RESEARCH

FECAL CONTAMINATION OF GROUNDWATER IN A SMALL RURAL DRYLAND WATERSHED IN CENTRAL CHILE

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ABSTRACT

Research on microbiological groundwater quality was conducted in Chile in a rural watershed that has almost no other water source. Forty-two wells were randomly selected and levels of indicator bacteria - total coliforms (TC), fecal coliforms (FC), and fecal streptococci (FS) - were repeatedly measured during the four seasons of 2005. The aim of this study was to characterize microbiological groundwater quality, relate indicator levels to certain watershed features and management characteristics which are likely to affect water quality. The dynamics of seasonal temporal contamination was determined with statistical analyses of indicator organism concentrations. Nonparametric tests were used to analyze relationships between bacterial indicators in well water and other variables. TC, FC, and FS were found in all samples indicating the wells had been contaminated with human and animal fecal material. The frequency distribution of microorganisms fitted a logistic distribution. The concentrations appeared to be temporal and levels varied between seasons with higher concentrations in winter. The cause of contamination could be linked to the easy access of domestic animals to the wells and to the permeable well casing material. Local precipitation runoff directly influenced the bacterial concentrations found in the wells.

Key words: biological contamination, bacteria, water quality, environmental pollution.

INTRODUCTION

Water quality is a key environmental issue involving natural watershed resources and local rural communities. The major environmental pressures have an impact on the quantity and quality of groundwater resources (Danielopol *et al*., 2003) which are generally perceived as being less vulnerable to contamination than surface water given the natural filtering ability of the subsurface. Although most groundwater is still thought to be free of disease-causing microorganisms, many systems are unprotected and contamination events could eventually occur because private groundwater wells are rarely, if ever, monitored. The risk of contaminated water for people was manifested in Lake Erie, Ohio, USA in 2004 when 1450 people became ill because of a pathogen in the well water (Fong *et al*., 2007). Furthermore, an estimated 750 000 to 5.9 million people are sick every year as a result of contaminated groundwater in the USA (Macler and Merkle, 2000).

One of the most frequent types of contamination in rural areas is fecal pollution from different sources, most frequently livestock and inadequate on-site human waste disposal systems (Conboy and Goss, 2001; Barnes and Gordon, 2004). The size and shape of pathogenic microorganisms, their surface density properties, and biological activities set them apart from other contaminants that are transported in surface and subsurface water environments (Pachepsky *et al*., 2006). Concentrations of microbiological contamination indicator organisms observed in groundwater are a function of the contamination sources active at that moment (Solo-Gabriele *et al*., 2000).

Microbiological contamination is dispersed, sporadic, and influenced by a range of interacting environmental factors such as the watershed's physical characteristics, climatic conditions, and agricultural management practices. Since the largest numbers of fecal coliforms and fecal streptococci are always present in manure

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(Chadwick and Chen, 2002), then the presence of either of these microbes in a well water sample is strong evidence of fecal contamination. One of the difficulties in tackling this problem is the fact that contamination is likely to come from various possible point and nonpoint sources (Mahler *et al*., 2000), thus obscuring its origins. It is important to detect fecal contamination in groundwater, especially if there are no pre-consumption water treatment systems (Atherholt *et al*., 2003). This is the case in some rural dryland areas of Chile where farmers obtain small amounts of water from private wells and face serious water supply problems for both human consumption and agricultural activities.

Improving the quality of groundwater resources offers an important economic opportunity for the gradual improvement of the quality of life in rural dryland communities. In order to develop strategies to diminish or eliminate microbiological contamination in groundwater wells, it is first necessary to assess the variability in its concentrations, and the relative importance of different factors affecting pollution.

The variability of microorganism concentrations in Chilean groundwater and the factors affecting them are not well-known at present. As rural communities continue to rely on shallow groundwater, it is important to improve the state of knowledge about the quality of this resource. To assess the presence of fecal contamination in a rural watershed, a study was undertaken to typify the quality of microbiological groundwater, describe its seasonal pattern, and look for probable characteristics exerting an influence on the quality of groundwater.

