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A geostatistical investigation of agricultural and infrastructural risk factors associated with primary verotoxigenic *E. coli* (VTEC) infection in the Republic of Ireland, 2008–2013

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SUMMARY

Ireland reports the highest incidence of verotoxigenic *Escherichia coli* (VTEC) infection in Europe. This study investigated potential risk factors for confirmed sporadic and outbreak primary VTEC infections during 2008–2013. Overall, 989 VTEC infections including 521 serogroup O157 and 233 serogroup O26 were geo-referenced to 931 of 18 488 census enumeration areas. The geographical distribution of human population, livestock, unregulated groundwater sources, domestic wastewater treatment systems (DWWTS) and a deprivation index were examined relative to notification of VTEC events in 524 of 6242 rural areas. Multivariate modelling identified three spatially derived variables associated with VTEC notification: private well usage [odds ratio (OR) 6·896, $P < 0·001$], cattle density (OR 1·002, $P < 0·001$) and DWWTS density (OR 0·978, $P = 0·002$). Private well usage (OR 18·727, $P < 0·001$) and cattle density (OR 1·001, $P = 0·007$) were both associated with VTEC O157 infection, while DWWTS density (OR 0·987, $P = 0·028$) was significant within the VTEC O26 model. Findings indicate that VTEC infection in the Republic of Ireland is particularly associated with rural areas, which are associated with a ubiquity of pathogen sources (cattle) and pathways (unregulated groundwater supplies).

Key words: *Escherichia coli* (*E. coli*), gastrointestinal infections, geographical information systems, infectious disease epidemiology, water-borne infections.

INTRODUCTION

Verotoxigenic *Escherichia coli* (VTEC), of which there are >100 serotypes, were first discovered in 1977 and are so called due to their ability to produce verotoxins similar to the AB5-type Shiga toxin produced by *S. dysenteriae* type 1 [1, 2]. Frequently encountered serotypes include O157, O26, O121 and O104, all of which are characterized by the presence of stx_1 and/or stx_2 genes [3]. Clinical presentation of VTEC infection ranges from mild diarrhoea to haemorrhagic colitis, thrombotic thrombocytopenic purpura and haemolytic uraemic syndrome (HUS), which is characterized by haemolysis, uraemia and thrombocytopenia [4]. Human VTEC infection became a statutorily notifiable disease in the Republic of Ireland (ROI) on 1 January 2004 under the Infectious Diseases (Amendment) Regulations 2003 [5].

Over the past decade, the ROI has repeatedly reported the highest incidence rates of symptomatic VTEC infection in the European Union (EU) [6],

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with national incidence rates exhibiting significant increases over the same period; notifications increased from 65 cases in 2004 to 702 in 2013, equating to a crude incidence rate (CIR) of 15·3 cases/100 000 persons in 2013 [7]. In the ROI, 4–7% of confirmed cases are associated with the occurrence of HUS and associated renal failure, particularly in younger subpopulations [7, 8]. To date, the most frequently identified VTEC serotype in both the ROI and several other regions has been *E. coli* O157 [8, 9]; this strain has been associated with a low infectious dose [10] and currently accounts for >200 notifications per annum in Ireland [7, 8]. Additionally, *E. coli* O26 has been increasingly detected in cases of diarrhoeal disease and HUS in the ROI [7]; an increasing clinical and analytical awareness of this verotoxigenic strain has coincided with the development and increasing use of novel detection techniques [11]. The most recently published national reports indicate a total of 721 notified cases of VTEC in Ireland in 2014, of which 24·8% ($n = 179$) and 31·9% ($n = 230$) were serogroups O157 and O26, respectively [12].

The main reservoirs of VTEC infection are considered to be agricultural animals, particularly cattle and other ruminants [13–15]. Consequently, annual infection peaks in Ireland typically occur during late summer [7], when the majority of livestock are grazing. About 80% of 'utilizable' land (68% of total area) in the ROI is designated pasture land, with mixed grazing of (beef and dairy) cattle and sheep being the dominant agricultural activity. The recent removal of EU dairy quotas (March 2015) is expected to result in a significantly increased national dairy herd. International studies have reported associations between human VTEC infection and cattle density [16–19].

The consumption of untreated groundwater from private wells represents another source of VTEC infection in Ireland [20]; at least 15 VTEC outbreaks in 2012 were linked with exposure to private groundwater supplies [8]. While ~13% of the Irish population were habitually exposed to water from private (unregulated) wells in 2011, 43% of confirmed VTEC cases were characterized by likely exposure [21]. Schets *et al.* [22] reported *E. coli* O157:H7 presence in 2·7% of private groundwater supplies in The Netherlands, with molecular analyses indicating cattle as the most probable source. Strachan *et al.* [16] reported a significant relationship between reliance on private water supplies and the incidence of human VTEC infection in Scotland.

