every 15 min, together with flow, by the hydrometric station logger. The *E. coli* storm-flow monitoring (and calculation of faecal loads for a year in which 25 out of 30 events were sampled) is discussed in more detail by Davies-Colley *et al.* (2008).

Microbiological analysis

The faecal bacterial indicator *E. coli* was measured using the Quantitray™ and Colilert® system, a multi-well (97) MPN enumeration system (IDEXX, USA) recommended for water analysis in New Zealand (MfE & MoH 1999).

Thermotolerant *Campylobacter* spp. (*Campylobacter* spp.) were measured by a three-tube multiple tube most-probable-number (MPN) with an increase in replicates to a five-tube MPN (to improve precision) for storm-flow samples. The analysis was a two-stage process: first, primary selective enrichment in Exeter broth (to enhance resuscitation, Exeter broth tubes were incubated for 24 h at 37°C prior to transfer to 42°C for a further 24 h); second, selective enrichment by sub-culture from Exeter broth tubes to mCCDA agar plates that were incubated in a microaerophilic atmosphere (5% O_2, 10% CO_2, 85% N_2 CampyGen™; Oxoid Ltd.) for 24 h at 42°C (Donnison 2002). Confirmation of *Campylobacter* spp. was by phase contrast microscopy (i.e. typical 'gull-shaped' cells). Concentrations were obtained from MPN tables (McBride 2003).

RESULTS

Routine monitoring

Escherichia coli were present in all water samples and *Campylobacter* spp. were detected in all but one sample collected fortnightly from the Toenepi over a period of a year. The overall median concentration of *E. coli* was 345 MPN 100 ml^{-1} and of *Campylobacter* spp. was 2.3 MPN 100 ml^{-1}; average stream flow was 329 l s^{-1}. Median concentration values for bacteria were also calculated for the 14 occasions when the stream was at an average base flow of 166 l s^{-1} (228 *E. coli* and 2.1 *Campylobacter* spp. MPN 100 ml^{-1}) and the seven occasions when flow was elevated

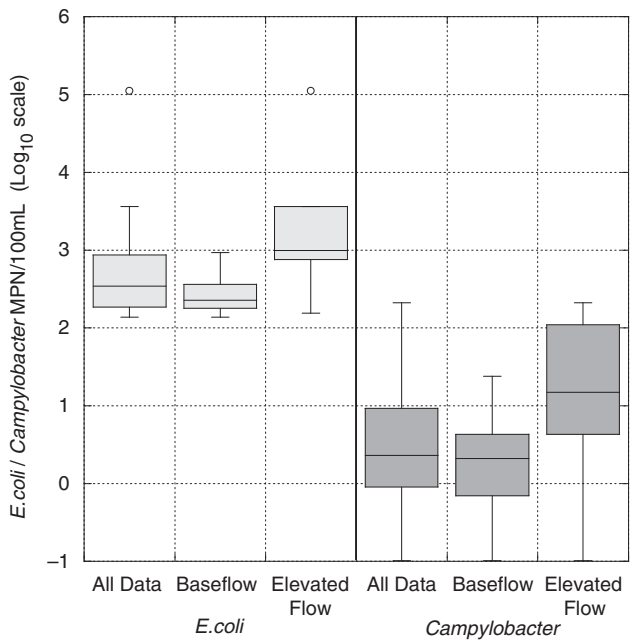

Figure 2 | Box plot of *E. coli* and *Campylobacter* spp. concentrations in the Toenepi Stream collected fortnightly from February 2001 to January 2002. All data is summarised together with separate plots for data collected at base flow and elevated flow.

