

Microbiological assessment of private groundwater-derived potable water supplies in the Mid-West Region of Ireland

Jean O'Dwyer, Aideen Dowling and Catherine C. Adley

ABSTRACT

Determining the likelihood that groundwater contains faecal coliforms can aid water resource management in facilitating the protection of drinking water supplies. This study assesses the incidence of the faecal indicator organism *Escherichia coli* in 125 private water supplies (PWSs) serving individual houses in the Mid-West Region of Ireland. Two factors, aquifer type and rainfall (mm), were chosen as independent variables that can affect the vulnerability of a groundwater body. Using a geographical information system, the relative hydrogeological and climatological features unique to each sampling location were derived. Utilising this information, a logistic regression (LR) model was used to predict the probability of contamination of PWSs with *E. coli*. The model contained two independent variables: rainfall (mm; $p < 0.001$) and aquifer characteristics ($p = 0.001$). The full model, containing both predictors, was statistically significant at $p < 0.001$, indicating that the model distinguished between the independent variables' relationship to the incidence of contamination. The likelihood of *E. coli* contamination is greater with increased rainfall and in areas where a bedrock aquifer is dominant. The LR model explained between 27.4% (Cox and Snell R squared) and 36.8% (Nagelkerke R squared) of the variance in contamination and correctly classified 75.2% of cases.

Key words | climatology, *E. coli*, geographical information system, groundwater vulnerability, hydrogeology, logistic regression

INTRODUCTION

A precondition for water supplies to qualify as safe drinking water sources is the absence of faecal coliforms (EU 1998; Schmoll *et al.* 2006; Irish Statute 2007). Globally, groundwater can be considered of good quality, which is mainly attributable to natural purification processes (UNEP 2003). For this reason, coupled with its accessibility, groundwater is a major source of potable water worldwide. However, despite its importance, groundwater is often misused, usually poorly understood and rarely well managed. Although groundwater is not easily contaminated, once this occurs it is difficult to remediate (UNEP 2003). For this reason it is important to identify which aquifer systems and settings are most vulnerable to degradation because the replacement

cost of a failing local aquifer will be high and its loss may stress other water resources looked to as a substitute.

This research uses geographic information system (GIS) technology to assess the microbiological contamination of groundwater as a function of both aquifer type and rainfall events in Ireland. This is achieved by the overlaying of the microbiological analysis results of 125 private wells, taken from numerous geographical locations, on national aquifer classification data in conjunction with precipitation data from local weather stations. The application of the statistical method 'binary logistic regression (LR)' assesses the impact of these factors on the likelihood of contamination.

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Groundwater as a drinking water source in Ireland

Approximately 26% of the public and private drinking water supply in Ireland is provided by groundwater or spring sources (EPA 2008a). These supplies can be categorised as individual private water supplies (PWSs) serving singular households or small private group schemes (SPGSs) which are defined by two or more households coming together to utilise a common water supply. As both PWSs and SPGSs are categorised as individual water supplies providing an average of less than 10 m³/day or serving less than 50 persons, they are excluded from the European Commission Drinking Water Directive 98/83/EC. As a result, the quality of each groundwater well is the sole responsibility of the user and is not legislated under the Irish Statute.

Groundwater contamination

Groundwater contaminated by pathogen-containing faecal waste may cause illness if consumed following inadequate treatment (Raina *et al.* 1999; Said *et al.* 2003). In Ireland, the occurrence of faecal contamination in groundwater is among the highest in Europe, with contamination reported in 34% of routine monitoring samples (EPA 2009; GSI 2009). Diffuse contamination sources, in the form of farming practices, are frequently acknowledged as the major sources of faecal pollution (Trevisan *et al.* 2002; Pachepsky *et al.* 2006; Goss & Richards 2008; Mosaddeghi *et al.* 2009). Similarly, as a consequence of rural infrastructure, a point pollution source in the form of domestic waste water treatment systems (DWWTSs) also poses a significant problem. Animal wastes have the potential to disperse pathogens, like bacteria, viruses and protozoa, if not properly contained, treated and managed (Sobsey *et al.* 2006). This poses a significant threat in Ireland as the utilisation of 191,799 groundwater-derived drinking water supplies (CSO 2006) are predominantly in rural areas where agricultural practices and DWWTSs are heavily employed. Public health authorities, in both developed and undeveloped countries, rely on the use of sanitary set back distances between animal and human waste disposal sites and drinking water wells, with the intention of protecting human health (Berger 2008). In Ireland these distances are not based on the unique hydrogeological properties of the

area, despite the well-documented knowledge that the risk of faecal contamination varies greatly in different aquifer types and under different climatological conditions; this suggests that many groundwater utilisers are at risk.