Additionally, the use of domestic wastewater treatment systems (DWWTS) has been proposed as a potential environmental source of human VTEC infection; Denno *et al.* [23] have documented an association between *E. coli* O157:H7 infection and the use of DWWTSs in Washington State (USA). Currently, about one third of Irish households rely on DWWTSs for domestic sewage treatment, the majority of which are rural or peri-urban [24].

Accordingly, this retrospective ecological study was undertaken to identify the spatial epidemiological patterns of human VTEC infection in the ROI via statistical analyses of geo-coded primary infections in concurrence with the geographical distribution of human population, livestock, private wells, DWWTSs and a (socioeconomic) deprivation index. This is the first study to examine the spatial patterns of primary human VTEC infection occurrence and potentially associated sources in the ROI, a country characterized by the highest rates of VTEC infection in the EU.

METHODS

In this study, a 'primary case' of VTEC infection was defined as a laboratory-confirmed case notified to a Department of Public Health between 1 January 2008 and 31 December 2013, with no proven epidemiological link to another notified case (i.e. a 'sporadic' or 'endemic' case), or, if an epidemiological link was identified by assignment of an outbreak code, the earliest notified case associated with the outbreak (i.e. 'index' case). Since most notified secondary cases are associated with childcare or other settings involving a high likelihood of person-to-person transmission [7], all secondary cases of infection (i.e. outbreak-related cases notified after the earliest case) were excluded from the developed database, as were cases associated with international travel (if the patient spent the incubation period of 10 days outside the ROI).

Data from five sources were used to develop the spatially linked database containing all primary VTEC cases in the ROI during the period 2008–2013, namely:

- Computerised Infectious Disease Reporting (CIDR) database (http://www.hpsc.ie/CIDR/);
- Central Statistics Office (CSO) Census 2011 (http://www.cso.ie/en/census/);
- Pobal Haase-Pratschke relative deprivation index (HPI) [25];
- CSO Census of Agriculture 2010 [26];
- Ordnance Survey Ireland (http://www.osi.ie/Products.aspx).

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The CIDR database comprises a national Irish database of notifiable infectious disease events notified to regional departments of Public Health in accordance with the Infectious Diseases (Amendment) Regulations 2011 (S.I. No. 452 of 2011). For inclusion in the CIDR database, a 'confirmed' VTEC case is defined as any person satisfying both clinical and laboratory criteria. Clinical criteria are primarily symptom-related (diarrhoea, abdominal pain and/or HUS), while laboratory criteria require at least one of the following: (i) isolation of an stx_1- or stx_2-positive *E. coli* strain, (ii) direct detection of stx_1 or stx_2 nucleic acid(s) (in the absence of strain isolation) or (iii) direct detection of verotoxins in stool sample.

Data regarding human population counts, primary domestic water supply data and domestic wastewater treatment type were extracted from the CSO Census of Ireland 2011 dataset. Census data were already compiled and spatially indexed using the Irish postal service's GeoDirectory geographic information system (https://www.geodirectory.ie/) to 18 488 pre-defined Census enumeration areas ('Small Areas'). Small Areas (mean land area = 3·8 km^2) have been developed by the National Institute of Regional and Spatial Analysis (NIRSA) on behalf of Ordnance Survey Ireland and CSO and comprise 50–200 domestic dwellings. Small Areas are nested within 3400 Electoral Divisions, the smallest legally defined administrative areas in Ireland, and are the highest-resolution geographical unit available for statistical compilation at the national (ROI) level in compliance with current data protection standards. Individual areas are assigned a unique numeric code, which were used to spatially derive and index the current dataset. The CSO's 14 urban/rural categories were merged to classify each Small Area as rural, urban or mixed, with population density and settlement size used to classify geographical census units as urban, rural or mixed; the current mean urban population density is ~1736 persons/km^2 compared to 26 persons/km^2 in rural areas [24].

The HPI score for each Small Area was extracted from the Health Atlas Ireland database. The HPI is an Irish deprivation index calculated by structural equation modelling involving ten census-derived measures of demographic profile, social class composition and labour market situation. It measures the relative affluence or disadvantage of each geographical area, assigning deprivation scores on a normal distribution with a national mean of zero and a range of −35 (−3·5 S.D., extremely disadvantaged) to +35 (+3·5 S.D., extremely affluent) [25]. Health Atlas Ireland (https://www.healthatlasireland.ie/) is a set of open-source software applications developed by OpenApp (http://open app.ie/).

