

Microbial groundwater quality and its health implications for a border-strip irrigated dairy farm catchment, South Island, New Zealand

Murray Close, Rod Dann, Andrew Ball, Ruth Pirie, Marion Savill and Zella Smith

ABSTRACT

Intensification of dairying on irrigated pastures has led to concern over the microbial quality of shallow groundwater used for drinking purposes. The effects of intensive dairying and border-strip irrigation on the leaching of *E. coli* and *Campylobacter* to shallow groundwater were assessed over a three-year period in the Waikakahi catchment, Canterbury, New Zealand. Well selection excluded other sources of contamination so that the effect of dairying with border-strip irrigation could be assessed. Groundwater samples (135) were collected, mostly during the irrigation season, with *E. coli* being detected in 75% of samples. *Campylobacter* was identified in 16 samples (12%). A risk assessment of drinking water with these levels of *Campylobacter* was undertaken. A probability distribution was fitted to the observed *Campylobacter* data and the @RISK modeling software was used, assuming a dose response relationship for *Campylobacter* and consumption of 1 L/day of water. The probability of infection on any given day in the study area was estimated at 0.50% to 0.76%, giving an estimated probability of infection during the irrigation season of 60% to 75%. An epidemiological assessment of the Canterbury region comparing areas encompassing dairy within major irrigation schemes (~55% border-strip irrigation) to two control groups was undertaken. Control group 1 (CG1) encompasses areas of dairying without major irrigation schemes, and a second larger control group (CG2) comprises the rest of the Canterbury region. Comparisons of the subject group to control groups indicated that there was a statistically significant increase in age-standardised rates of campylobacteriosis (CG1 Relative Risk (RR) = 1.51 (95% CI = 1.31-1.75); CG2 RR = 1.51 (1.33-1.72)); cryptosporidiosis (CG1 RR = 2.08 (1.55-2.79); CG2 RR = 5.33 (4.12-6.90)); and salmonellosis (CG2 RR = 2.05 (1.55-2.71)).

Key words | *Campylobacter*, epidemiology, *E. coli*, groundwater, irrigation, risk assessment

INTRODUCTION

Increasing populations, access to international markets and improvement in agricultural technology have created a catalyst for intensification of land-use in the farming sector in New Zealand and throughout the world. Accompanying this intensification is an increase of diffuse contamination sources and associated pollution of surface and groundwater

resources. Groundwater is a major source of drinking water in New Zealand and its pollution by pathogens and elevated concentrations of nitrate is of concern due to its use for drinking and its effect on the quality of surface water bodies into which groundwater discharges. The dairy industry is an extremely important part of the New Zealand economy.

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However, the intensification of dairy farming has created new pressures on the environment, mainly through the effects of dairy effluent on fresh water and the contamination of groundwater (Ministry of Agriculture & Forestry 2003).

Sustainable agricultural production is reliant on water and, in areas without consistent or sufficient rainfall, irrigation is used. One method of irrigation common in some areas of New Zealand close to large rivers is 'border-strip' irrigation. This involves the controlled flooding of contoured, bordered paddocks with generally around 100 mm of water. The flooding can lead to significant macropore or bypass flow where cracks and other macropores exist in the soil profile. There is concern that in dairy farming situations, where pathogens are being released to the ground surface continually through animal faeces and, in parts of the farm, effluent irrigation, macropore flow could lead to significant contamination of shallow groundwater resources. This is due to decreased filtration and residence time through the soil and vadose zone (profile between the bottom of the soil and the groundwater table), reducing the time for pathogen die-off to occur.

Waterborne pathogens of concern include (but are not limited to) protozoan parasites such as *Cryptosporidium* and *Giardia* species, bacteria such as pathogenic *Escherichia coli* (*E. coli*) and *Campylobacter*, and a variety of viruses. Pathogens are released into the environment through a number of point and diffuse sources including septic tanks, effluent irrigation schemes, and animal (eg. cattle, sheep, birds) defecation onto the land surface or directly into water bodies. Once in the environment they can be transported by water across the land surface and into rivers or through soils and into groundwater systems (Ginn *et al.* 2002; Unc & Goss 2004).

Many factors affect the likelihood of groundwater resources becoming contaminated by pathogens. These include the number of pathogens released to the environment initially (dependant on stocking rates, type of animal, and the infection rate of the animals); the persistence of the pathogens in the environment; filtration of pathogens through the soil and vadose zones; and the hydrological regime. Lower temperatures, higher moisture contents, neutral pH, high organic matter content and low densities of predator species generally enhance persistence of pathogens in the environment. The size of the pathogens,

soil depth, and pore size distribution are the main factors influencing filtration, with larger organisms such as protozoa (~3–20 µm) being filtered out more rapidly than smaller bacteria (~1–6 µm) and viruses (~100 nm). There is greater filtration of pathogens in finer grained soils compared to coarser-stony soils, however, macropores may enable rapid transport of pathogens through otherwise fine-grained soils. Deeper soils will generally filter out more pathogens than similarly structured shallow soils, minimizing groundwater contamination. The hydrological regime determines the amount of water available for leaching and transport of the pathogens. It impacts the travel time through the soil and vadose zone to the aquifer. The travel time is also affected by (a) the water content and structure of the soil and vadose zone materials; (b) geochemical properties and organic matter content of the soil and the properties of the microbial cells (affecting the adsorption and desorption of pathogens); and (c) the depth to groundwater table; deeper groundwater tables provide more time for pathogens to die off and/or be filtered before entering the groundwater system.

Assessment of the risk to human health is pertinent for studies such as this, where the people living in these environments often use groundwater without treatment for household purposes, including drinking, on a daily basis. The disease outbreak in Walkerton, Canada is a case where microbial contamination of groundwater in a small community without adequate treatment lead to disastrous results (Unc & Goss 2004). Much investigation has been carried out on the probability of infection due to consumption of pathogens, including *Campylobacter* (Teunis *et al.* 1999). There have been a mixture of study approaches, including analysis of outbreaks (Hanninen *et al.* 2003; Teunis *et al.* 2004) and feeding trials (Black *et al.* 1988). The assessment of risk of illness related to pathogens in relation to drinking water is relatively limited (Teunis *et al.* 1997; Carrique-Mas *et al.* 2005). Schets *et al.* (2005) suggest in their investigation of drinking water from private wells that a risk assessment should be performed as the monitoring of both faecal indicators and pathogens does not predict the health effects of microbial contamination of drinking water on a population.

The objectives of this study were to assess the microbial quality of groundwater in a border strip irrigation dairy catchment on the South Island of New Zealand; to

determine any relationship between timing of stocking, border strip irrigation and contamination of the groundwater using an indicator bacteria (*E. coli*) and the pathogen *Campylobacter*; and to carry out a preliminary assessment of the health risk related to drinking groundwater sourced from shallow aquifers in this area. The risk assessment involved combining the observed *Campylobacter* data and likely consumption with a dose response model to give a risk of infection, and an epidemiological assessment of notified cases of disease in areas with this land use pattern compared to areas with a different land use pattern. Information gained from this investigation has also formed the basis of recommendations for more effective land management practices which are reported elsewhere (Close *et al.* 2005), with the goal that groundwater microbial contamination is minimized in dairying areas.