Escherichia coli as an indicator organism of faecal contamination

Of all bacterial species, *E. coli* has had a dominant role in water microbiology. It was introduced as an indicator bacterium, not because of the inherent pathogenicity of certain serotypes but rather it was a convenient indicator of faecal pollution. *E. coli* is found in the bowels of all warm-blooded animals including human beings and is almost always present in water which has been contaminated with human or animal faeces. The presence of a solitary faecal coliform in a drinking water supply is a breach of the Irish Drinking Water Regulations (Irish Statute 2007) as adopted from the EU Council Directive 98/83/EC (EU 1998). While *E. coli* is a useful reference tool in relation to overall contamination, the pathogenicity of the actual organism should not be overlooked. Pathogenic *E. coli* threatens human health and may cause kidney problems and failure, cramps, diarrhoea and even death (Kuntz & Kuntz 1999). The most dangerous *E. coli* bacteria contain the gene for producing Shiga toxin. *E. coli* O157:H7 is the most widespread, but at least 81 serotypes have been identified (Prager *et al.* 2005). Release of this toxin into the body can result in chronic bloody diarrhoea, kidney failure and can be fatal even in normally healthy individuals and particularly in immuno-compromised individuals, children and the elderly (Jones 1999).

METHODS

Study area and microbiological sampling regime

Area and demographics

The Mid-West Region of Ireland is made up of counties Clare, Limerick and North Tipperary, as shown in Figure 1. The region extents 8,248 km², 11% of the total area of the state and has a population of 361,028. The population of

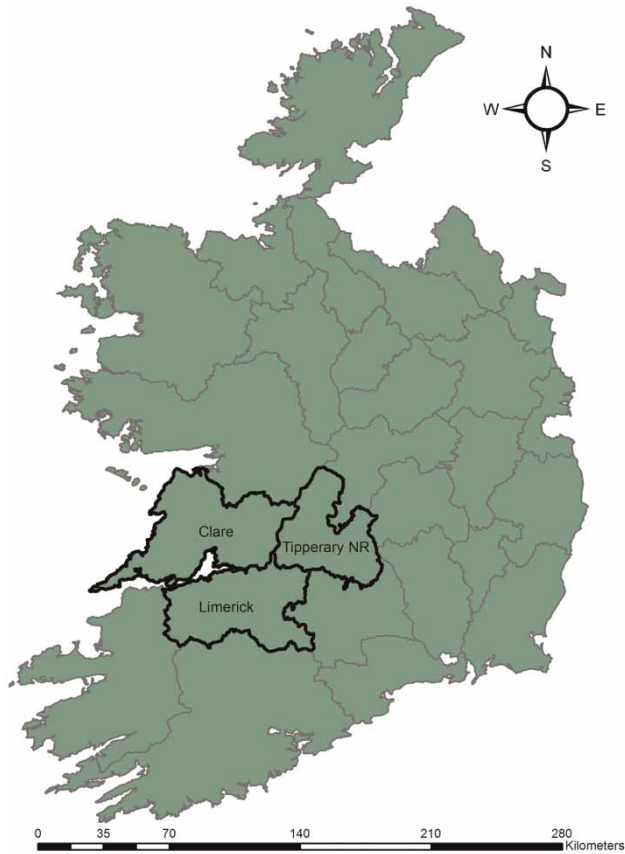


Figure 1 | Map of the research area: the Mid-Western Region of Ireland; including counties Clare, Limerick and North Tipperary.

the area is divided between town (163,620) and rural aggregate (197,408) areas with 21,332 households which are solely dependent on the abstraction of groundwater by means of private boreholes and wells (CSO 2006).