Based upon previous international studies [16–18] and resulting study hypotheses, the following variables were derived and assigned to each Small Area:

$$\text{population density}\left(/\text{km}^2\right) = \frac{\text{small area population}}{\text{small area land surface}},$$

$$\frac{\text{private well usage}}{\left(\text{per population unit}\right)} = \frac{\text{well number}}{\text{small area population}},$$

$$\text{septic tank density}\left(/\text{km}^2\right) = \frac{\text{septic tank number}}{\text{land surface area}}.$$

The Census of Agriculture [26] was completed in 2009 for all agricultural holdings in the State with a 'farmed area' of $\geqslant$1 hectare (2·47 acres), in compliance with Regulation (EC) No. 1166/2008; analogous censuses were conducted in all EU member states during 2009/2010 [26]. Data from the agricultural Census were accessed and used to aggregate and calculate cattle and sheep densities for each Small Area as follows:

$$\text{livestock density}\left(/\text{km}^2\right) = \frac{\text{livestock count}}{\text{land surface area}},$$

Anonymized cases extracted from the CIDR database were geo-referenced to individual Small Areas using the Health Atlas Ireland geo-referencing tool; a look-up routine was used to match an address list with identical or similar addresses in the An Post (Irish postal service) GeoDirectory (https://www.geodirectory.ie/), and link these with corresponding Small Areas. Both automatic and manual matching was used to geo-reference address data. Once unique identifiers for individual cases were linked to a Small Area, address data were discarded (i.e. case/event depersonalization). Case-specific clinical data (age, gender, serogroup (where available), date of onset/notification) were subsequently linked to associated Small Areas. In Small Areas associated with >1 VTEC infection, clinical data were numbered (i.e. age-*n*, gender-*n*, serogroup-*n*, date-*n*) and series-linked. For case and variable distribution mapping (dot-and-choropleth), Health Atlas Ireland was used to map each individual VTEC case to its associated Small Area centroid, and predictor variable (e.g. livestock density) interquartile ranges were mapped by Electoral Division for visual clarity.

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Table 1. *Geo-referenced VTEC cases in the Republic of Ireland 2008–2013 categorized by gender and age range (n = 989)*

	Age range, years	Female (%)	Male (%)	Total (%)
All serotypes	0–4	201 (20·3)	219 (22·1)	420 (42·5)
	5–14	81 (8·2)	90 (9·1)	171 (17·3)
	15–64	161 (16·2)	112 (11·3)	273 (27·6)
	⩾65	83 (8·4)	42 (4·2)	125 (12·6)
	Total	526 (53·2)	463 (46·8)	989
VTEC O157	0–4	94 (18)	88 (16·9)	182 (34·9)
	5–14	50 (9·6)	66 (12·7)	116 (22·3)
	15–64	98 (18·8)	73 (14)	171 (32·8)
	⩾65	38 (7·3)	14 (2·7)	52 (10)
	Total	280 (53·7)	241 (46·3)	521
VTEC O26	0–4	76 (32·6)	88 (37·8)	164 (70·4)
	5–14	17 (7·3)	15 (6·4)	32 (13·7)
	15–64	17 (7·3)	10 (4·3)	27 (11·6)
	⩾65	8 (3·4)	2 (0·9)	10 (4·3)
	Total	118 (50·6)	115 (49·4)	233

Prior to analyses, all independent variables were assessed for normality using the Kolmogorov–Smirnov test, in concurrence with Q-Q plots. Pearson's χ^2 test was used to test whether observed differences in cumulative incidence rates between urban and rural areas was statistically significant. Significant rates of skewness and kurtosis were both significantly associated with the urban/rural classification variable, thus informing the decision to deselect urban/rural classification as a model variable and focus multivariate analyses on categorically rural VTEC cases. Exclusion of urban cases was found to significantly minimize independent variable skewness (i.e. exclusion of zeros and very low values for livestock density, septic tank density, and private well reliance, in concurrence with exclusion of very high values for population density). While independent variables were still associated with slightly skewed distributions, this was minimal and within reasonable bounds for ordinary logistic regression [27], i.e. neither skewed logistic regression nor independent variable transformation were considered necessary. Logistic regression models were constructed using the spatial presence/absence of ⩾1 confirmed case of VTEC infection in a Small Area during the study period as the dichotomous dependent variable. The Mann–Whitney *U* test was used to test for associations between non-parametric predictor variables, with Pearson's χ^2 test used to test for associations with the HPI deprivation score. The collinearity diagnostic test for tolerance (<0·1) and the variance inflation factor (>10) were used to assess collinearity between independent variables prior to regression modelling. The independent variables for logistic regression modelling were selected based on biological plausibility, as documented in international literature. The 'forced entry' method was used: all variables were tested simultaneously, with backward elimination of variables that contributed least to the model. The Hosmer–Lemeshow test and Nagelkerke's R^2 were used to assess model goodness-of-fit and effect size, respectively. SPSS v. 22 (IBM Corp., USA) was employed for all statistical analyses.

The protocol for linking clinical attributes of depersonalized VTEC events to Small Areas was submitted to the Office of the Data Protection Commissioner and approved under the Data Protection Acts. The study protocol received ethical approval from the Research Ethics Committee of the Royal College of Physicians of Ireland.

RESULTS

During the 6-year study period of 2008–2013, 2210 VTEC cases were submitted to the CIDR surveillance database; 902 cases were excluded on the basis of being outbreak-associated secondary infections, while 44 cases were associated with international travel and similarly excluded. Of the remaining 1264 cases, 989 cases (mean 164·8 cases/year) were successfully geo-referenced to 931 individual 'Small Areas' using the Health Atlas. As shown (Table 1), 46·8% ($n = 463$) of geo-matched cases were associated with a male patient, with 42·5% ($n = 420$) and 12·6% ($n = 125$) of cases occurring within the vulnerable 0–4 and ⩾65-year subpopulations, respectively.