MATERIALS AND METHODS

The small rural Estero San José (ESJ) watershed (10.8 km^2) is located in the Bío-Bío Region, Chile (Figure 1). The catchment area is sparsely inhabited by families dedicated to traditional agriculture. The ESJ watershed is characterized by a Mediterranean climate with a long dry season leading to water shortages and a short wet season.

The watershed soils have low permeability and capacity to provide underground water. Moisture accumulation in the watershed takes place between April and June. The major runoff period of the year is from July to October when the ground is saturated and almost all the precipitation that falls in the watershed runs off. Precipitation is scarce between November and March, with practically no base flow in the watershed. Farmers obtain small amounts of water from private wells. On the average, these are 7.0 m deep and yield a median of 1.1 L min^{-1}. Groundwater is used as drinking water, for other domestic purposes, orchards, gardens, greenhouses, and livestock production. Agricultural production in the area

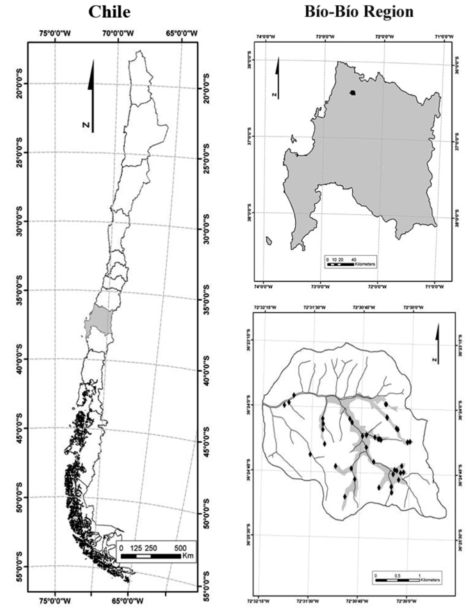


Figure 1. Location of the Estero San José Watershed and sampling sites.

is mostly wheat (*Triticum aestivum* L.) and lentils (*Lens culinaris* Medik.). The density of domestic animals is low.

A 10-month monitoring study was undertaken. Forty-two wells were chosen with the Stratified Random Sample (Murray, 2002) and site-location data were determined with global positioning system units (Garmin 12XL, Garmin International Inc., Kansas, USA). Water pH was measured in the field with Hanna Instruments® HI9025, whereas electrical conductivity (EC) and temperature were measured with Hanna Instruments® HI9835. The sampling periods were defined in accordance with the precipitation regime and variations in the hydrologic levels in the wells. Based on these criteria, four sampling seasons were established (March, June, September, and December).

Water samples were analyzed for total coliforms (TC), fecal coliforms (FC), and fecal streptococci (FS). Although TC is widespread in the environment, it was included in order to meet the Chilean standard requirement (NCh 409. Of 70). Aseptic sample collections were taken in sterilized flasks. Samples were held at 5 ºC after being collected and for no more than 6 h until reaching the laboratory. Results were expressed in colony forming units (CFU) per 100 mL. TC, FC, and FS concentrations were analyzed with a membrane filtration technique following standard methods (Clesceri *et al*., 1998). Aliquots (100, 10, and 1 mL) of each water sample were filtered through a 0.45 μ Millipore membrane filter. All samples were tested in triplicate. Results were reported as CFU 100 mL^{-1}. Samples that were overgrown were considered to contain > 1000 CFU 100 mL^{-1}. Colonies forming a green metallic sheen were counted as TC on m-Endo agar (Difco®, Detroit, MI, USA). To count FC, filters were placed on Petri dishes containing m-FC agar (Difco®, Detroit, MI, USA) which gave the selected colonies a blue color, whereas the selective FS count was carried out by incubating the filters in m-Enterococcus agar (Difco®, Detroit, MI, USA). Water sample analyses were performed in the microbiology laboratory of the Centro de Ciencias Ambientales (EULA) at the Universidad de Concepción.

Count data analyses were performed with STATISTICA™ StatSoft 6.0. The median was used rather than the mean to analyze the microbiological data because it basically eliminates extreme values (Smith *et al*., 1996).