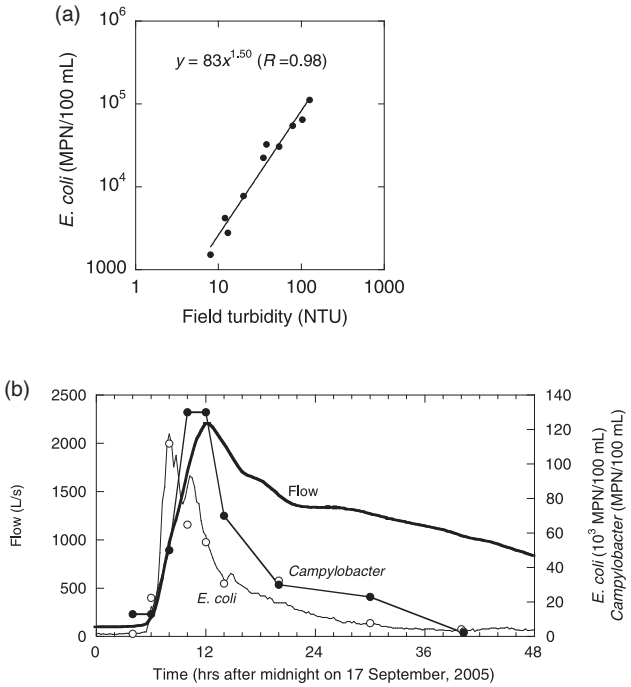

Figure 3 | Microbial concentrations measured on auto-samples taken over a flood event in the Toenepi Stream peaking on 18 September 2005. (a) *E. coli* vs. field turbidity (measured continuously by a Greenspan TS100 instrument; (b) time series of flow, *E. coli* and *Campylobacter* spp. Flow, continuous solid line; *E. coli* as simulated from field turbidity (using the relationship shown in (a)) is shown as a thin line with measured *E. coli* superimposed (open circles); *Campylobacter* spp. are shown as closed circles (note that *E. coli* concentrations are graphed as *E. coli* $\times 10^3$ MPN 100 ml^{-1}).

(987 *E. coli* and 15 *Campylobacter* spp. MPN 100 ml^{-1}), on average to 655 l s^{-1} (Figure 2).

The highest concentrations of *E. coli* and *Campylobacter* spp. were obtained on an occasion (5 September 2001) when the Toenepi stream was sampled at high flow on the rapidly rising limb of a large flood event (Figure 1(a)). Other comparatively high concentrations were measured on samples obtained mainly on rising stages or near the peak of storm-flows. Concentrations measured at base-flows were appreciably lower than those associated with storm-flows for both *E. coli* and *Campylobacter* spp. (Mann–Whitney, one-sided tests: *E. coli* W = 118, p = 0.004; *Campylobacter* spp. W = 124, p = 0.014) and this is reflected in the lower median concentrations under base-flow as shown in Figure 2.

In general, substantial loads of faecal bacteria were transported downstream during the course of the study increasing by three orders of magnitude from base-flow to elevated flow for both indicator and pathogenic bacteria. On average, base-flow loads were 4×10^5 *E. coli* MPN s^{-1} and 6×10^3 *Campylobacter* spp. MPN s^{-1} rising to 5.5×10^8 *E. coli* MPN s^{-1} and 1×10^6 *Campylobacter* spp. MPN s^{-1}.

Storm-flow monitoring

Four storm events were followed to define the dynamics of *E. coli* and *Campylobacter* spp. in relation to flow hydrographs. A typical time series for *Campylobacter* spp. and *E. coli* showing the relationship to the flow hydrograph and turbidity record is shown in Figure 3 for the storm-flow sampling event in September 2005. *E. coli* correlated closely with turbidity (measured continuously at the hydrometric site; Figure 3(a)) which was used to simulate continuous concentrations of *E. coli* (Figure 3(b)). Peak *E. coli* concentrations generally preceded the flow peak as has been reported previously (Nagels *et al.* 2002). *Campylobacter* spp. in contrast, seemed to lag somewhat behind *E. coli* (and turbidity), and peaked closer to the flow peak (Figure 3(b)). A similar pattern was observed for the remaining storm events with peaks arriving in relative order of turbidity $\geq$ *E. coli* $>$ *Campylobacter* spp. $\geq$ peak stream flow (Table 2).