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Table 2. *Cumulative incidence (CI) (per 100 000 population) of geo-matched VTEC cases in the Republic of Ireland 2008–2013 by urban, mixed and rural classification*

Spatial category	All serotypes		VTEC O157		VTEC O26	
	N	CI	*N*	CI	*N*	CI
Urban	339	12·6	170	6·3	61	2·3
Rural	565	38·0	282	19·0	153	10·3
Mixed	85	20·9	53	13·1	15	3·7

Autumn (37·8%, $n = 374$) and summer (33·1%, $n = 327$) months were associated with highest levels of VTEC case notification, followed by spring (15·6%, $n = 156$) and winter (13·3%, $n = 132$).

Overall, 853 (86·2%) of 989 primary cases were assigned a laboratory-confirmed serotype; of the remaining 136 cases, 92 were not serotyped ('ungroupable', 9·3% of total), and 44 cases were associated with a missing serotype (4·4% of total cases). Positively confirmed VTEC O157 (61·1%, $n = 521$) and O26 (27·3%, $n = 233$) cases accounted for 88·4% of serotype cases; of the remaining cases, only VTEC O145 (25 cases, 2·9%) and O103 (18 cases, 2·1%) comprised >10 cases, with 29 VTEC serotypes encountered overall (i.e. All VTEC). Accordingly, ecological analyses focused on VTEC O157 and O26 infections within the Irish population, as these collectively comprised almost 90% of primary cases for which a serotype existed during the study period. Just over 5% of 18 488 Small Areas were associated with $\geqslant 1$ notified instance of VTEC infection ($n = 931$), while 2·7% ($n = 505$) and 1·2% ($n = 229$) of Small Areas had $\geqslant 1$ case of O157 and O26, respectively.

The 6-year cumulative incidence of VTEC O157, VTEC O26 and all VTEC cases (irrespective of serotype) was calculated for each spatial category (Table 2). The 6-year cumulative incidence of VTEC O157 infection associated with rural areas was three times that of urban areas ($\chi^2_1 = 131{\cdot}047$, $P < 0{\cdot}001$) (Fig. 1), while the cumulative incidence associated with mixed areas was twice that of urban areas ($\chi^2_1 = 28{\cdot}687$, $P < 0{\cdot}001$). Nationally, 58·3% (10 777), 33·8% (6242) and 7·9% (1469) of Small Areas are categorically urban, rural and mixed, respectively; however, during the study period 33·7% ($n = 170$), 55·8% ($n = 282$) and 10·5% ($n = 53$) of geo-coded VTEC O157 cases corresponded with these categories. Similarly, the 6-year cumulative incidence of all VTEC infections was three times greater in rural areas than urban areas ($\chi^2_1 = 226{\cdot}251$, $P < 0{\cdot}001$) (Fig. 1), while the 6-year cumulative incidence of O26 infection was 4·5 times greater in rural areas than urban areas ($\chi^2_1 = 113{\cdot}13$, $P < 0{\cdot}001$) (Fig. 1).

As shown (Table 3), in all cases, lower human population densities and higher cattle densities concurred with a higher instance of notified infection; for example, rural Small Areas associated with VTEC O157 infection during the study period had a mean cattle density of 119·11 head/km^2, compared to 101·41 head/km^2 in areas where no confirmed infections had occurred ($P < 0{\cdot}001$) (Fig. 2). It is considered likely that the significant association between VTEC O26 infection and sheep density is due to higher sheep densities typically coinciding with higher levels of annual precipitation and increasingly permeable, thinner subsoil layers. As shown in Figure 2, areas characterized by a mean cattle density within the first (<65 cattle/km^2) and second (65–105 cattle/km^2) quartile ranges had a markedly level of VTEC O157 infection occurrence during the study period. The number of private wells per head of population was higher in Small Areas characterized by $\geqslant 1$ case of VTEC infection (any strain) and VTEC O157 infection ($P < 0{\cdot}001$). DWWTS density was also associated with VTEC infection ($P = 0{\cdot}006$) and VTEC O26 infection ($P = 0{\cdot}004$) occurrence; however, contrary to expectation, mean DWWTS densities were 14–16% lower in areas characterized by notified infection. Small Areas' HPI scores exhibited no statistical association with the occurrence of VTEC infection.

Results of Hosmer–Lemeshow goodness-of-fit tests ($P > 0{\cdot}05$) indicate that all of the logistic regression models were well calibrated (Table 4). As shown, the final model for VTEC O157 and O26 included three variables, namely private well usage ($P < 0{\cdot}001$), cattle density ($P < 0{\cdot}001$) and septic tank density ($P = 0{\cdot}002$), thus corresponding with results of bivariate analyses (Table 3). Interpretation of resulting odds ratios indicate an increased likelihood of VTEC infection occurring in parallel with increasing private well use and cattle density and decreasing DWWTS density. Similarly, private well usage ($P < 0{\cdot}001$) and cattle density ($P = 0{\cdot}007$) were associated with VTEC O157 infection in rural Small Areas, while DWWTS density was significant within the VTEC O26 logistic regression model.