Results for TC, FC, and FS obtained in the four seasons were analyzed by looking for spatial correlations with spatial S-PLUS software using Geary's and Moran's Index (Cai and Wang, 2006). Statistical analyses were conducted to determine the relationship between bacterial concentrations and pH, electrical conductivity, temperature, and factors expected affecting concentrations or associated with the presence of indicator bacteria. These variables were treated as binomial categorical data. To further the analysis, the variables were transformed from continuous to categorical. Data included different land use activities (prairie, gardens, orchards, bare soil) within the proximity of the monitoring well (*ca*. 10 m radius); well condition (good, average, and poor); well location (highlands or lowlands); well cover (wood or cement); border height (to 15, 50, and 100 cm); casing (cement or brick); slope (to 15%, between 15% and 60%), latrine characteristics (location uphill or downhill from the well, casing); animal access in the vicinity of the well; type of animal (horses, pigs, sheep, poultry, cattle, dogs), and well-to-latrine distance (to 30 m, to 80 m). Parameters such as soil and geology were assumed to be constant because of the small differences detected at each sample site. Data were analyzed statistically by nonparametric Mann-Whitney rank-sum and Kolmogorov-Smirnov tests (Rohatgi, 1984) to determine significant differences in mean concentrations and indicator distribution found in well groups presenting specific characteristics. Factors were ordered dichotomously. Rainfall data were collected as an additional factor likely to exert an influence on microbiological quality. The environmental variables were selected because of their expected impact on the numbers of microorganisms detected in the samples.

RESULTS AND DISCUSSION

Groundwater indicator bacteria concentrations exceeded Chilean water quality regulations in all samples (NCh 409 Of. 70). The three indicators had a detection rate of 100%, finding at least 1 CFU 100 mL^{-1} in all tested samples. These concentrations indicated degraded groundwater quality. The existence of both FC and FS provided strong evidence of fecal contamination (Atherholt *et al*., 2003). The presence of indicators in all four sampling seasons denoted frequent, if not continuous, fecal contamination in the ESJ watershed. There seemed to be a permanent source of fecal bacteria regularly entering the wells. Microbial data (Table 1) revealed marked variations throughout the year.

The most frequent indicator was TC. Seasonal variations in the microbial quality of water were evident, with peaks in winter for TC, FC, and FS. Variations in FC were less dramatic than in FS. Median concentrations of TC, FC, and FS increased in June (as compared to March), decreased in September, and increased again in December (Figure 2). This last increase can be attributed to higher demands on the wells during the later part of the year, combined with minimal water yields. Environmental persistence or growth of bacterial indicators during the summer months could confound the interpretation of baseline dynamics (Shanks *et al*., 2006).

The wells exhibited a high proportion of low counts and a small number of very high counts that exerted a significant influence on the median. Indeed, bacterial indicators from natural sources do not usually occur in elevated concentrations since they come from disperse sources such as waste of warm-blooded animals (Ortiz, 2004). During transport and after retention in the soil, microorganisms are affected by environmental conditions

Table 1. Median and range of indicator bacteria concentrations in the four sampled months (CFU 100 mL^{-1}).

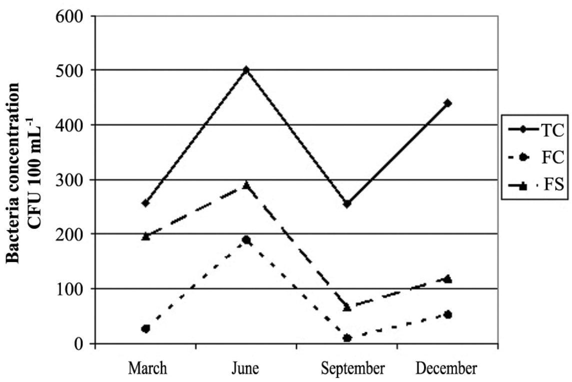
Sampled month	Nº samples	Total coliforms		Bacterial indicator Fecal coliforms		Fecal streptococci	
		Median	Range	Median	Range	Median	Range
March	41	257	16 – 4.71×10^{3}	27	1 – 1.16×10^{3}	196	9 – 1.12×10^{3}
June	41	501	14 – 5.00×10^{3}	190	1 – 5.80×10^{3}	290	20 – 1.17×10^{3}
September	42	255	11 – 1.06×10^{4}	10	1 – 3.00×10^{2}	67	9 – 1.28×10^{3}
December	39	440	22 – 3.60×10^{3}	53	1 – 1.38×10^{3}	120	5 – 1.10×10^{3}