MATERIALS AND METHODS

Site characteristics

This groundwater study was undertaken in the Waikakahi catchment, which is a largely dairying catchment in South Canterbury characterized by border strip irrigation. This catchment area is typical of many areas throughout the South Island of New Zealand (Figure 1). The Waikakahi catchment forms part of the Glenavy aquifer basin that covers approximately 157 km². The hydrogeology comprising highly permeable post-glacial gravels (hydraulic conductivities ~600–1,700 m/day) adjacent to the Waitaki River and less permeable Pleistocene gravels (hydraulic conductivities ~40–90 m/day) situated above the terrace of the Waitaki River (URS 2003a). Using an estimate of the groundwater gradient of 0.0042 and an assumed porosity of 0.2, the groundwater velocities are likely to range from approximately 1 m/day to 36 m/day. All the sites chosen for the groundwater study are located within the more permeable post-glacial gravels relatively close to the river, with the exception of one well (J41/0134), which is located further from the river. The closest distance between the wells and the river was about 750 m, and the gradient was slightly towards the river. The estimated average saturated aquifer thickness throughout the catchment is 15 m. The Glenavy basin has an estimated rainfall infiltration rate

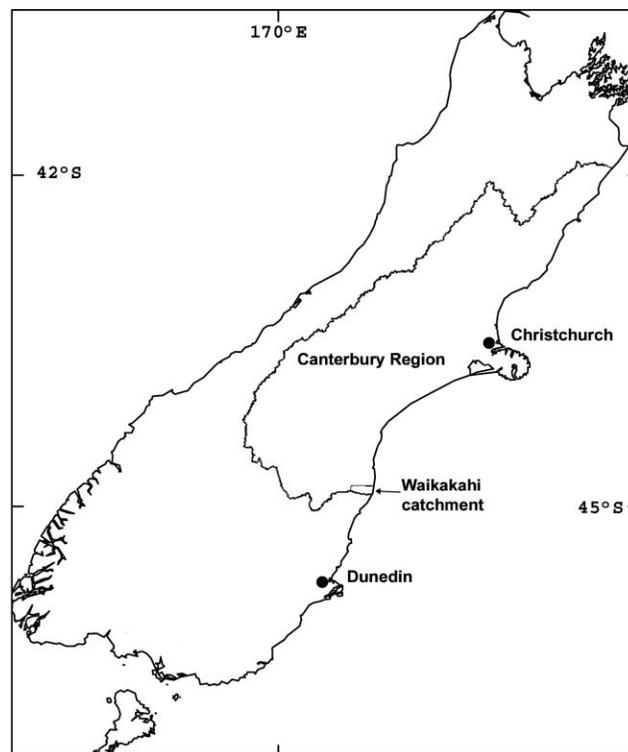


Figure 1 | Location of the Waikakahi catchment, Canterbury, in the South Island of New Zealand.

(rainfall minus evapotranspiration) of 54 mm/year, with an estimated infiltration rate of 902 mm/yr from both rainfall and irrigation infiltration (URS 2003b). Rout *et al.* (2002) in their report on irrigation efficiency in the nearby Ashburton region indicated that application depths for contour border-strip irrigation were typically 100–130 mm with lower levels for laser-levelled borders (70–100 mm).

In the first year of the study six wells were selected in the Waikakahi catchment (Figure 2) with well depths ranging from 4.6 to 11 m below ground level. The wells were selected to avoid possible contamination from septic tanks and to ensure good wellhead protection. Three wells were slightly modified to improve the wellhead protection. Details for each well are given in Table 1.

Farm management data collection

Information on stocking and irrigation practices was collected from the six well owners, and climatic data was collated. There were occasional periods where the stocking and irrigation data were not recorded. For these periods the

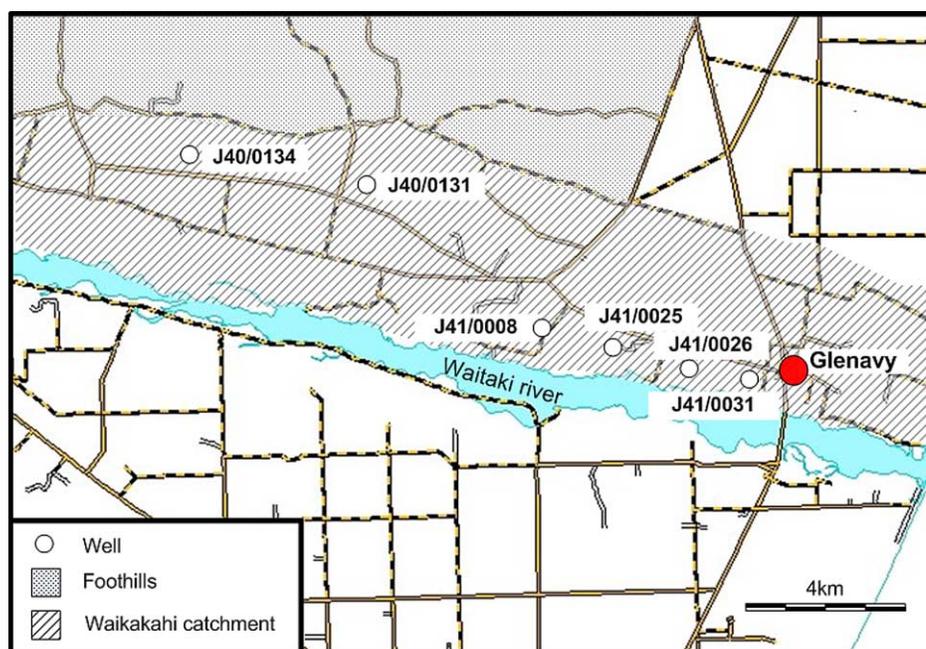


Figure 2 | Location of monitoring wells within the Waikakahi catchment. Well J40/0131 was decommissioned in the second year of the study.

landowners or farm managers indicated that irrigation occurred approximately every 14 days and grazing occurred approximately every 21 days. This is consistent with the recorded data. Farm maps were obtained where possible and the groundwater flow direction was estimated based on local topography. Information was collected for the four fields up-gradient of the well which were considered to be most likely to impact the well. Wells for the study were

selected to avoid septic tank contamination and wellhead protection issues, which meant that the microbial contamination was most likely related to dairying combined with border strip irrigation. At the end of the first year one of the wells was replaced with a much deeper well by the farm owner and was no longer available for sampling, leaving 5 wells for the remainder of the study.

Table 1 | Details of wells used in the study

Site	Well diameter (mm)	Well Depth (m)	Usage
J40/0134	200	4.6	Stock
J40/0131 [#]	200	6.1	Domestic/Dairy
J41/0008	150	15	Dairy/Stock
J41/0025	150	6.5	Domestic/Stock/Dairy
J41/0026	200	5.7	Domestic/Stock
J41/0031	150	7.1	Domestic/Stock

Notes: # Well decommissioned by land owner at the start of the 2nd year of study.