Table 2 | Arrival times (h) for peaks in flow, turbidity, *E. coli* and *Campylobacter* spp. relative to the start of increase in stream flow hydrograph (Time 0) during storm events and associated flow, amount or concentration as appropriate in parentheses

Storm event		**1**	**2**	**3**	**4**
Date		**18 May 05**	**18 July 05**	**18 Sept 05**	**17 Dec 05**
Peak flow	(l s^{-1})	17 h (273)	12 h (3,112)	9 h (2,207)	9 h (835)
Turbidity peak	(NTU)	2 h (58)	7.5 h (186)	5 h (126)	5.5 h (23)
E. coli peak	(MPN 100 ml^{-1})	3 h (8.4×10^3)	7 h (10.2×10^3) & 13 h (12.2×10^3)	5 h (112×10^3)	8.5 h (11×10^3)
Campylobacter spp. peak	(MPN 100 ml^{-1})	10 h (50) & 26 h (30)	10 h (50)	7 h (130)	10.5 h (50)

DISCUSSION

The annual median *E. coli* concentration in the Toenepi Stream (345 MPN 100 ml^{-1}) during routine monitoring over one year was similar to that reported earlier by Wilcock *et al.* (1999) and Davies-Colley & Nagels (2002) (420 and 280 MPN per 100 ml, respectively). Median *E. coli* concentrations of this order appear to be fairly typical of dairy-dominated catchments (Wilcock *et al.* 2007) in which direct stream access by dairy cattle occurs frequently. Only about 46% of the Toenepi stream network is fenced (Wilcock *et al.* 2006) so there are places that can receive direct deposition of faeces. The direct deposition of fresh faeces has a high potential to contribute viable pathogens to a stream. Davies-Colley *et al.* (2008) determined a total annual export of 1.0×10^{13} cfu *E. coli* yr^{-1} km^{-2} from the Toenepi catchment. They also calculated that the contribution of direct deposition to this exported load was very small ($\sim$4% of total export of *E. coli*) with overland flow and sub-surface drain flow during storm events making the greatest contribution. Direct deposition, however, can have a significant impact during base-flow conditions that occur for more than 75% of the year (Muirhead *et al.* 2008).

The export of faecal contamination to downstream waters during base-flow and particularly during episodic fluxes generated by storm runoff has important implications for ensuring compliance in downstream waters with health-based standards for recreation, shellfish harvesting and source water for drinking (Signor *et al.* 2005; Kay *et al.* 2007). In New Zealand, freshwater used for recreation has alert and action guideline level values for *E. coli* (for single samples) in the range of 260–550 *E. coli* 100 ml^{-1} as an indicator of increased risk from *Campylobacter* spp. infection (MfE & MoH 2003). Environment Waikato has also produced standards for contaminants in contact recreation water for which the median concentration of *E. coli* in dry weather (throughout the bathing season) shall not exceed 126 *E. coli* 100 ml^{-1} and a single sample maximum shall not exceed 235 *E. coli* 100 ml^{-1} (EW 2006). Under base-flow conditions, the median concentration (228 *E. coli* 100 ml^{-1}) exceeded 126 *E. coli* 100 ml^{-1} in the Toenepi Stream and 43% of samples exceeded acceptable single sample levels. Under storm-flow conditions, *E. coli* concentrations were very high (e.g. 1×10^5 MPN 100 ml^{-1} on 5 September 2001) when sampling coincided with the rising stage of the hydrograph of a large storm event (Figure 1(a)). Similarly, other comparatively high *E. coli* concentrations ($>10^3$ MPN 100 ml^{-1}) were observed during elevated flow (mainly on rising stage or near-peaks of floods), while at base-flow, concentrations were typically much lower at $\sim 10^2$ MPN 100 ml^{-1}.

Campylobacter spp. were ubiquitous in the Toenepi stream water (93% positive samples) but at concentrations typically less than 10 MPN 100 ml^{-1}. On several occasions, concentrations exceeded 10^2 MPN 100 ml^{-1}, which coincided with those periods for which flows exceeded the 90th percentile flow rate of $\sim$500 l s^{-1}. Seasonality of *Campylobacter* spp. in rivers has been reported in several studies: for example Hudson *et al.* (1999) and Jones (2001) reported higher recovery in winter than in summer while, in contrast, Eyles *et al.* (2003) found concentrations were highest in summer. Till *et al.* (2008) found that the highest concentrations (in 25 New Zealand rivers) occurred in autumn. Similarly, in the present study, concentrations of *Campylobacter* spp. were on average highest in autumn although no significant seasonal effect was identified.