such as nutrient availability and predation (Pachepsky *et al*., 2006). Moreover, traditional monitoring and research programs quantify the microorganism concentrations in samples using standard methods. These methods are designed to target public health and do not completely measure either clumped organisms or those associated with particles, and may not fully specify organism concentrations (Borst and Selvakumar, 2003).

Indicator concentration data fit a logistic distribution, showing a parallel evolution in the distribution of FC and FS (Figures 3, 4, 5). A descriptive criterion was chosen for this distribution.

Statistical analyses showed that FC was better correlated with TC in March, June, and December, and with FS in September. In June (winter), the three indicators showed the highest correlation. FC and TC were highly correlated. Correlation analyses revealed a strong, significant, and positive correlation between TC and FC in June (Table 2). A strong relationship between two indicators may provide some evidence that both indicators originate from the same or similar contamination sources (Francy *et al*., 2000). Correlations between indicators, without considering the season, were very low ($r = 0.35$ between TC and FC, $r = 0.34$ between FC and FS, and $r = 0.21$ between TC and FS, $p < 0.05$). Strong correlations between indicators were obtained only when the analyses considered the season. Analysis of the annual pattern showed almost no correlations. This confirmed the importance of carrying out seasonal analyses.

By comparing indicator medians in different seasons (Kruskal-Wallis test for comparing medians), it was possible to obtain results for FC (p-value = 2.95×10^{9}) which infer that seasonal medians were not equal, although FC did not change drastically with the seasons. There were differences (with a significance level of 5%) between the medians of: March/June, March/September, June/September, and September/December. FS had a p-value = 7.95×10^{7}. Differences had the same significance level between the medians of: March/September, June/September, and June/December. The significant differences observed between the median concentrations of June with respect to September and December for FC and FS showed a temporal change. Median concentrations of TC did not differ significantly between seasons. Persistence of bacteria in the aquatic environment depends on various parameters, especially on the existing nutrients and temperatures (Leclerc *et al*., 2002). The prevalence of FS, which die off more rapidly in the environment than other bacterial indicators, shows either relatively recent contamination of a source by fecal



CFU: Colony Forming Units; TC: Total Coliforms; FC: Fecal Coliforms; FS: Fecal Streptococci.

Figure 2. Median concentrations of indicator bacteria.

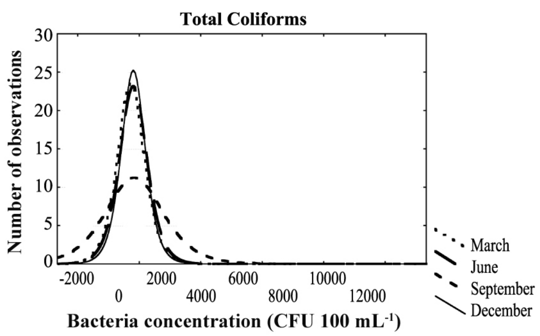


Figure 3. Logistic distribution of TC (Total Coliforms).

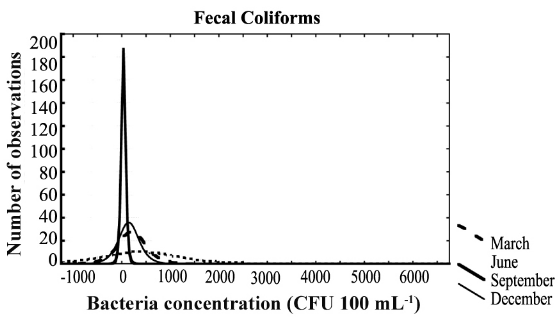


Figure 4. Logistic distribution of FC (Fecal Coliforms).