Sampling regime

The focus in this study is mainly the *Campylobacter* and *E. coli* concentrations in groundwater and *Campylobacter* in cowpat samples. Total coliforms were also analysed as part of the *E. coli* analysis. A study of *Campylobacter* within cow faecal samples within the catchment herds was undertaken in the first year of the study. The study comprised the collection of samples of cow faecal material from three sites on each sampling occasion, with the other three sites being sampled on the following sampling occasion, with each sample a composite of 5 cowpats.

The groundwater sampling regime varied throughout the three years of the study. Groundwater contamination was quantified in the first year by monitoring wells at monthly intervals during the irrigation season. In addition a

sample was collected in September 2003 to obtain an indication of microbial water quality in the winter, non-irrigation period. In the second and third years of the study the impact of timing of stocking, irrigation and climatic factors on pathogen contamination of groundwater in border-strip and spray irrigation farming systems was assessed. Sampling in the second year consisted of approximately monthly groundwater for three of the wells with more intensive (twice weekly) sampling undertaken on two wells (J41-0026 & J41-0008) for 2 months of the irrigation period. The third year sampling regime comprised of a winter/spring sample in late September followed by an intensive weekly sampling program for the 5 wells extending for 9 weeks from the start of the irrigation period. A total of 50 samples were collected in the third year program giving a total of 135 samples for the entire study period.

Analytical techniques

Well samples were analysed for *E. coli* and *Campylobacter*. *E. coli* were enumerated using the Colilert system with the Quantitray 96 well enumeration. This analysis also provides counts of total coliform bacteria. *Campylobacter* were quantitatively analysed using a MPN/PCR format for *C. jejuni* and *C. coli*, these being the 2 major pathogenic species. The PCR method for *Campylobacter* as validated by Wong *et al.* (2004) was used with a 3 tube MPN format (volumes of 1000 ml, 500 ml, and 100 ml). This gave a quantification range of <0.6 to >3.1 MPN per litre. It should be noted that there is significant uncertainty associated with a 3 tube MPN but this was used due to budgetary constraints. Briefly the PCR method involved enrichment in two sequential selective enrichment broths. Positive, negative and sterility controls were included to ensure that contamination of the broth media had not occurred and that selective enrichment of *Campylobacter* was occurring. The secondary enrichment step ensured that only viable cells were detected by PCR, and this avoided any false positives caused by dead *Campylobacter*. After enrichment cells were recovered from a portion of the broth, washed to remove residual broth and other inhibitors, then lysed to release the DNA. Contamination controls were included in the washing process. The DNA was amplified in a multiplex PCR targeting three regions of

DNA (Wong *et al.* 2004). These target regions were specific for 1) all thermophilic *Campylobacter*; 2) *C. jejuni*; and 3) *C. coli*.

Health risk assessment methodology

An assessment of the health risk associated with these numbers of *Campylobacter* to consumers of this water was undertaken. The risk was taken to be equal to the dose or exposure combined with a dose response relationship and was calculated using a Monte Carlo approach with the @RISK modelling programme (Palisade Corporation v4.5.3). Two approaches were used to estimate the concentration, which was then combined with likely consumption to give the dose. In the first an exponential distribution was fitted to the observed *Campylobacter* data and used to calculate the concentration distribution in the shallow groundwater. The effects of truncation of the exponential distribution were tested and found to be insignificant for truncation above levels of 4. The second approach followed that described by McBride (2005). Briefly, a Geometric distribution was fitted to the data to obtain the Geometric distribution θ value, i.e. probability of trials in the Geometric model. The Geometric distribution output is termed the *bin selector*. The possible $1 \times 1 \times 1$ MPN occurrence probabilities are binned into defined ranges such that each internal bin contains one MPN with occurrence probabilities greater than 0.2 using exact MPN values (McBride 2005). The boundary conditions are defined and a bins assigned such bin 0 = all zero values, bin 3 = values >3.1 with the maximum value of 5 (although rather arbitrary, it is assigned based on expert opinion). The effects of setting the roof of bin 3 to 4, 5, or 10 was tested and there was very little effect on the resulting distribution parameters. During one iteration, a random sample is drawn from the geometric distribution which determines the bin to be selected. A random sample is then drawn from the respective bin which is defined by a uniform distribution whose bounds are the lower and upper limits of the bin. This returns the *Campylobacter* concentration in the sample. A Monte Carlo simulation in @RISK using this approach was run for 10,000 iterations.

Dose response data for one strain of *Campylobacter jejuni* have been obtained in human feeding trials

(subjects were healthy adults) undertaken by Black *et al.* (1988). Medema *et al.* (1996) derived a beta-Poisson model [$P_{\text{inf}} = 1 - (1 + N/\beta)^{-\alpha}$] to fit the experimental data, with maximum likelihood estimates for model parameters being: $\alpha = 0.145$ and $\beta = 7.59$, where P_{inf} is the probability of infection and N is the (Poisson) mean number of bacteria ingested. The dose required for a 50% risk of infection from this model is 896 bacteria. However, the smallest number of bacteria given to volunteers in the Black *et al.* (1988) trial was 800 bacteria. As such, there is considerable uncertainty around the effects of lower doses on the infection rates (Teunis & Havelaar 2000). For very low doses (less than 1) the use of a hypergeometric dose-response model gives more acceptable 95% confidence limits (Medema *et al.* 1996), but the predicted probability of infection values are not very different from those predicted using the beta-Poisson model.

In the World Health Organization Guidelines for Drinking-Water Quality, values for chemical contaminants are based on the assumption of a 60 kg adult consuming 2 litres per day from drinking water, which would be equivalent to 3 litres per capita per day including food consumption (World Health Organisation 1993). As a proportion of the water consumed is boiled, which may not affect chemical toxicity but is likely to greatly reduce microbial loads, the consumption of water which could contain viable *Campylobacter* will be lower. Accordingly a value of 1 litre was used as the daily consumption. This will probably give a conservative estimate of risk.

Epidemiological assessment

An epidemiological survey of the effects of dairy and irrigation in the greater Canterbury region was undertaken using the ESR EpiSurv notifiable disease database (as at April 2006), combined with dairy farm location and major irrigation scheme data. In New Zealand medical practitioners are required under the Health Act 1956 to report cases of notifiable disease to their local Medical Officer of Health. This data is collected into a national database (EpiSurv). Enteric diseases with an average of more than 400 cases reported in New Zealand each year over the time period 1997 to 2005 were selected for this analysis. The dairy farm location data comprises a map of the boundary of all

dairy farms in the Canterbury region. Most dairy farms in Canterbury use irrigation but border strip and flood irrigation is mainly confined to major irrigation schemes. Areas of major irrigation schemes (defined as irrigating over 500 hectares) throughout Canterbury were taken from Dommissie (2005). These areas comprise approximately 55% border strip and flood irrigation and 45% spray irrigation. The remaining dairy farms in Canterbury would be predominately (>90%) irrigated using spray irrigation. It was not possible to separate individual dairy farms into those irrigating using border strip compared to those using spray irrigation.