Aquifer classification and rainfall

The aquifer classification system used in Ireland, which was developed by the Geological Survey of Ireland, is defined in the Groundwater Protection Schemes publication (DELG/EPA/GSI 1999). It is intended to reflect the groundwater flow type (flow through fissures, flow through karst systems and intergranular flow through gravels) and to describe the resource potential (e.g. regionally important, locally important and poor). For the purpose of this study, the aquifer systems have first been defined as either bedrock or a sand/gravel aquifer. The bedrock aquifers are then

subsequently subdivided into ‘Fissure’ and ‘Karstic’ systems, the aim being to predict groundwater vulnerability as a function of local hydrogeological features.

In relation to rainfall, the region has a typical west maritime climate with relatively mild, moist winters and cool, cloudy summers. The prevailing winds are westerly to south-westerly and the average relative humidity is high. Average annual precipitation is highest on the west coast and in inland areas of high relief. Compared with the east coast of Ireland, average annual rainfall and relative humidity are higher, mean annual temperature is lower, and annual duration of bright sunshine and frost-free periods are shorter (Irish Meteorological Office 2013).

Microbiological sampling and enumeration

Participants in the study were obtained by broadcasting an announcement on a popular Irish discussion website (www.boards.ie) offering microbiological analysis of drinking water for households supplied by groundwater abstracted via a PWS serving an individual household. These private water wells ($n = 125$) were each sampled three times between 12 September 2011 and 12 November 2012 from all over the research area as shown in Figure 2. Particular consideration was given to resampling after a period of successive rainfall events. The water sample was taken by the researcher from the kitchen tap after first sterilising the spout using 70% ethanol and allowing the water to run for 60 s. As the maximum volume of sample (100 mL) was required for zero-dilution bacteriological analysis, well water samples were collected directly on site in disposable 120 mL sterile vessels containing sodium thiosulphate to neutralise any chlorine that may be present in the sample. All samples were stored in a cool environment and were analysed within 4 hours.

The most probable number of *E. coli* was assayed using a standard US Environmental Protection Agency (EPA)-approved commercial culture kit (Colilert, IDEXX Laboratories Inc., Westbrook, ME, USA) according to the manufacturer’s directions. The stated amount of the Colilert reagent was added to the 100 mL water samples. The reagent contains two carbon sources which are selectively metabolised by either all coliforms present in the sample or solely by *E. coli* colonies. The resulting reaction when an indicator is cleaved through metabolism is a colour