DISCUSSION

The current study sought to examine associations between confirmed primary VTEC infections in the ROI

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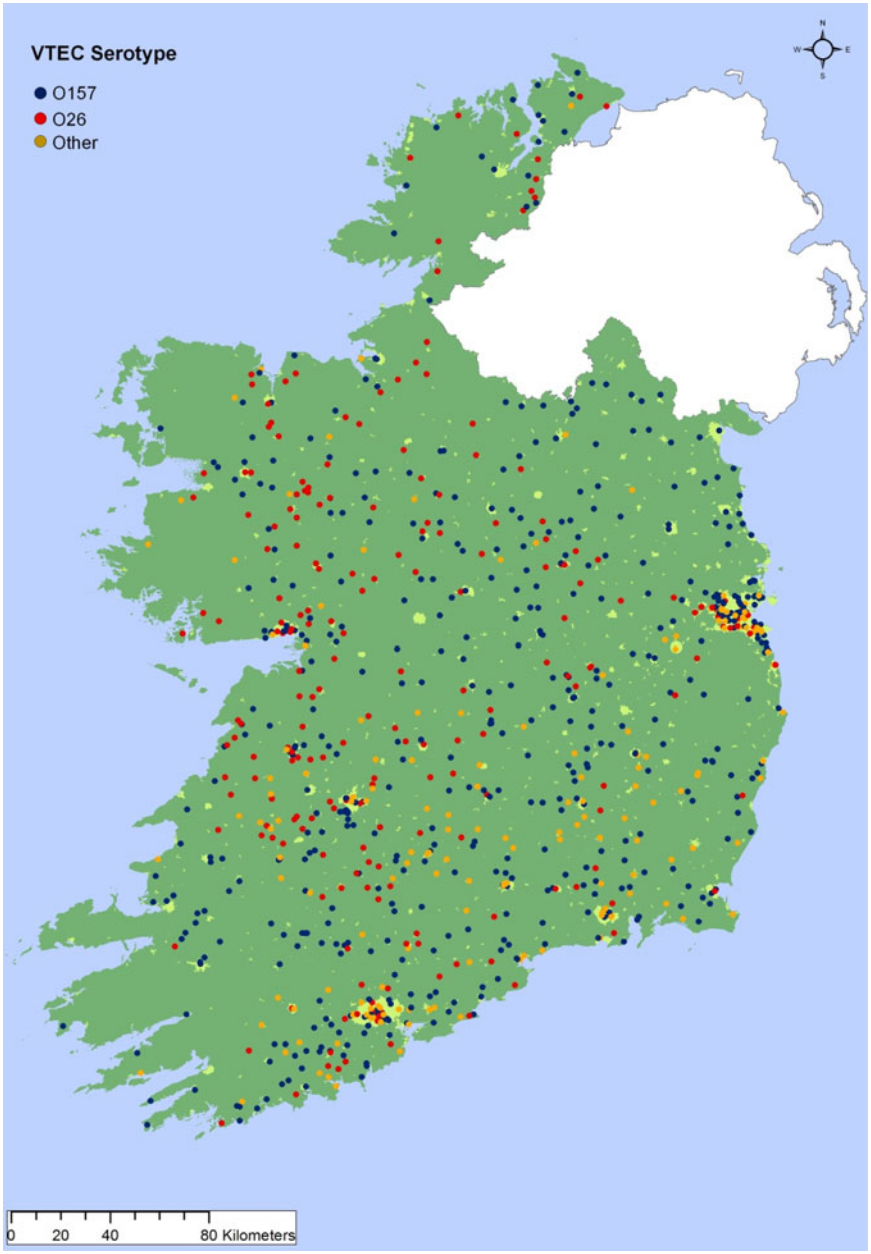

Fig. 1. Confirmed VTEC O157, VTEC O26 and VTEC (other) cases in the Republic of Ireland during the period 2008–2013 overlain on Central Statistics Office urban/rural spatial classification for each electoral Small Area (Note: lighter shades signify urban areas).

during the period 2008–2013 and a number of potentially causative spatially derived variables. A database geo-referenced and linked by census enumeration area was developed and employed for this purpose. This is the first ecological study to investigate primary VTEC infections in the ROI, a country characterized by a long-term CIR significantly greater than the current EU mean. To date, the spatial distribution and potential sources of VTEC infection in the ROI have remained unquantified, thus impeding the development of effective preventive strategies and more focused surveillance.