An analysis of the dairy and irrigation data was undertaken to produce three subset groups comprising a varying number of meshblocks. The meshblock is the smallest geographic unit for which statistical data is collected and processed by Statistics New Zealand, it defines a geographic area, varying in size from part of a city block to large areas of rural land. Each meshblock abuts against another to form a network covering all of New Zealand including coasts and inlets. The three subsets investigated were 'Subject Group' (SG) all meshblocks that intersect dairy farms and major (>500 hectares) irrigation schemes (population @ 2001 of 5,088); 'Control Group 1' (CG1) all other dairy farms (i.e. not within major irrigation schemes) (population @ 2001 of 30,903); and 'Control Group 2' (CG2) the remainder of the meshblocks in Canterbury (population @ 2001 445,149). The effect of having a mixture of border strip and spray irrigation in SG would be to minimize the differences between SG and CG1, compared to what would be expected if SG was entirely border strip irrigation.

Data were extracted from EpiSurv by meshblock for the 9-year period, aggregated and then divided by the number of years to produce an annualised crude rate for incidence of campylobacteriosis, giardiasis, salmonellosis, cryptosporidiosis, yersiniosis, and gastroenteritis (various known and unknown causal agents but excluding those diseases otherwise specified in this list). Age-standardised disease rates were calculated using the New Zealand 2001 Census meshblock population data for the three study groups and the total New Zealand population for the standard population. There were too few cases for giardiasis, yersiniosis, and gastroenteritis in the study group for age-standardisation so only crude rates are presented. Relative risk (RR), which is calculated as the mean incidence of illness

for one population/mean incidence of illness for another, was calculated for both crude and age-standardised rates. A RR value of one suggests no difference in risk while a RR value of 2 suggests twice as much risk of illness compared to another population set. The 95% confidence intervals were calculated using the total number of cases over the 9 year period using the procedure set out by Morris & Gardner (1994).

RESULTS

Campylobacter in cow faeces

Campylobacter was detected in 19 of 21 (90%) faecal samples analyzed. It should be noted that these were from composite samples (5 cow pats) and so do not give a true carriage rate but the results indicate that *Campylobacter* was generally present in the dairy herds throughout the study area.

E. coli and *Campylobacter* in groundwater

The groundwater results indicated *E. coli*, *Campylobacter*, and total coliforms were detected in the groundwater, with higher concentrations of total coliforms and *E. coli*, as expected. The results from the groundwater samples are summarised in Table 2 and Figure 3. For the calculation of mean concentrations and correlations between bacteria, values less than the detection limits were replaced by 0 and values greater than maximum value were replaced by twice the maximum value. There were 14 samples with >2400 MPN/100 ml for total coliforms, no samples with >2400 MPN/100 ml for *E. coli*, and 2 samples with >3.1 MPN/L for *Campylobacter*. Total coliforms were detected on 98.5% of sampling occasions at concentrations ranging from <1 to >2,400 MPN (most probable number)/100 ml with an average for all wells of 757 MPN/100 ml and a median of 80 MPN/100 ml.

E. coli was detected in all wells, with the concentrations ranging from <1 to 2400 MPN/100 ml with an average for all wells of 40 MPN/100 ml and a median of 2 MPN/100 ml. The mean level of *E. coli* in the individual well samples ranged from 6 to 137 MPN/100 ml, which is significantly above the New Zealand drinking water standard for *E. coli* of <1 MPN/100 ml. The overall detection rate for *E. coli* in all samples was 75%.

Over the three years *Campylobacter* has been detected in samples from each of the wells on at least two sampling occasions with levels ranging from 0.6 to >3.1 MPN/L. Of the five wells sampled over the three years *Campylobacter* were detected in 14 out of a total of 126 samples. In addition, relatively elevated levels (3.1 and >3.1 MPN/L) of *Campylobacter* were also detected in 2 samples taken from the well which was decommissioned in year two of the study, resulting in a total of 16 positive *Campylobacter* results and giving an overall detection rate of 12%. *Campylobacter jejuni* were isolated in 11 samples and the remaining 5 were thermophilic *Campylobacter* sp. other than *C. jejuni* or *C. coli*.

There were two sampling rounds carried out in the winter, one in 2003 and one in 2004. There was little difference in the detection rates for the total coliforms, with 99% being positive during the irrigation season compared to 91% positive in the winter, with an overall detection rate of 98%. Only 2 samples out of 129 did not contain total coliform bacteria. There was a much larger difference with *E. coli*, with 77% of samples containing *E. coli* during the irrigation season compared to 55% in the winter (overall detection rate of 75%). There was little difference in the *Campylobacter* detection rate with season, with 12% of samples being positive during the irrigation season compared to 9% during the winter, giving an overall detection rate of 12%. It should be noted that the 9% detection rate in winter is derived from one positive detection out of a total of 11 samples, so this rate would be likely to vary if more samples were collected during the winter period.

Over the three years of the study, there was a poor linear correlation between both *Campylobacter* and *E. coli* ($r = 0.035$; $p = 0.69$, $n = 129$) and between *Campylobacter* and total coliforms ($r = -0.02$; $p = 0.82$, $n = 129$). However, there was a significant nonlinear correlation, using the Spearman rho rank correlation coefficient, between both *Campylobacter* and *E. coli* ($\rho = 0.260$; $p = 0.0035$, $n = 129$) and between *Campylobacter* and total coliforms ($\rho = 0.187$; $p = 0.038$, $n = 129$). This is probably due to the relatively low number of *Campylobacter* detections (16) in comparison to *E. coli* (97) and total coliform detections (99%), and the much wider range of values for the coliform data.

A graphical analysis of time lag between stocking and irrigation and elevated microbial counts in the down-gradient

Table 2 | Summary of groundwater quality data for each well for the entire study period (2002–2004). Mean and ranges of water levels are shown, as are number of detections for *Campylobacter*, and median values for *E. coli* and total coliforms, with ranges displayed in brackets

Site	Water level (m bgl)	<i>C. jejuni</i> (MPN/L)	<i>C. therm</i> (MPN/L)	<i>E. coli</i> (MPN/100 ml)	Total coliforms (MPN/100 ml)
J40/0134	1.77 (1.23–2.6)	2 detections (< 0.6 – > 3.1)	0 detections (< 0.6)	1 (< 1 –53)	41 (< 1 – > 2400)
J40/0131	2.47 (1.29–3.29)	1 detection (< 0.6 – > 3.1)	1 detection (< 0.6 –3.1)	12 (2–120)	550 (20–2400)
J41/0008	7.43 (7.29–7.52)	4 detections (< 0.6 –0.6)	1 detection (< 0.6 –3.1)	5 (< 1 –1100)	180 (2– > 2400)
J41/0025	2.1 (1.81–2.50)	1 detection (< 0.6 –0.6)	2 detections (< 0.6 –0.6)	3 (< 1 –81)	62 (14– > 2400)
J41/0026	3.72 (3.31–4.04)	2 detections (< 0.6 –0.6)	0 detections (< 0.6)	2 (< 1 –100)	66 (20– > 2400)
J41/0031	3.29 (3.0–3.64)	1 detection (< 0.6 –0.6)	1 detection (< 0.6 –0.6)	2 (< 1 –2400)	71 (25– > 2400)

Notes: 1/ *C. Therm* are thermophilic *Campylobacter* that are probably not *C. jejuni* or *C. coli*. 2/ There were only 7 samples collected from well J40/0131.

wells was undertaken (data for two of the wells shown in Figures 4–7). Irrigation events were ranked according to their likely risk of contributing to contamination of the aquifer. For the purposes of the graphical analysis, risk was taken as inversely proportional to the number of days prior to the irrigation since stock had grazed the paddock, and the size of the circle is proportional to the estimated risk. The largest circles indicate that stock were grazing in conjunction with irrigation on days just prior to irrigation (denoted ‘high risk’), while smaller circles indicate that stocking of paddocks occurred numerous days before irrigations (‘low risk’).