Throughout the routine monitoring period, concentrations of *Campylobacter* spp. were always lower than *E. coli*. Overall, the median ratio *E. coli*:*Campylobacter* spp. was 93, which is higher than the ratios of 30–40 observed in other rural streams (authors' unpublished data). The mean ratio was 220 and comparable to that of 184 reported for rivers in predominantly dairy-impacted catchments (Till *et al.* 2008). A higher level of *E. coli* relative to *Campylobacter* spp. in the Toenepi may be the result of a greater contribution of *E. coli* faecal bacteria mobilised from distant parts of the catchment together with the ability of *E. coli* to survive longer in the environment.

From results obtained in both laboratory (Korhonen & Martikainen 1991) and field studies (Sinton *et al.* 2007a, b), it is generally believed that *Campylobacter* spp. do not survive as well as *E. coli* in the environment (Jones 2001). The presence of thermotolerant *Campylobacter* spp. in streams reflects relatively recent faecal contamination and a persistent presence suggests constant inputs (Bolton *et al.* 1987) from sources including direct defecation into streams or tributary drains. Under wet conditions, overland or sub-surface flows will also transport faecal bacteria from land stores (faecal pats and soil) into streams, although transfer of *Campylobacter*

spp. is expected to be strongly dependent on how recently contributing areas were grazed. Vereen *et al.* (2007) found that levels of *Campylobacter* spp. in streams were significantly influenced by increasing precipitation. In the present study, high concentrations of *Campylobacter* spp. in the stream water coincided with peak flow (5 September 2001, Figure 1(a)), with peak concentrations up to two orders of magnitude greater than pre-flood conditions, indicating extensive wash-in of land stores from the catchment. For the Toenepi stream it has been determined that about 7% of the annual rainfall flows as surface runoff into the stream (Thorrold *et al.* 1996). This overland flow only contributes around 23% to stream-flow; the rest is derived from percolation through the soil (Thorrold *et al.* 1996). The extensive network of sub-surface drains in the Toenepi catchment reduces the overland flow but facilitates the contribution of sub-surface flow as a transmission route of microorganisms to streams. Connolly *et al.* (2004) observed little difference in *Campylobacter* spp. concentrations in drainage water compared with surface runoff in experiments carried out under wet conditions at comparable sites, suggesting that, when soils are very wet, attenuation in soil infiltration can be limited and broadly comparable to attenuation in overland flow through pasture. A similar finding was observed for mole tile drainage collected after farm dairy effluent was irrigated onto wet soils (Ross & Donnison 2003).

Previous studies (Nagels *et al.* 2002; Muirhead *et al.* 2004) have demonstrated that *E. coli* concentrations in streams may increase by several orders of magnitude under conditions that generate storm-flow. Storm-flow can thus have a significant influence on the mobilisation of faecal contamination and consequent impact on downstream water quality. A detailed study quantifying faecal pollution loads in the Toenepi stream reported that storm-flow contributed 95% of the total annual export of *E. coli* from the catchment. In a natural event (i.e. rain-generated) *E. coli* are derived from both land stores transported from surrounding grazed pasture and in-stream stores (Muirhead *et al.* 2004). In studies carried out in hill country catchments (authors' unpublished data), *E. coli* numbers increased substantially under high flow conditions when cattle had recently grazed the surrounding paddocks. Consistent with this, Collins *et al.* (2005) reported very high concentrations of *E. coli* in overland flows generated on pasture slopes by a rainfall simulator and that *E. coli* concentrations in the overland flow were strongly negatively associated with the age of the faecal matter measured as days since grazing. Much of the *E. coli* contamination mobilised by floods comes from streambed sediments. The importance of in-stream stores as a reservoir of *E. coli* has been demonstrated with artificial flow events in the absence of rainfall (Muirhead *et al.* 2004; Wilkinson *et al.* 2006) which produced water column concentrations of the same order of magnitude as those observed in natural floods (Nagels *et al.* 2002).