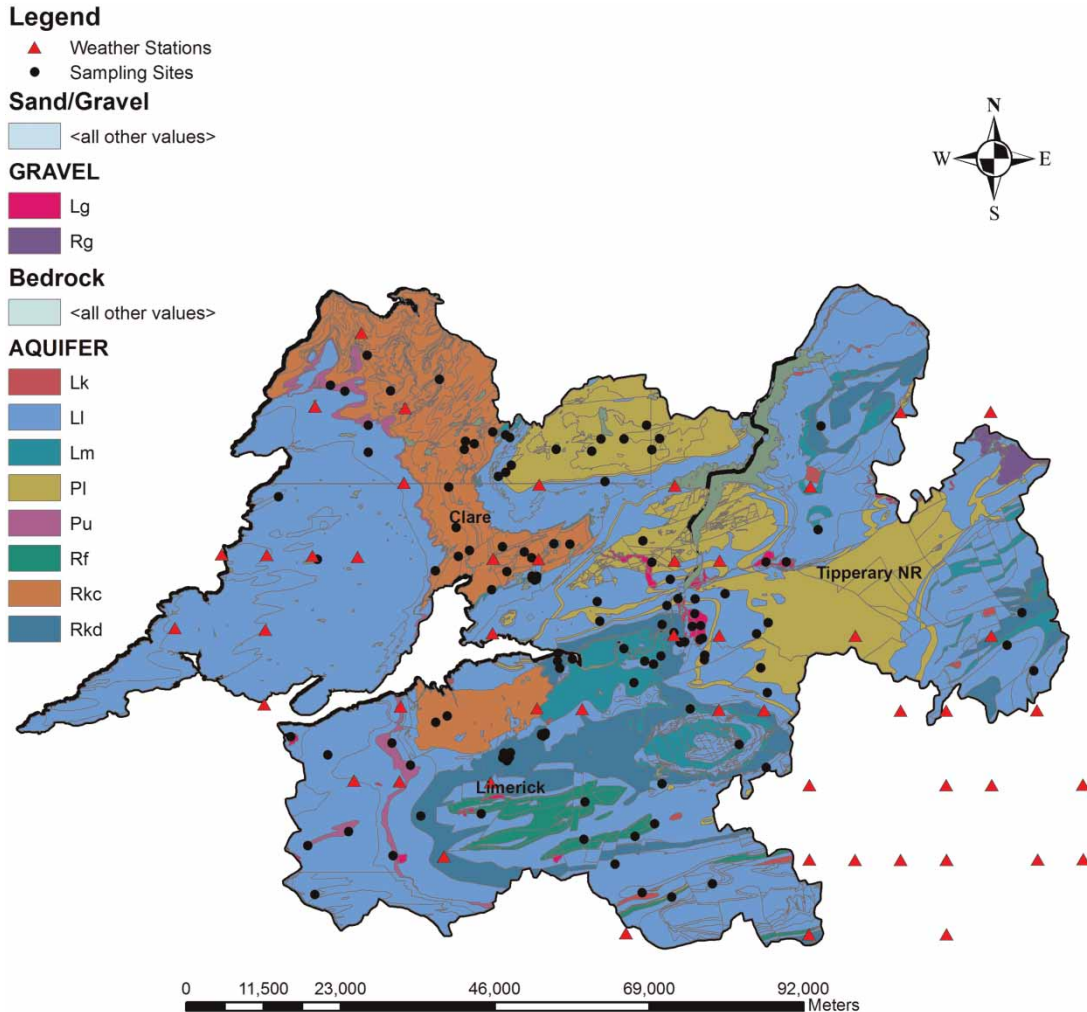


Figure 2 | Location of the 125 sampling sites overlaid on the hydrogeological characteristics of that research area as obtained from geographical information system. Also shown is the spatial distribution of weather stations utilised in the study.

differentiation; yellow for total counts and fluorescent for *E. coli*. The samples were poured into a 51-well 'Quanti-Tray', heat sealed and incubated for 24 hours at 35 °C. After the incubation period, the number of positive fluorescent compartments was counted; each compartment indicated that a minimum of one indicator organism was initially present. As a control, a sterile water sample was analysed periodically.

Acquisition and interpretation of data

The aquifer characteristics were obtained from the Geological Survey of Ireland. Two datasets were utilised in this

study: the National Draft Gravel Aquifer Map and the Bedrock Characteristics Aquifer Map. These two parameters were uploaded into a GIS using ArcGIS ArcMap10. The result is a map integrating geological information to defined areas of the Republic at a scale of 1:100,000 (Figure 2). Average daily rainfall data were obtained from the Irish Meteorological Office; 65 weather stations were plotted onto the GIS using the location coordinates (Figure 2). The station in closest proximity to the sampling location was chosen as representative of that area. The location of each individual microbiological sample analysis performed in this study was input directly to the GIS by using the global positioning system (GPS) coordinates recorded at

each sampling location via a GPS navigational system (Garmin Nuvi 40LM). The location of each sampling point was merged with the geological and rainfall data to create a new dataset detailing the specific parameters unique to each monitoring location. The frequency of each data category within the study is summarised in Table 1.

Logistic model

LR has been used extensively in the health sciences since the late 1960s to predict a binary response from explanatory variables (Lemeshow et al. 1988) and more recently in the environmental sciences to identify variables that significantly affect groundwater quality, as in this study. Binary LR is used to predict a binary response, such as the absence of a specific contaminant above a given concentration threshold, in this instance >0 , from independent explanatory variables. The probability of being in one of the response categories (contaminated or non-contaminated) is modelled. The predicted probability is thus a prediction of the response being above the concentration threshold (response equal 1), while $1 - \text{probability}$ is the predicted probability of the response being a 0. The odds ratio is based on the probability of exceeding the given concentration threshold value. The main assumption of LR is that the natural logarithm of the odds ratio or probability of

being in a response category is linearly related to the explanatory variables (aquifer type and rainfall).