Figure 4 shows an elevated *E. coli* count (accompanied by a positive *Campylobacter* result) after a series of ‘high risk’ irrigation events up-gradient of well J41-0008. It is difficult to determine whether there is one paddock or a combination of paddocks contributing to the contamination, or determine the lag period. There are only small amounts of rainfall during this period indicating that the irrigation is the main cause of the contamination.

Unusually high *E. coli* counts (up to 1100 MPN/100 ml) accompanied by positive *Campylobacter* results from well J41-0008 are shown in Figure 5. In the period leading up to the contamination event there was a ‘high risk’ irrigation

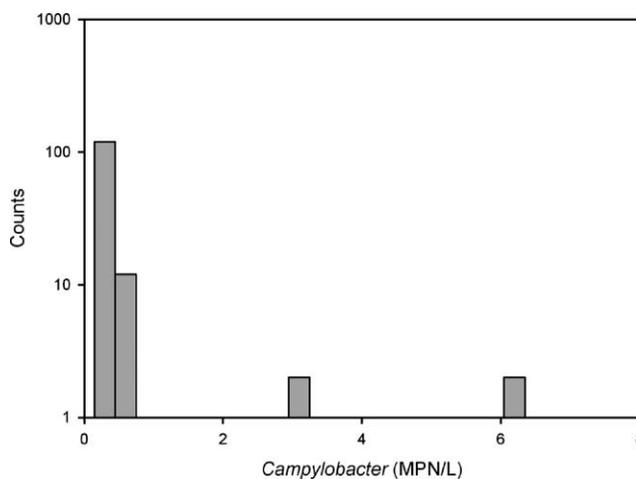


Figure 3 | Distribution of *Campylobacter* counts from groundwater samples. Values < 0.6 MPN/L were plotted at 0.3 MPN/L and values of > 3.1 MPN/L were plotted at 6.2 MPN/L for display purposes. Note the log scale for the y axis.

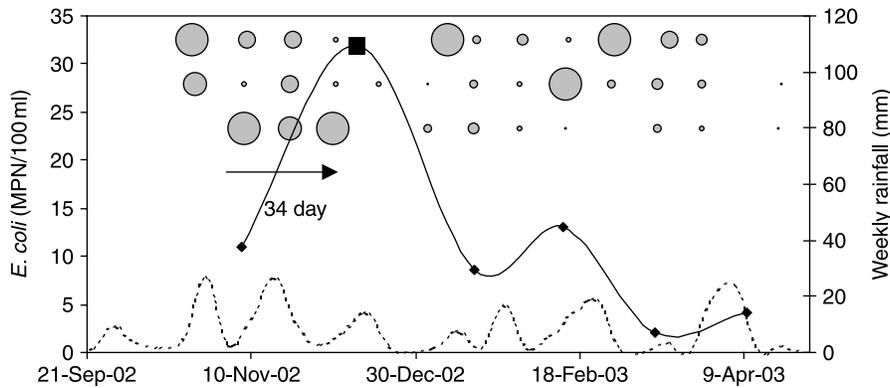


Figure 4 | A graphical analysis for J41-0008 in 2002/03, showing potentially 'higher' risk irrigation events (soon after stocking) for each paddock, represented by larger area circles, and potentially 'lower' risk irrigation events (smaller circles). Also shown are *E. coli* counts (solid line) and weekly rainfall totals (dashed line). A solid square indicates a positive *Campylobacter* result. The lag is the time between a high risk irrigation event and contamination in the well.

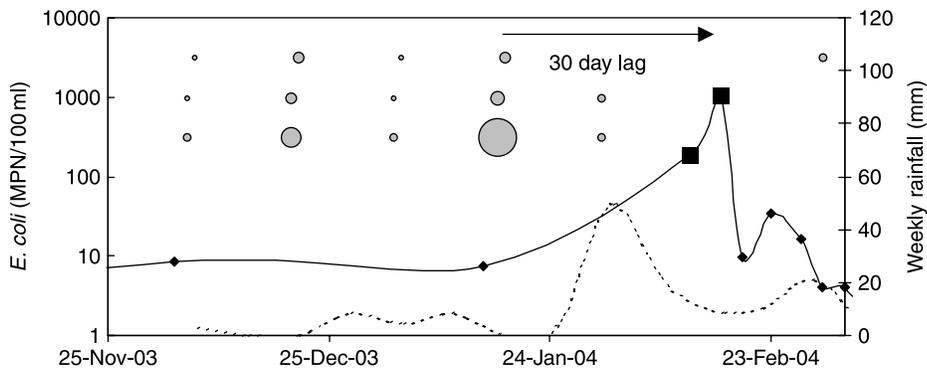


Figure 5 | A graphical analysis for J41-0008 in 2003/04, showing potentially 'higher' risk irrigation events (soon after stocking) for each paddock, represented by larger area circles, and potentially 'lower' risk irrigation events (smaller circles). Also shown are *E. coli* counts (solid line) and weekly rainfall totals (dashed line). A solid square indicates a positive *Campylobacter* result. The lag is the time between a high risk irrigation event and contamination in the well. Note the log scale on the *E. coli* axis.

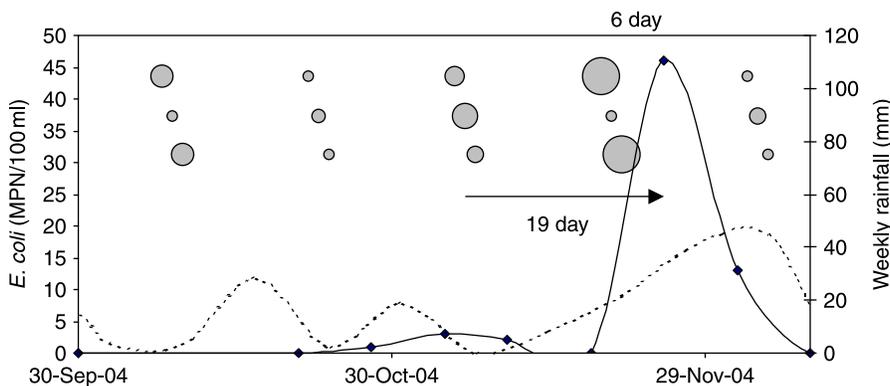


Figure 6 | A graphical analysis for J41-0026 in 2003/04, showing potentially 'higher' risk irrigation events (soon after stocking) for each paddock, represented by larger area circles, and potentially 'lower' risk irrigation events (smaller circles). Also shown are *E. coli* counts (solid line) and weekly rainfall totals (dashed line). A solid square indicates a positive *Campylobacter* result. The lag is the time between a high risk irrigation event and contamination in the well.