As shown (Table 1), a disproportionately high number of children aged <5 years were associated with confirmed VTEC infection over the study period; based upon the most recent Irish census, this demographic comprises ~7·8% of the national population, but represents 42·5% of VTEC cases (annual mean CIR 19·7 cases/100 000) during the study period. This finding is of particular significance due to the

Table 3. *Bivariate tests of association between spatially derived predictors and VTEC infection in categorically rural areas in the Republic of Ireland, 2008–2013 (n = 565)*

	N	Mean DWWTS density*	Mean population density*	Mean well usage*	Mean HPI†	Mean cattle density*	Mean sheep density*
All areas	18 488	16·16	3199·55	0·035	0·000	48·371	26·539
All serotypes							
Yes	524	7·768	70·26	0·118	−1·544	118·389	54·885
No	5718	9·069	116·57	0·092	−1·890	100·729	63·090
Sig.		$P = 0·006$	$P < 0·001$	$P < 0·001$	$P = 0·374$	$P < 0·001$	$P = 0·683$
VTEC O157							
Yes	282	7·917	83·95	0·126	−1·885	119·110	57·834
No	5960	9·009	114·04	0·093	−1·86	101·412	62·617
Sig.		$P = 0·269$	$P = 0·035$	$P < 0·001$	$P = 0·760$	$P < 0·001$	$P = 0·814$
VTEC O26							
Yes	153	7·558	62·97	0·096	−1·504	112·295	48·710
No	6089	8·995	113·93	0·094	−1·870	101·958	62·745
Sig.		$P = 0·004$	$P = 0·003$	$P = 0·524$	$P = 0·424$	$P = 0·022$	$P < 0·001$

DWWTS, Domestic wastewater treatment systems; HPI, Pobal Haase-Pratschke relative deprivation index.
* Wilcoxon rank-sum (U) tests.
† χ^2 tests.

reported association between paediatric VTEC infection and HUS [17, 28].

The 6-year cumulative incidence of all sporadic or outbreak primary cases of VTEC and of VTEC O157 infections was three times higher in rural areas ($P <$ 0·001), while the incidence of VTEC O26 was 4·5 times greater in rural areas ($P < 0·001$) thus highlighting the contribution of the rural population to the national disease burden. Michel *et al.* [29] have previously observed a significantly higher incidence of VTEC O157 infection in rural areas of Ontario, while Innocent *et al.* [30] have reported a decreasing cumulative incidence in concurrence with increasing population density in Scotland. Likewise, previous studies by Strachan *et al.* [16] and Pearl *et al.* [31] provide evidence of the influence of geographical location on VTEC infection incidence, with reported VTEC incidence rates 1·7–4 times greater in rural areas than in urban areas.

Results of bivariate and multivariate analyses indicate that within categorically rural areas, private (unregulated) well usage and cattle density were both associated with previous occurrence of a confirmed primary VTEC infection. Based upon previous studies [16, 17, 19, 20, 29, 31, 32] and application of the source-pathway-receptor (SPR) model of environmental risk assessment, it may be concluded that cattle and private wells potentially represent the primary pathogen source and environmental pathway for rural VTEC transmission, respectively, in the ROI. Multivariate analysis indicates that an increased stocking rate of 100 cattle/km^2 (1 animal/ha) concurs with a 10% increased likelihood of a confirmed VTEC O157 infection occurring in a rural area. Previous studies have reported associations between human VTEC infection and cattle density relative to land surface area [17, 31] or local cattle:human ratios [18, 19, 30, 33]. However, this finding is particularly notable within the Irish context due to (i) existing high levels of agriculture in the ROI in comparison with other EU regions and (ii) recent amendments to Irish agricultural policy and practices, including the removal of EU dairy quotas in 2015 and the initiation of Food Harvest 2020: a draft strategy for the development of the Irish agri-food sector, which envisages increased national beef and dairy herds, including a target of 50% increase in milk production (2·75 billion litres), for 2020.

The most significantly associated variable with respect to the occurrence of all confirmed VTEC and of VTEC O157 infections was private well usage. The use of private wells, which are currently unregulated in the ROI, has been previously implicated as a likely route of waterborne enteric infections [8, 20]. A recent quantitative risk assessment estimates an overall VTEC CIR of 28·3/100 000 within the Irish population served by private wells, i.e. 3–4 times