In comparison, the land stores of thermotolerant *Campylobacter* spp. are considered to be smaller than those for *E. coli* because, although all cattle shed *E. coli*, only infected animals shed *Campylobacter* spp. Prevalence rates of about 50% have been reported for New Zealand dairy cows (Adhikari *et al.* 2004; Ross *et al.* 2008). In addition, field studies (Sinton *et al.* 2007b) have demonstrated shorter survival of *Campylobacter* spp. than *E. coli* in cow pats in all seasons. The land stores of both bacteria will contribute to water column concentrations and replenish sediment stores. Different time-concentration patterns between *E. coli* and *Campylobacter* spp. during flood events in our studies indicate that sediment stores were relatively depauperate for entrainment of *Campylobacter* spp. as the sediment stores were apparently insufficient for the accelerating flood wave to produce a *Campylobacter* spp. peak before the peak water flow. Consistent with this interpretation, preliminary data (not reported here) suggest low levels of *Campylobacter* spp. in stream sediments despite abundant levels of *E. coli*. The high concentrations of *Campylobacter* spp. observed under flood conditions are considered to be principally due to wash-in of (recent) faecal matter in overland flow generated by rainfall.

Bacterial concentrations were measured in this study by the laboratory culture methods that were used in a large-scale New Zealand freshwater microbiology study that assessed health risks (Till & McBride 2004; Till *et al.* 2008). Conventional culture for *Campylobacter* spp. provides isolates that can be subjected to multi-locus sequence typing (Carter *et al.* 2009), a topic of ongoing research in the Toenepi catchment. Culture-independent methods (Haugland *et al.* 2005) have the potential to allow rapid and sensitive analysis of large numbers of samples and are likely to be increasingly used in microbial water quality assessment. These methods directly enumerate both the culturable and non-culturable target population and can be used for source tracking

(Meays *et al.* 2004; Shanks *et al.* 2007). At present, however, culture-independent methods require validation against health risks (Haugland *et al.* 2005) for regulatory acceptance (Noble & Weisberg 2005).

Wilkinson *et al.* (2006) proposed three distinct mechanisms mobilising faecal microbes in streams during storm-flows. Mechanism 1 involves entrainment of faecal bacteria from stream sediment stores (particularly from streambed areas immediately upstream of the monitoring site) by the rapidly accelerating water currents on the flood wave front. These bacteria arrive on the rising limb of the hydrograph, well ahead of the peak flow. Mechanism 2 involves bacteria being entrained at distant points in the stream network (by storm-flow wave passage, but also, potentially, by wash-in and artificial drain-flow from contributing areas of the catchment), followed by travel at the mean water velocity to produce a peak concentration occurring close to the peak water flow (and considerably later than the bacteria mobilised from within the stream). A third mechanism involves stochastic erosional processes associated with turbulent bursting, to explain persistence of elevated bacterial concentrations at steady flows. In the Toenepi Stream, Mechanism 1 (entrainment from stream stores) appears to be the dominant process for mobilisation and transport of *E. coli*, which consistently peaks close to the mid-rise of the hydrograph; that is, *E. coli* arrives *ahead* of the flood peak. In contrast, the *Campylobacter* spp. peak is appreciably later and close to the time of peak flow, which may be the signature of dominance by Mechanism 2 for this microorganism. We postulate that mobilisation of *Campylobacter* spp. is mainly as wash-in to channels or artificial drains of (fresh) faecal material from contributing areas where there has been recent grazing, followed by movement to the monitoring site at the mean water velocity (McBride 2011). Further studies are being done to support this with investigations on reach scale (rather than point) storage of microbes in stream sediments using flume experiments and field sampling to determine streambed sediment areal densities and entrained *E. coli*:*Campylobacter* spp. ratios in the absence of wash-in from upstream contributions.