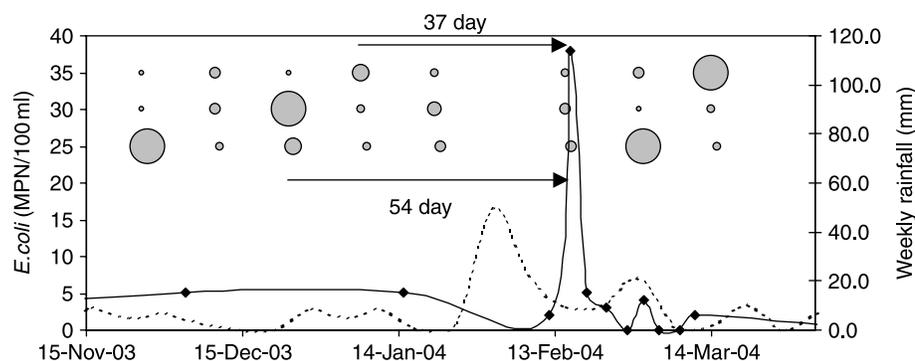


Figure 7 | A graphical analysis for J41-0026 in 2004, showing potentially 'higher' risk irrigation events (soon after stocking) for each paddock, represented by larger area circles, and potentially 'lower' risk irrigation events (smaller circles). Also shown are *E. coli* counts (solid line) and weekly rainfall totals (dashed line). The lag is the time between a high risk irrigation event and contamination in the well.

event 30 days prior followed by a significant amount of rain (49 mm in a week). It is likely that the combination of these two factors led to saturated soils, macropore flow and significant release of microbes from cowpats, which resulted in the high microbial counts in the groundwater sample.

Figure 6 shows a short peak of contaminants in well J41-0026 on 16 February 2004. Prior to this event there is a coincidental irrigation event (stock on paddock the same day as irrigation) 54 days prior to the peak and a relatively 'high risk' event (stock on paddock 3 days prior to irrigation) 37 days prior to the *E. coli* peak. In addition there is a rainfall peak of (49 mm in a week) in the lead-up time.

There are a number of 'high risk' irrigation events leading up to the peak shown in Figure 7. Two irrigation events occurred on the 19 November only 1 day after the paddocks were stocked and 19 days prior to the peak (6 November) a relatively 'high risk' irrigation event occurred 3 days after the stocking of a paddock. There was no significant rainfall during this period indicating that the irrigation events were likely to be the major factor in the contamination of groundwater at this time.

Health risk assessment

The first approach using an exponential distribution fitted to the observed *Campylobacter* data from all wells gave a good fit to the data with $\beta = 0.28$. Using a Monte Carlo approach in the @RISK modeling program with the assumptions stated above (water from shallow unconfined groundwater

underlying dairying with border strip irrigation; dose response relationship from Medema *et al.* (1996); and consumption of 1 litre per day), the probability of infection on any given day in the study area was found to be 0.50%.

Using the approach of McBride (2005) and the same assumptions concerning the dose response curve and consumption, a distribution is obtained for the probability of infection on any given day in the study area. Two curves were fitted to this distribution, a lognormal and an exponential curve. The mean probability of infection was 0.762% and 0.759% from the lognormal and exponential distributions, respectively. The expected daily probabilities of infection from both approaches were fairly similar, 0.50% compared to 0.76%, with the more precise estimate being slightly higher.

If P_d is the probability of daily infection, then treating each day's risk as independent, the annual probability of infection (P_a) is:

$$P_a = 1 - (1 - P_d)^{365}$$

For $P_d = 0.50\%$ and 0.76% , $P_a = 84\%$ and 94% , respectively. As the measured groundwater concentrations are predominantly from the irrigation season and were more contaminated than the winter samples, it is more appropriate to estimate the probability of infection during the irrigation season. If the irrigation is taken as October to March inclusive (6 months or 182 days), then the probability of infection during the irrigation season is estimated as 60% and 75% from a P_d of 0.50% and 0.76%, respectively.

It should be noted that infection does not necessarily result in illness or clinical expression. A Food and

Agriculture Organisation (FAO) report estimated that the probability of illness following infection is a beta distribution with a mean of approximately 33%, determined from the Black *et al.* (1988) feeding trial data. Also noted in the FAO report is an outbreak investigation in which 53% of those infected became ill (FAO/WHO 2007). This would result in an irrigation season probability of illness of between 20% and 40%.

Epidemiological assessment

Episurv data for the six diseases queried are presented in Table 3. There is only a slight difference between the crude and age-standardised rates, with the rates for SG decreasing slightly and the rates for CG1 and CG2 slightly increasing. There is an apparent gradient of notified cases of cryptosporidiosis, salmonellosis, and yersiniosis per 100,000 with the greatest number of cases in areas of dairying and major irrigation schemes followed by areas of dairying with no

major irrigation, with generally the least number of cases in the main control group, taken from the rest of the region. Rates of campylobacteriosis were much higher in SG compared to CG1 and CG2.

The epidemiological assessment indicated significant differences in RR based on the crude rates for four of the six pathogens investigated, either for one or both comparison groups (Table 4). The results for the age-standardised rates were similar. Using the crude rates, the RR for campylobacteriosis was 1.57 and 1.56 for SG versus CG1 and SG versus CG2, respectively, indicating that people in SG were about 56% more likely to have a notified illness of campylobacteriosis compared to the rest of the population. The RR values for cryptosporidiosis of 2.32 (comparison with CG1) and 6.03 (comparison with CG2) indicate significantly higher risks in scheme irrigated dairy areas (SG). The RR values for salmonellosis indicate the risk compared to CG2 (non-dairy control) to be about two times, whilst in comparison to the dairy with no irrigation the RR is 1.43 but was not significantly different from one for the age-standardised rates. There was a significant RR of 1.82 for gastroenteritis cases for comparisons between dairy with major irrigation and dairies without major irrigation. Even though the RR values for yersiniosis were around 1.5, they were not significant differences because of the low number of yersiniosis cases (Table 3). The RR values for giardiasis were around 0.9 and not significantly different from one. The RR values calculated from the age-standardised rates were slightly lower than those calculated from the crude rates but were still significant at the 95% confidence level with the exception of salmonellosis between SG and CG1.