RESULTS AND DISCUSSION

Gross microbial contamination

For ease of communication, where contamination was present, the analytical results were categorised based on the severity of contamination ranging from moderate to acute. This approach was adapted from a method utilised by the Irish EPA (EPA 2006, 2008a, b). This allows for comparisons to be made with existing national data for the bacteriological quality of groundwater. The classification assigned to each contamination class was, however, arbitrarily chosen and may not conform to EPA reports.

The results outlined in Table 2 show that contamination was above the national statistic of microbiological contamination in groundwater of 34% of routine samples (EPA 2009). The results demonstrate that 58.4% of wells ($n = 73$) were positive for *E. coli* at least once and hence failed to meet the legislative microbiological standards of the Drinking Water Directive 98/83/EC. Of the 125 PWSs analysed, 41.6% ($n = 52$) were found to contain none of the indicator bacterium *E. coli*. Samples were further categorised to show the frequency of positive results over multiple sampling events as detailed in Table 3. Of the 73 samples, 83.6% ($n = 61$) tested positive for *E. coli* at every sample event.

Table 1 | The frequency of climatological categorisation, contamination with *E. coli* and the hydrogeological characteristics of the sites sampled in the research area

Variable	Category	n	%	Cumulative %
Rainfall (mm)	0–10	86	68.8	68.8
	>10 < 20	28	22.4	91.2
	>20	11	8.8	100
	Total	125	100	
<i>E. coli</i>	0	52	41.6	41.6
	01–10	35	28	69.6
	10–100	17	13.6	83.2
	101–200	14	11.2	94.4
	>200	7	5.6	100
	Total	125	100	
Aquifer	Bedrock	86	68.8	68.8
	Sand/gravel	39	31.2	100
	Total	125	100	
Bedrock	Karst	42	48.8	48.8
	Fissured	44	51.2	100.0
	Total	86	100.0	

Table 2 | Gross microbial contamination results of 125 private wells characterised based on contamination categories adapted from the Environmental Protection Agency (EPA 2006, 2008a, b)

Microbiological contamination category	Category classification (colony forming units/100 mL)	No. of wells	Proportion of wells (%)
Uncontaminated	<1	52	41.6
Contaminated:			
Moderately	1–10	35	28.0
Seriously	11–100	17	13.6
Severely	101–200	14	11.2
Acutely	>200	7	5.6
Cumulative contamination	> 1	73	58.4

Table 3 | Frequency of microbiological contamination of private water supplies analysed

Contamination category (most probable number)	No. of wells	Wells positive for 100% of sampling events		Wells positive for 75–99% of sampling events		Wells positive for 50–74% of sampling events	
		<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Moderately (1–10)	32	29	90.6	31	96.9	32	100
Seriously (11–100)	20	16	80	20	100	20	100
Severely (101–200)	13	9	69.2	9	75	13	100
Acutely (>200)	8	7	87.5	8	100	8	100
Total	73	61	83.6	68	93.2	73	100

The variability of the levels of microbiological contamination coupled with the frequency of contamination in this study suggests that faecal contamination of groundwater is an important issue within the region.

Regression analysis: LR based on aquifer type and rainfall

Binary LR was performed to assess the impact of climatological, namely rainfall, and hydrogeological factors on the likelihood that private well water supplies would be contaminated with the indicator organism *E. coli*. The first model contained two independent variables: rainfall (mm) and aquifer characteristics (bedrock or sand/gravel). The full model, containing both predictors was statistically significant $p < 0.001$, indicating that the model was able to distinguish between the independent variables' relationship to the incidence of contamination. The overall model explained between 27.4% (Cox and Snell R squared) and 36.8% (Nagelkerke R squared) of the variance in contamination and correctly classified 75.2% of cases. As shown in Table 4, both the independent variables (rainfall and aquifer type) made a unique statistically significant contribution to the model. The strongest predictor of reporting

contamination was rainfall (mm), with odds ratio of 1.173. This indicated that contamination was 1.173 times more likely with an increase in rainfall, controlling for the other factor in the model. The odds ratio of 0.224 for aquifer type was less than 1, indicating that sand/gravel aquifers were 0.224 times less likely to contain *E. coli*.