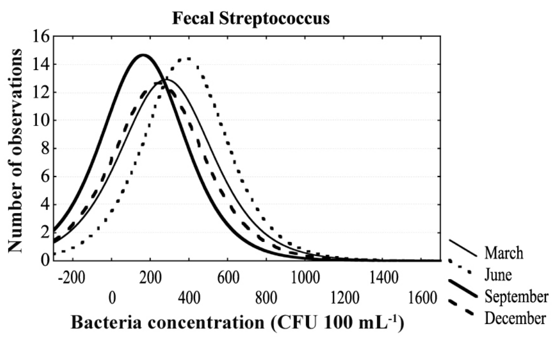


Figure 5. Logistic distribution of FS (Fecal Streptococci).

Table 2. Correlation coefficients (r) between different indicator organism concentrations.

	Sample month											
	March			June			September			December		
Organism	TC	FC	FS	TC	FC	FS	TC	FC	FS	TC	FC	FS
TC	1	0.26	0.28	1	0.91*	0.60*	1	0.23	0.41**	1	0.40**	-0.06
FC		1	0.11		1	0.53*		1	0.24		1	0.12
FS			1			1			1			1

*p < 0.001. **p < 0.05. TC: Total coliforms. FC: Fecal coliforms. FS: Fecal streptococci.

material or a very high level of contamination possibly associated with organic matter (Conboy and Goss, 2001); the latter could have been the case in September. FC was more persistent in freshwater than FS (Anderson *et al.*, 2005). Nevertheless, in an experiment of some treatments in simulated groundwater environments by Conboy and Goss (2001), FS was able to survive for over 140 d.

Concentrations of TC, FC, and FS were not correlated with well temperature, conductivity, and pH ($p < 0.001$). Rainfall measured over the sampling period was 23.6 mm until March, 447.7 mm between March and June, 302.5 mm between June and September, and 59.9 mm between September and December (Figure 6).

The highest rainfall was recorded between May and July. FC and FS median concentrations varied over time and showed a pattern similar to that of rainfall. However, FS were more affected by rainfall than FC, although the variation patterns of FC were highly influenced by two extreme concentrations. Correlation coefficients between indicators and rainfall showed a significant relationship with FC ($r = 0.84$) and FS ($r = 0.81$). This relationship was weak for TC ($r = 0.23$) and not coupled with other factors. The high temporal variance in the collected data means that precipitation can exert an influence by providing transport energy for the potential sources. The median demonstrated that microbial water quality changes following a rainfall runoff pattern for microbial source inputs, with a marked annual cycle (Figure 6). Results revealed a strong association between bacterial concentrations in groundwater wells and rainfall through elevated concentrations in samples taken after precipitation. It can be assumed that the higher concentrations recorded in June are partly attributable to the fact that it is the wettest month of the year. These correlations suggest that bacteria were largely associated with suspended particulate materials and transported by runoff, since some coliforms in runoff are associated with particles (George *et al.*, 2004). Characteristics of the initial fecal material deposition site on the soil surface influence the infiltration, runoff, and retention rate of the microorganisms in the feces (Ferguson *et al.*, 2003). Soil surrounding wells was eroded at almost all the sites, thereby preventing interaction between bacteria that could be transported by runoff and allowing them to eventually reach the well.

Moreover, no spatial correlations were found according to Geary's and Moran's Index. Neighboring wells were hydrologically independent. Spatial variability in the concentrations of TC, FC, and FS was not significant (Kolmogorov-Smirnov test, $p < 0.05$) between sampling sites in the highlands and lowlands of the watershed. Fecal contamination due to surface runoff implied that the phenomenon is highly responsive to rainfall intensity and duration, and will display a high degree of temporal variability. The fact that there is no significant difference between concentrations of indicators in highlands and lowlands suggests that local runoff produced the contamination rather than a landscape level phenomenon.

The analysis of the relationship between bacterial indicator levels and environmental characteristics presents several statistical challenges. Due to the complex nature of FC destination and transport, empirical methods such as regression models are unable to build up reliable load-concentration relationships (Bai and Lung, 2006). However, factors (Table 3) were recorded which were expected to affect concentrations or be associated with the presence of indicator bacteria since these offer preliminary insight into the causes of well contamination.