Table 3 | Crude and age-standardised rates of pathogen illness per 100,000 for the three meshblock groups assessed. There were not enough cases to carry out age-standardisation for gastroenteritis, giardiasis and yersiniosis. Data collection period was 1997–2005

Illness	Dairy with major irrigation (SG)	Dairy without major irrigation (CG1)	Rest of Canterbury (CG2)
	Cases per 100,000		
Crude Rates			
Campylobacteriosis	513.2	326.8	328.5
Cryptosporidiosis	135.4	58.2	22.5
Gastroenteritis	52.4	28.8	48.4
Giardiasis	28.4	31.6	35.4
Salmonellosis	111.4	78.0	49.9
Yersiniosis	26.2	18.7	15.8
Age-standardised Rates			
Campylobacteriosis	501.2	331.3	331.6
Cryptosporidiosis	126.7	60.9	23.8
Salmonellosis	106.7	80.4	52.0

DISCUSSION

Groundwater contamination

The results indicate that both *E. coli* and the pathogenic bacteria *Campylobacter*, were consistently detected in the groundwater samples collected from wells within the catchment of border strip irrigation with dairying, with *E. coli* often being detected at concentrations in excess of the New Zealand drinking water guideline of < 1 /100 ml.

Table 4 | Relative risk (RR) calculations comparing areas with dairy and major irrigation schemes (SG), areas with dairy and no major irrigation scheme (CG1), and the remainder of the Canterbury region (CG2). Significant RR at the 95% CI are indicated with an asterisk

Illness	Comparison	Relative risk	95% Confidence limits	
			Lower	Upper
Crude rates				
Campylobacteriosis	SG:CG1	1.57 *	1.36	1.81
	SG:CG2	1.56 *	1.37	1.78
Cryptosporidiosis	SG:CG1	2.32 *	1.74	3.11
	SG:CG2	6.03 *	4.66	7.80
Gastroenteritis	SG:CG1	1.82 *	1.16	2.87
	SG:CG2	1.08	0.72	1.62
Giardiasis	SG:CG1	0.90	0.50	1.61
	SG:CG2	0.80	0.47	1.39
Salmonellosis	SG:CG1	1.43 *	1.05	1.94
	SG:CG2	2.23 *	1.69	2.95
Yersiniosis	SG:CG1	1.40	0.75	2.63
	SG:CG2	1.66	0.94	2.95
Age-standardised rates				
Campylobacteriosis	SG:CG1	1.51 *	1.31	1.75
	SG:CG2	1.51 *	1.33	1.72
Cryptosporidiosis	SG:CG1	2.08 *	1.55	2.79
	SG:CG2	5.33 *	4.12	6.90
Salmonellosis	SG:CG1	1.33	0.98	1.80
	SG:CG2	2.05 *	1.55	2.71

The specific selection of the wells to avoid septic tank contamination and wellhead protection issues means that the microbial contamination is associated with intensive dairying combined with border strip irrigation. The actual mechanism is likely to be infiltration via macropores from

the border strip irrigation and large rainfall events. The possible exception to this is well J41-0031, where bull calves were kept in the small paddock, which houses the well, between October and December 2003. It is possible that very high concentrations (2400 MPN/100 ml) of *E. coli* observed in January 2004 were related to the impact of the bull calves in this paddock at this time.

The occurrence of *Campylobacter* in groundwater samples is an important finding from this study. *Campylobacter* have previously been reported in groundwater in New Zealand (Savill *et al.* 2001) but in that study samples were collected from infiltration galleries, so the *Campylobacter* were most likely derived from the infiltrating river water. This is the first study to the authors' knowledge to show *Campylobacter* in groundwaters in New Zealand related directly to intensive farming. The numbers of *Campylobacter* assayed ranged from <0.6 to >3.1 MPN/L. *Campylobacter* has been found in groundwater in England associated with a dairy farm in the catchment (Stanley *et al.* 1998).

There is a general trend throughout the data set of high concentrations of *E. coli* being detected in groundwater approximately 20–30 days after a coincidence of stocking and irrigation (or a large rainfall event) in the nearby paddocks, as shown in Figures 3–6. The relationship between concurrent stocking and irrigation/high rainfall and high *E. coli* counts was difficult to assess in the October–December 2004 sampling period due to the unusually high rainfall resulting in a lack of irrigation events.

Groundwater velocities are likely to be quite variable from approximately 1 m/day to 36 m/day. For an approximate average distance of 200 m to travel from the paddock to the well this works out to be from 6 to 200 days travel time. *E. coli* are able to survive for relatively long periods in the aerobic alluvial aquifer environment, whereas *Campylobacter* are likely to die off more rapidly. Sinton *et al.* (2007) reported that the survival of *E. coli* was about 6 times greater than *Campylobacter* in dark river water (with similar chemistry to the shallow groundwater in this study). *Campylobacter* counts are also likely to be lower ($\sim 10^3$ g⁻¹ faeces; Hutchison *et al.* 2004) than *E. coli* counts in cowpats ($\sim 10^7$ g⁻¹ faeces; Avery *et al.* 2004). These factors probably account for the less regular *Campylobacter* positive results.

There appears to be a trend of *Campylobacter* contamination of wells after periods of major rainfall events. This is generally in conjunction with increases in *E. coli* counts. It appears that rainfall may contribute significantly to leaching of microbes with the energy from the raindrops assisting in the release of pathogens from the cowpat into the soil profile. Kress & Gifford (1984) noted that high intensity rainfall led to a greater release of bacteria from 20 day old cowpats in contrast to low intensity rain. Thelin & Gifford (1983) stated that increasing age of the cowpats extended the time taken to thoroughly wet it and there was a significant difference in release concentrations of faecal coliforms (depending on time of saturation) from relatively fresh 2-day-old cowpats to older cowpats.

Analysis of all the contamination events indicated most lags were between 20 and 40 days but could range wider than these values. There was not a consistent lag response of groundwater contamination in wells after 'high risk' flood irrigation events and the combination of significant rainfall and 'high risk' irrigation events appears to have induced more severe microbial contamination. There are a number of factors that could result in this variability. The size of the up-gradient paddocks is generally quite large and thus microbial contaminants would be sourced from a large area with differing travel distances to the well. The wells are only drawing water from a relatively small down-gradient section of the aquifer, and there is uncertainty about the precise groundwater flow direction. Groundwater sampling was undertaken on a twice-weekly basis for 2 wells for some of the study period, but mostly sampling was on a monthly basis. It is possible that a number of contamination events were not detected due to their occurrence between sampling events. The survival and subsequent leaching of bacteria from cowpats is likely to be quite variable, with longer time intervals between deposition and irrigation, higher temperatures and greater sunlight leading to faster drying of cowpats and greater die-off prior to leaching through soils. A combination of rainfall and flood irrigation on relatively new cowpats with moist soils appears to be the worst-case scenario for facilitating infiltration of microbes resulting in contamination of groundwater resources in this catchment.

Concurrent studies of microbial contamination under dairy farms using different irrigation methods, spray irrigation using Briggs Rotarainer and a centre pivot systems,

have identified only very low levels of contamination in contrast to this area of border-strip irrigation (Close *et al.* 2005). This highlights the impact of irrigation methods and poor irrigation efficiencies on leaching of microbes and contamination of groundwater.