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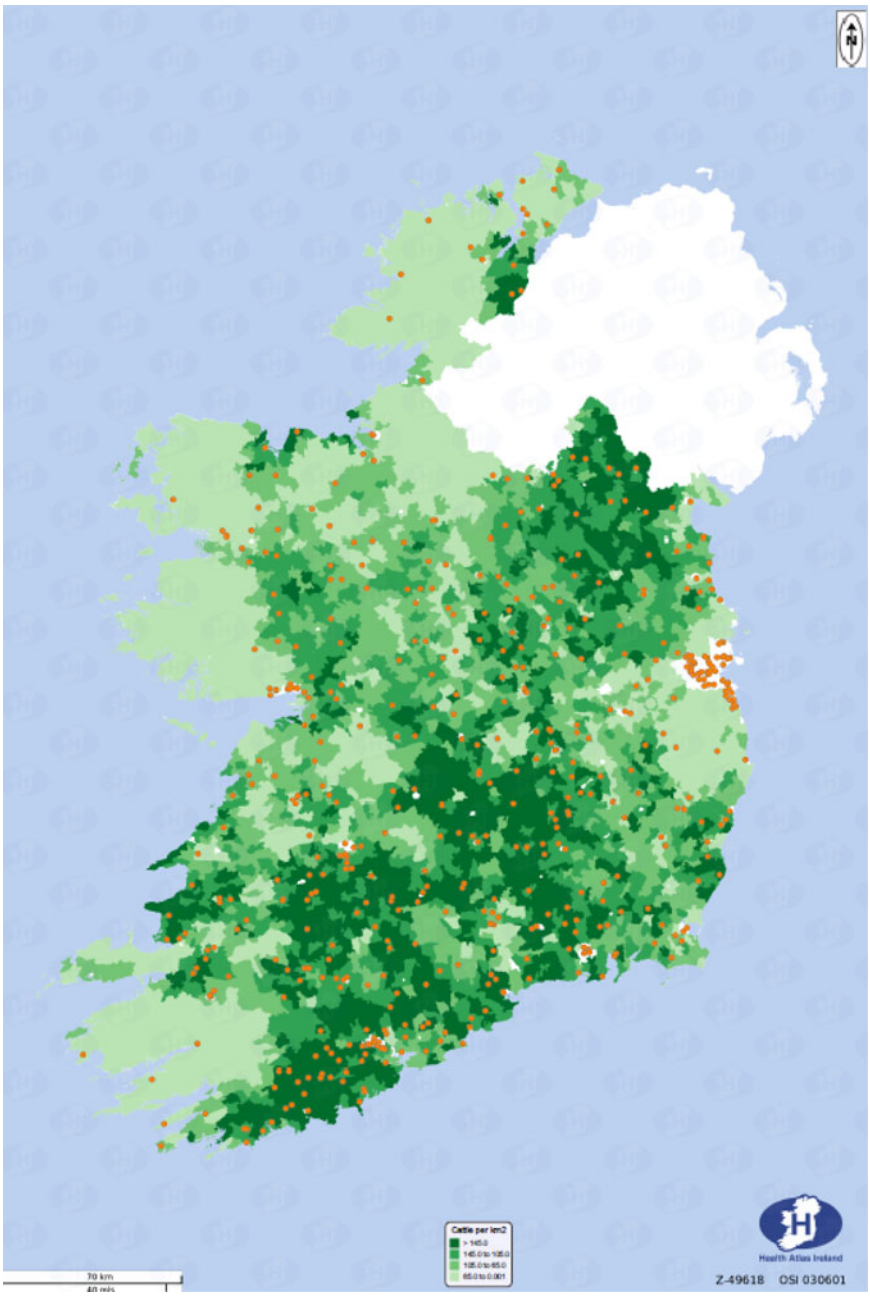

Fig. 2. Confirmed VTEC O157 cases in the Republic of Ireland during the period 2008–2013 overlain on calculated cattle density (cattle/km^2) quartiles for each electoral Small Area (Q1: <65; Q2: 65–105; Q3: 104–145; Q4: >145).

higher than CIRs within the general population and up to 25 times higher than the current EU mean [34]. The inclusion of a large control group (i.e. areas with no notified case) in the current study provides strong empirical evidence for the existence of this association. Internationally, formal quantification of an association between confirmed VTEC infection (particularly sporadic infection) and private well use in the published literature has been limited. Where the relationship between private well usage and VTEC infection has been examined, findings are consistent with those of the current study; Strachan *et al.* [16] reported that VTEC O157 infection in north-east Scotland was associated with reliance on private water supplies. Within the Irish context and in other regions characterized by high levels of groundwater reliance, particularly in rural areas, these findings are particularly notable. Hynds *et al.* [35] have previously

Table 4. *Logistic regression models for VTEC infections VTEC O157 and VTEC O26 in categorically rural areas in the Republic of Ireland, 2008–2013 (n = 6242)*

Model	Predictor	*B*	*P*	OR	95% CI
VTEC (O157)	Private well density	2·930	<0·001	18·727	6·231–56·283
	Cattle density	0·001	0·007	1·001	1·001–1·003
VTEC (O26)	DWWTS density	0·033	0·028	0·967	0·94–0·996

OR, Odds ratio; CI, confidence interval; DWWTS, domestic wastewater treatment systems.

reported low levels of awareness among groundwater users in rural Ireland, in concurrence with low levels of water treatment, testing and well maintenance. Accordingly, current findings reinforce the clinical significance of previous individual-level studies which have identified risk factors for *E. coli* contamination of private wells in several regions of Ireland [20, 36, 37], thus highlighting the need for local and regional public-health risk assessment, interventions and risk communication. It is important to note that the majority of private well users reside in rural areas; thus, it is possible that observed risk differences are partially due to urban/rural differences. Direct animal contact, consumption of different foods and/or different food-handling practices could lead to varying exposure risks in rural communities [38]; however, these exposure data were not available for inclusion in the current study, thus representing a study limitation.