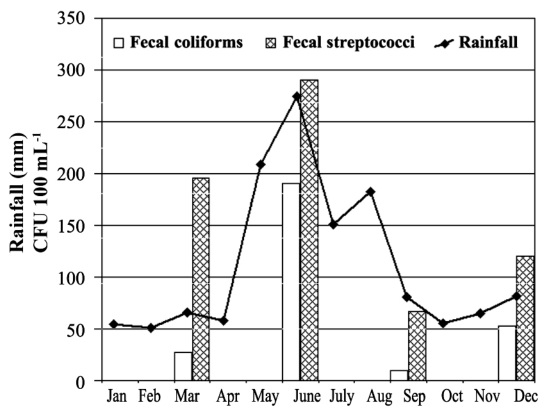


Figure 6. Rainfall and seasonal variability of indicator bacteria concentrations.

Table 3. Landscape and management factors expected to affect concentrations or be associated with the presence of indicator bacteria.

Well	Land use	Slope	AA	Animal type	Well condition	Well cap	Well cover	B h	LU	D	L casing
1	Prairie	5	Yes	H	Good	Wood	Cement	45	Yes	85	Cement
2	Orchard	15	Yes	Pi	Good	Wood	Cement	100	Yes	11	Cement
3	Garden	5	No	-	Good	Wood	Brick	30	No	42	None
4	Orchard	5	Yes	Po	Good	Cement	Cement	60	No	84	None
5	Orchard	9	No	-	Regular	Cement	Brick	30	Yes	44	Cement
6	Prairie	15	Yes	S	Regular	Wood	Cement	30	No	40	None
7	Prairie	10	Yes	S	Poor	Wood	Cement	40	No	52	None
8	Bare soil	26	No	-	Good	Cement	Cement	3	Yes	70	None
9	Orchard	10	Yes	Po	Regular	Cement	Cement	70	Yes	28	None
10	Orchard	18	No	-	Regular	Wood	Cement	75	Yes	83	None
11	Orchard	40	Yes	Po	Poor	Wood	Cement	40	Yes	62	Cement
12	Orchard	35	Yes	Po. C	Poor	Wood	Cement	40	Yes	85	None
13	Orchard	35	Yes	Po	Regular	Cement	Cement	40	Yes	50	None
14	Orchard	40	Yes	Po, pi	Good	Cement	Cement	60	Yes	62	Cement
15	Bare soil	18	No	-	Regular	Wood	Cement	50	Yes	82	None
16	Prairie	30	Yes	Po	Poor	Wood	Brick	18	Yes	120	None
17	Bare soil	45	Yes	Po, C	Good	Cement	Cement	40	No	86	None
18	Prairie	5	Yes	Po, C	Good	Cement	Cement	50	No	86	None
19	Prairie	53	Yes	Po, pi	Regular	Wood	Cement	5	Yes	60	None
20	Orchard	45	No	-	Good	Cement	Brick	15	Yes	48	None
21	Orchard	25	No	-	Good	Cement	Brick	60	Yes	43	None
22	Bare soil	5	Yes	H	Good	Cement	Cement	20	Yes	70	None
23	Orchard	0	No	-	Regular	Cement	Brick	40	No	22	None
24	Prairie	18	No	-	Regular	Cement	Brick	60	Yes	18	None
25	Prairie	15	Yes	Po, D	Good	Cement	Cement	70	Yes	50	None
26	Garden	10	Yes	Po	Good	Cement	Cement	40	No	23	Cement
27	Orchard	23	Yes	C	Good	Cement	Cement	60	Yes	62	Cement
28	Orchard	4	Yes	Po	Regular	Wood	Brick	80	Yes	79	Cement
29	Orchard	17	Yes	Po	Regular	Wood	Cement	55	Yes	80	Cement
30	Prairie	18	Yes	Po, C	Poor	Wood	Cement	10	Yes	91	None
31	Prairie	40	Yes	Po, C	Good	Wood	Cement	50	Yes	133	None
32	Orchard	22	Yes	Po, S	Regular	Wood	Cement	50	Yes	10	Cement
33	Orchard	12	Yes	Po	Good	Cement	Cement	20	Yes	42	None
34	Bare soil	13	Yes	S	Poor	Cement	Cement	5	Yes	54	None
35	Garden	20	No	-	Good	Wood	Cement	50	Yes	26	None
36	Orchard	15	Yes	D	Good	Cement	Cement	60	Yes	39	None
37	Prairie	5	No	-	Good	Cement	Cement	100	No	10	Cement
38	Orchard	18	Yes	Po	Poor	Wood	Cement	90	Yes	41	None
39	Prairie	35	No	-	Regular	Cement	Brick	120	No	45	None
40	Orchard	40	Yes	Po, D	Poor	None	Cement	45	Yes	11	None
41	Orchard	5	No	-	Poor	None	Cement	12	Yes	63	None
42	Orchard	15	No	-	Regular	Wood	Cement	30	No	82	None