Water may also be important in establishing and maintaining *Campylobacter* spp. infection on farms, thereby supporting the persistence of land-based sources. For example, Humphrey & Beckett (1987) reported that herds of dairy cows that drank stream water from which *Campylobacter* spp. could be isolated had high rates of infection. Similarly Hänninen *et al.* (1998) found more *Campylobacter*-positive cows when the animals used a lake as a source of drinking water than when drinking tap water; these workers reported that the same subspecies types of *C. jejuni* occurred in both the lake water and the cows. In contrast, herds that drank treated mains water or water sourced from deep wells, in which no *Campylobacter* spp. were detected, did not have any evidence of infection.

Bacterial concentrations in streams are influenced by animal numbers in the catchment, carriage and shedding rates, the extent of direct access to the stream and/or its tributaries and the potential for live bacteria in cowpats and soil to be transported from 'contributing areas' into the stream. Analysis of *Campylobacter* spp. isolates (data not reported here) demonstrated that most of the *Campylobacter* spp. recovered were *C. jejuni* although urease positive thermotolerant (UPTC) were present from time to time under base-flow in summer, demonstrating a contribution of *Campylobacter* spp. sourced from wild animals, in this case birds, as well as pastoral animals. French (2005) also reported the dominance of avian *Campylobacter* isolates in pastoral streams under low flow conditions, while at high flow *Campylobacter* spp. were dominated by ovine/bovine isolates lending credence to wash-in of land stores as a major source and delivery pathway for *Campylobacter* spp. to waterways. Further work to determine the contribution of birds to *Campylobacter* spp. loads in the Toenepi is the subject of ongoing work in the catchment.

The timing difference in catchment dynamics between *E. coli* and *Campylobacter* spp. found in our studies indicates that mitigations applied on-farm to reduce faecal microbial losses may not have the same effect on reducing the concentrations of different faecal microbes in streams. Modelling indicates that mitigations to reduce *E. coli* losses by 50% may have a greater effect on reducing *Campylobacter* levels (McBride & Chapra 2011). For *Giardia* and *Cryptosporidium*, which produce environmentally resistant cysts, there is a risk that mitigations may be less effective. Thus there is a need to understand the impacts of stream sediment reservoirs of microbes on both base- and storm-flow loads from catchments, and the source of the microbes found in sediments (Muirhead *et al.* 2004; Davies-Colley *et al.* 2008). A greater understanding of stream channel dynamics with respect to

faecal microbes is required before the catchment-scale ramifications of mitigations applied at a farm-scale can be determined in a similar way as has been done for nutrients, for example (Kronvang *et al*. 2005; Monaghan *et al*. 2009).

CONCLUSIONS

Thermotolerant *Campylobacter* spp., which cause high rates of reported disease in New Zealand, were consistently present in water samples from a stream in an intensively dairy-farmed catchment in the Waikato Region. Both the faecal indicator *E. coli* and *Campylobacter* spp. were elevated, sometimes markedly so, in storm-flows by comparison with base-flow conditions.

The behavioural dynamics of *Campylobacter* spp. during storm events in the Toenepi Stream contrasted with that of *E. coli*. *E. coli* consistently peaked on the rising limb of storm hydrographs while *Campylobacter* spp. tended to peak later, close to the time of peak flow. This difference in peak timing implies contrasting environmental behaviour which we interpret to reflect different predominant sources of faecal contamination for microbial mobilisation and transport in streams. The *E. coli* peak reflects mobilisation primarily from in-stream stores (particularly stream sediments) by the flood wave-front. In contrast, we hypothesise that wash-in from recent faecal deposits on contributing areas of the catchment is more important for *Campylobacter* spp., which, once mobilised, travel at the mean velocity of the water to arrive close to the water peak. This interpretation is supported both by: 1) modelling of kinematic wave disturbance and transport of microbes from different sources ; and 2) preliminary findings of high *E. coli* concentrations, but very low *Campylobacter* spp., in stream sediments. Further studies are being done to extend our understanding of the relative importance of in-stream versus land-based sources of these faecal bacteria.

The consistent presence in agricultural streams of thermotolerant *Campylobacter* spp., and the mobilisation by storm flows, implies a risk both to recreational users of streams and to downstream waters (including shellfish harvesting) that are episodically contaminated by flood plumes. A better understanding of the stream channel dynamics of faecal microbes is required before the effects of on-farm mitigations on catchment-scale water quality can be predicted.

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