A significant negative association was found between septic tank density and VTEC O26 infections; this finding was not expected and has not previously been reported. Septic tank effluent is an acknowledged source of groundwater contamination in Ireland and further afield [20, 39]. Hynds *et al.* [20] have demonstrated that on-site septic tanks are significantly associated with (both pathogenic and non-pathogenic) *E. coli* presence within a cohort of Irish wells, while a case-control study undertaken by Borchardt *et al.* [39] found that both viral and bacterial diarrhoea were associated with septic tank density in Wisconsin. Although not possible to substantiate, a potential reason for the negative correlation in this study may be septic tank density acting as a surrogate or indicator of the social demographic and/or infrastructural capacity associated with specific areas. More specifically, due to the decentralized nature of rural Ireland, which is characterized by peri-urban clustered developments and 'one-off' dwellings in more rural areas, septic tank density may be expected to increase with proximity to urban centres. Clustered peri-urban or mixed (commuter) developments typically comprise a younger demographic and higher household income, thus resulting in improved quality of dwellings and associated infrastructure (i.e. domestic water supply and wastewater treatment) in parallel with a higher concentration of vulnerable individuals (particularly children and infants). Conversely, lower septic tank densities indicate more sparsely populated (and increasingly agricultural) areas, an older demographic and lower standard of water and wastewater infrastructure.

No association was evident between the occurrence of VTEC infection and the HPI; accordingly, this indicates that the occurrence of VTEC infection in Ireland is not significantly related to socioeconomic status (SES). While Auger *et al.* [40] have previously reported that lower SES is often associated with higher rates of illness, Uhlmann *et al.* [38] more recently found that SES was not significantly associated with the instance of several waterborne enteric infections within a Canadian cohort ($n = 814$). Overall, it may be concluded that, in terms of VTEC infection, a health inequality based upon SES is not evident in Ireland, instead replaced by one based upon rural/urban residential classification or 'place'.

Based upon findings from the current study, it is concluded that VTEC infection in Ireland is a characteristically rural illness, and significantly associated with cattle density and private well reliance. Due to the diverse agricultural, infrastructural, geological and meteorological profile of the ROI, it constitutes a unique region in which to study the overarching mechanisms and effects of waterborne enteric pathogens and resulting infections. Accordingly, further work will seek to elucidate enteric pathogen hydrodynamics by inclusion of spatially derived geological, subsoil, precipitation, temperature and agricultural cycle data to the current dataset. The results from this study, along with future research, will aid in the development of more proficient disease surveillance and will subsequently facilitate the implementation of systematic, preventive public-health interventions.

It is important to note that the current study, like all retrospective ecological studies, comprises a number of inherent limitations [41]. As previously outlined (see Methods section), the use of a dichotomous independent variable (i.e. infection present/absent), while justified due to the low number of census areas associated with >1 VTEC case during the study period, negates the potential effect influence of multiple infections within one area, i.e. higher pathogen source density may (or may not) be associated with higher infection numbers. Moreover, ecological studies are disposed to 'ecological fallacy', i.e. individual exposure is assumed to be equal to the group mean exposure, which is almost certainly not the case for all individuals [41]. Moreover, the retrospective nature of the study allows for quantification of association, but not causation; therefore, while study results have strengthened the overarching research hypotheses, they have not proven them. The current study employed data pertaining to laboratory confirmed VTEC infections; however, as previously described by Wilson *et al*. [42], 6·3% of faecal samples from dairy farm residents in Ontario tested positive for the presence of VTEC in the absence of any symptomatic illness. Thus, the current study may not be used to develop or further any existing hypothesis regarding asymptomatic VTEC infection in rural Ireland, which may be considerable. Wilson *et al.* additionally concluded that rural residents likely experience subclinical immunizing VTEC infections at a young age, which frequently involve non-O157 VTEC found in cattle [42]; further research is required in Ireland to elucidate the rates and mechanisms associated with acquired immunity to VTEC infection. Last, the current study involved integration of datasets from several Health Service Executive areas; thus a measure of data fusion may be present.

Understanding how VTEC O157 may be changing relative to non-O157 serotypes, particularly VTEC O26, remains a challenge. Marked increased VTEC incident rates which started in 2012 appear to be levelling off, suggesting that much of the increase is due to the introduction of PCR testing. The number of HUS cases also increased, although numbers are small, suggesting some of this increase is real. What is most interesting is that in 2013 and 2014 the incident rate of non-O157 serotypes, particularly VTEC O26 appear to be replacing VTEC O157, which may be reducing. It is too early to determine if this is a trend or to examine the data for an interaction. A repeat of this analysis in a few years' time, when methods are consistent across the country is warranted, particularly to determine if the strong association with private well density and VTEC O157 is sustained and the absence of this association with VTEC O26 remains. Reproducing this will be the most useful public health finding.

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DECLARATION OF INTEREST

None.

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