AA: animal access to the well. Bh: well border height. LU: latrine uphill from the well. D: distance between well and the closest latrine. L casing: latrine casing. Animal type: H: horses, Pi: pigs, Po: poultry, S: sheep, C: cattle, D: dogs.

The distance between wells and latrines is highly variable, ranging from a minimum of 10 to 133 m. Seventy-four percent of the latrines in the sampled households had brick casing. Hence, they were not sealed. On at least one of the four sampling dates, animals were observed around approximately 67% of the wells. Table 4 demonstrates that characteristics with $p < 0.052$ were considered to be statistically significant. Statistical analysis of the data showed that five factors are likely to influence the concentration of bacteria in groundwater: animal access close to the wells (specifically pigs and poultry); land use; bricks used for well casing; latrine-to-well distance; and a slope up to 15%. Only two of these factors showed a highly significant ($p < 0.01$) association with the presence of the bacterial indicators: animal access close to the well in June and a latrine-to-well distance of < 80 m in December.

These results suggest that the most important factors affecting well vulnerability to bacterial contamination were those related to the well itself: construction and site management. In the month when the indicator concentrations are the highest, the factors potentially influencing these levels are animal access (specifically poultry) and well casing. Some wells have a brick casing instead of cement, which does not seal them sufficiently and allows water runoff from the surroundings to enter. Statistically, contamination levels were more closely tied to animal access in the vicinity of wells and the well casing material than to land use or distance between wells and latrines. Livestock grazing practices creates a diffuse source of fecal contamination to watersheds (Tian *et al*., 2002; Harter *et al*., 2002). Pathogens from animal feces may enter waterways by direct deposition or as a result of overland runoff containing fecal material deposited in the watershed. The FC:FS ratio as used by (Donderski and Wilk, 2002; Troussellier *et al*., 2004) showed that the source of indicator bacteria is mostly animal, followed by mixed sources. Considering that a great number of wells have fences to prevent animal access, wildlife cannot be disregarded as a source. Cox *et al*. (2005) showed that poultry fecal samples have a higher FC concentration (median 1.1×10^8 CFU g^{-1} wet wt) than those of other domestic animals (median for adult cattle 1.8×10^5 CFU g^{-1} wet wt, pigs 7.1×10^6 CFU g^{-1} wet wt, and sheep 6.6×10^5 CFU g^{-1} wet wt). This could explain the significance of poultry access to the wells as a factor affecting indicator counts. Furthermore, Wheeler *et al*. (2002) demonstrated that *Enterococcus faecalis* had a limited host range and was found in humans, dogs, and chickens.

Land use in the watershed also affected the extent of fecal contamination, but not as strongly as the other factors described above. A pattern did not emerge in spite of the fact that three different land uses were significant. Latrines appear to have little influence on the presence and level of bacterial indicators, suggesting that latrines can also be a potential source of microbial contamination in groundwater. Other factors not considered in this study may also affect bacterial concentrations in well water.

These data provide new information by relating indicator bacteria loads for certain factors at specific times of the year. The fact that the most significant indicator related to a factor was TC in March and in December, FC in June, and FS in September, suggests that fecal contamination is mostly a winter phenomenon.

Table 4. Factors with significant differences between the means of indicator bacteria concentrations.