LR based on bedrock type and rainfall

The second LR model focused again on rainfall; however, it was inclusive of an independent variable based on the categorisation of bedrock aquifers: fissured system or karst systems. The full model, containing both predictors was statistically significant at $p < 0.05$, indicating that the model was able to distinguish between the independent variables' relationship to the incidence of contamination. The overall model explained between 28.1% (Cox and Snell R squared) and 39.5% (Nagelkerke R squared) of the variance in contamination and correctly classified 77.9% of cases.

As shown in Table 5, both the independent variables (bedrock and rainfall) made a unique statistically significant contribution to the model. The strongest predictor of reporting contamination was the bedrock type, with odds ratio of

Table 4 | Logistic regression predicting likelihood of contamination based on the independent variables rainfall (mm) and aquifer type

	B*	Standard Error	Wald	Degrees of freedom	<i>p</i>	Odds ratio	95.0% confidence interval for odds ratio
Rainfall (mm)	0.16	0.041	15.080	1	<0.001	1.173	1.082–1.271
Aquifer type	–1.5	0.461	10.510	1	0.001	0.224	0.091–1.554

*B = coefficient for the constant in the null model.

Table 5 | Logistic regression predicting likelihood of contamination based on the independent variables rainfall (mm) and bedrock type

	B*	Standard error	Wald	Degrees of freedom	p	Odds ratio	95.0% confidence interval for odds ratio
Bedrock	1.9	0.636	8.779	1	0.003	6.581	1.892–22.887
Rainfall (mm)	0.14	0.047	8.793	1	0.003	1.148	1.048–1.258
Constant	−0.8	0.386	3.416	1	0.065	0.490	

*B = coefficient for the constant in the null model.

6.58. This indicated that contamination was 6.58 times more likely to be present in a karstified bedrock aquifer, controlling for the other factor in the model. In this model, rainfall (mm) demonstrates a higher odds ratio of 1.148. This suggests that in relation to bedrock aquifers containing either karstic or fissured flow regimes, the probability of contamination with *E. coli* is 1.15 times higher with an increase in rainfall.

CONCLUSION

A selection of 125 private domestic drinking water supplies in the Mid-Western Region of Ireland was found to demonstrate a microbiological contamination incidence rate of 58.4% in 2011–2012. This rate is higher than the national statistic of microbiological contamination in groundwater of 34% of routine samples (EPA 2009). The occurrence of microbiological contamination was related to hydrogeological and climatological variables using LR. The LR model proved to be a potentially effective tool for the prediction of bacterial contamination in PWSs in Ireland and in similar regions, from a geological and meteorological perspective. The model found that aquifer material plays a dominant role in vulnerability to microbial contamination. Where bedrock is present as the parent material, the model finds that the flow paths within the system have a significant influence on vulnerability. This research suggests that households utilising a well water supply in areas where the underlying aquifer is overtly permeable, as in karst aquifer systems, are statistically more vulnerable to microbiological contamination and therefore appropriate guidance and source protection are recommended. Furthermore, it has been shown that precipitation also has a significant impact on the extent of faecal contamination with the likelihood of contamination

increasing with successive rainfall episodes. The significance of the findings demonstrate the need for increased awareness for the consumer; through appropriate risk management and the incorporation of treatment systems in vulnerable areas, the potential human health impacts attributable to private wells in Ireland could be reduced.

Limitations of the study

LR analysis is a robust statistical analysis for predictability; however, there are limitations with its use. As stated, 75.2% of cases of contamination were correctly classified; and thus not all categories could be predicted by the model. It is also noteworthy to assert that as this model focused on the indicator organism *E. coli*, it may not predict the presence of other pathogens that may be more robust than *E. coli* in the environment. Rather, the model is a tool to highlight areas where faecal contamination is more likely and thus an indication that a groundwater system is being polluted and requires remediation.

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