Health risk assessment

Both methods used for assessing the daily probability of infection from the observed concentrations of *Campylobacter* in the groundwater samples gave similar results. There was concern that fitting the exponential distribution to the observed data would lead to sampling of unrealistically high values by the @RISK programme. This was tested using truncation of the exponential distribution at various values and there was no difference observed for truncation at values above 4, which is fairly similar to the maximum observed value (on 2 occasions) of >3.1 MPN/L. The approach used for fitting the exponential distribution to the data focused on matching the number of observations in the first two concentration levels from the MPN (<0.6 and 0.6 MPN/L). Most of the observations (97%) fell into these levels. The second approach used the information from the exact MPN distribution and should be a more accurate approach. Both approaches are probably limited by the underlying uncertainty implicit in a 3 tube MPN, which was used due to budget constraints. It should also be noted, as discussed earlier, that there is significant uncertainty about the infective response of humans to low levels of *Campylobacter* ingested in drinking water, as the lowest dose used in the feeding trial of Black *et al.* (1988) was around 800 bacteria and only one strain of *C. jejuni* was used.

The assessment of the health risk associated with these numbers of *Campylobacter* in the shallow groundwater to consumers of this water indicates a significant risk of infection. It is likely that other areas of New Zealand which have similar hydrogeological and land-use conditions (i.e. dairying and border strip irrigation with shallow unconfined groundwater) may have a similar probability of infection. It is consistent with the epidemiological evidence presented in a previous section, where areas in Canterbury with these land-use practices showed increased incidence of campylobacteriosis and cryptosporidiosis compared to control groups. This risk assessment allows

for different susceptibility of individuals within a population but takes no account of any immunity which may be gained by an individual or community through continued exposure to these microbes. Recently an approach of considering this type of immunity response within a risk assessment has been developed (McBride & French 2006) although this immunity response was not included in this present analysis as the input parameters were not available. In conversations with farm owners there is anecdotal evidence that it tends to be new workers and visitors who tend to get sick but there is no documentation of this. An immunity response would tend to decrease the risk of illness compared to that estimated in this study.

Discussions with the well owners indicate that there was a concern over water quality for drinking purposes. Four of the six wells sampled in this study had been used for household drinking purposes. One landholder indicated the occurrence of intermittent stomach complaints from a resident, and there was concern that it was related to water quality. Following an elevated *E. coli* count (>2400 MPN/100 ml) in one of the groundwater samples from his well, the landholder was informed and has since installed a filter system on that particular well and also on a well at his adjacent property. At the end of the first year one of the wells in the study was replaced with a much deeper well by the farm owner and was no longer available for sampling, leaving 5 wells for the remainder of the study. It is interesting to note that this well was the most contaminated of all wells and it appears that the well owner deepened the well to obtain cleaner water in response to the first year study results.

Epidemiological assessment

Socioeconomic status is a determinant of health with people living in more deprived conditions generally having poorer health outcomes. Enteric diseases notifications, however, can show the opposite effect with more disease cases reported from areas of low deprivation. To identify potential confounding caused by socioeconomic status, the deprivation index value for each meshblock was included for the analysis. NZDEP01 scores indicate the level of a population's social deprivation and living conditions with the degree of deprivation increasing with increasing NZDEP scores

(Salmond & Crampton 2002). Mean NZDEP01 scores from SG, CG1, and CG2 were 904, 925, and 975, respectively. These scores correspond to deciles 2, 3, and 5 for SG, CG1, and CG2, respectively, and showed that SG (dairy with major irrigation) was the least deprived, followed by CG1 and CG2. Although there is generally a link between socioeconomic status and health, there is also the possibility of under-reporting of health problems in the rural sector due to access issues (Panelli *et al.* 2006). This would tend to reduce the reported rates of disease and, together with the NZDEP01 scores, would indicate that any increase in notified levels of disease in SG compared to CG1 and CG2 would not be a result of deprivation but would be related to other factors.

The epidemiological assessment is consistent with the risk assessment discussed in the previous section and shows a significant increase in notifications of campylobacteriosis for the dairying with major irrigation groups compared to the other control groups. As stated previously it was not possible to completely separate border strip irrigation from spray irrigation but the majority of the border strip and flood irrigation was contained in SG, with about 55% of the irrigation being border strip in SG compared to <10% in CG1. Both SG and CG1 would have animal contact as a possible transmission route so the difference in disease notifications could be the irrigation practice. It is possible that if the irrigation practices had been able to be better separated between the 2 groups an even larger effect would have been observed. The higher levels of campylobacteriosis in CG1 compared to CG2 (Table 3) may reflect the increased risk from animal contact.

There were significant RR values for salmonellosis for SG compared to CG2 (Table 3) and for cryptosporidiosis for SG compared to both CG1 and CG2. The levels of salmonellosis were higher in both SG and CG1 compared to CG2 (Table 3) which may reflect a higher exposure to animals in the rural compared to the urban environment. The significant differences in the rates of cryptosporidiosis (also between CG1 and CG2 – Table 3) suggest that there are factors associated with the irrigation practice as well as general farming and contact with animals. Animal contact has been implicated in cryptosporidiosis in New Zealand by Learmonth *et al.* (2004), who found that the spring seasonal peak in notifications of cryptosporidiosis matched the high levels of shedding from animals, particularly calves, in

spring. Groundwater samples from this study were not analysed for *Salmonella* or *Cryptosporidium* and this could be worthwhile in future studies.

Concern over potential health effects in the study area was supported from discussions with landholders (for whom the groundwater is their sole supply of drinking water), which provided anecdotal evidence of unexplained intermittent stomach complaints. As the majority of samples collected had *E. coli* values above the drinking water MAV of <1 MPN/100 ml, the shallow groundwater from this region does not meet the drinking water quality criteria, and should be treated prior to consumption.

CONCLUSIONS

Microbial contamination of groundwater beneath border strip irrigation is occurring in the study area. The faecal indicator *E. coli* was found in 75% of groundwater samples at concentrations up to >2400 MPN/100 ml, while *Campylobacter* was identified in 16 groundwater samples (12%) over the three-year study period at low concentrations.

Graphical analysis of the lag period between 'high risk' irrigation events and elevated numbers of *E. coli* in groundwater samples indicated a variable time delay, which was mostly in the range of 20–40 days. Several factors would have contributed to this variability including the varying distances, and hence travel times, of the up-gradient paddocks from the well; variability in the age of cowpats prior to irrigation and rainfall events, and different sunlight and temperature exposures; variability in the soil moisture deficit; and fluctuations in the bacterial load deposited on the ground.

Concurrent studies of microbial contamination under dairy farms using different irrigation methods, spray irrigation and a centre pivot systems, have identified only very low levels of contamination in contrast to this area of border strip irrigation.

Health risk assessment methodologies used in this study indicate that there is potentially a probability of infection from the pathogen *Campylobacter* during the irrigation season which is estimated at between 60% and 75%. It is likely that there are other areas of New Zealand which have similar hydrogeological and land-use conditions (i.e. border

strip irrigation practices with shallow unconfined groundwater) which may have a similar probability of infection. Epidemiological analysis indicated that there were significantly higher notifications of illness in areas of dairying with major irrigation schemes from campylobacteriosis, salmonellosis and cryptosporidiosis.

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