Month	Indicator	Factor	P	Test
March	Fecal streptococcus	Land use: bare soil	0.038	Mann-Whitney
March	Total coliforms	Pig access	0.015	Mann-Whitney
June	Fecal coliforms	Animals close to well	< 0.005	Kolmogorov-Smirnov
June	Fecal coliforms	Animals close to well	0.021	Mann-Whitney
June	Fecal coliforms	Poultry access	< 0.05	Kolmogorov-Smirnov
June	Fecal coliforms	Poultry access	0.052	Mann-Whitney
September	Fecal streptococcus	Land use: orchard	0.051	Mann-Whitney
September	Fecal streptococcus	Well casing material (brick)	< 0.05	Kolmogorov-Smirnov
September	Fecal streptococcus	Well casing material (brick)	0.041	Mann-Whitney
September	Fecal streptococcus	Latrine-to-well distance < 80 m	0.044	Mann-Whitney
December	Fecal streptococcus	Land use: garden	0.039	Mann-Whitney
December	Fecal streptococcus	Slope < 15%	0.041	Mann-Whitney
December	Total coliforms	Well casing material (brick)	< 0.05	Kolmogorov-Smirnov
December	Total coliforms	Well casing material (brick)	0.033	Mann-Whitney
December	Total coliforms	Latrine-to-well distance < 80 m	< 0.05	Kolmogorov-Smirnov
December	Total coliforms	Latrine-to-well distance < 80 m	0.007	Mann-Whitney

CONCLUSIONS

There is widespread groundwater contamination in the ESJ watershed. The microbiological quality of the sampled wells was impaired with regard to Chilean standards.

A seasonal trend was identified. Concentrations of FC and FS varied over time and showed a pattern similar to rainfall which appeared to exert a local influence on the indicator concentrations. FS were more affected by rainfall than FC.

The lack of a significant difference between wells located uphill and downhill suggests that contamination is not a result of surface runoff from upgradient areas. Our results indicate that one cause of microbial contamination in well water is manure bacteria entering directly through local surface runoff.

There was no spatial correlation between wells, showing that there were no identified groups of wells which maintained certain concentration tendencies.

The present study shows that the analysis of microbial data in combination with basic environmental and management data can provide preliminary insight into the causes of fecal contamination in groundwater. In fact, indicator counts turned out to be significantly related to certain watershed features during specific months. Inherent well site characteristics and its surroundings, as well as rainfall are the main factors that affect groundwater quality in the ESJ watershed.

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RESUMEN

Contaminación fecal en agua subterránea en una pequeña cuenca de secano rural en Chile Central. Se realizó una investigación de la calidad microbiológica de las aguas subterráneas en una cuenca rural chilena. En esta cuenca prácticamente no había otra fuente de agua disponible. En 42 pozos seleccionados al azar, se midieron niveles de bacterias indicadoras en cuatro temporadas distintas durante el año 2005. Las bacterias incluyeron coliformes totales (TC), coliformes fecales (FC) y Estreptococos fecales (FS). El objetivo fue caracterizar la calidad microbiológica del agua subterránea y relacionar los indicadores con ciertas propiedades y el manejo de la cuenca que pueden afectar la calidad del agua. La dinámica temporal de la contaminación fue determinada mediante análisis estadístico de la concentración de organismos indicadores. Las relaciones entre indicadores bacteriales presentes en el agua de los pozos y otras variables fueron analizadas con pruebas no paramétricas. En todas las muestras se detectaron TC, FC y FS, indicando que los pozos han estado contaminados con material fecal de humanos y animales. La distribución de frecuencia de los microorganismos se ajustó a una distribución logística. Las concentraciones muestran una base temporal con niveles variables entre temporadas, con una mayor concentración en invierno. La causa de la contaminación se puede asociar al fácil acceso de los animales domésticos a los pozos, y a su material de revestimiento permeable. La escorrentía local de las precipitaciones mostró tener una influencia directa sobre la concentración de los microorganismos en los pozos y en la concentración de los indicadores bacteriales encontrados en los pozos.

Palabras clave: contaminación biológica, bacteria, calidad del agua, contaminación ambiental.

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