

Spatial Effects of Livestock Farming on Human Infections with Shiga Toxin-Producing *Escherichia coli* O157 in Small But Densely Populated Regions: The Case of the Netherlands

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Key Points:

- Results indicate a potential transmission of STEC O157 from small ruminants, and not cattle, to humans via the environment.
- However, the underlying mechanisms warrant further investigation and corresponding results could offer new targets for control.
- The newly proposed exposure metric has potential to improve similar spatial modelling studies, especially in densely populated countries.

Abstract

The role of environmental transmission of typically foodborne pathogens like Shiga toxin-producing *Escherichia coli* (STEC) O157 is increasingly recognized. To gain more insights, we assessed the spatial association between sporadic STEC O157 human infections and the exposure to livestock (i.e. small ruminants, cattle, poultry, and pigs) in a densely populated country: the Netherlands. This was done for the years 2007-2016, using a state-of-the-art spatial analysis method in which hexagonal areas with different sizes (90, 50, 25 and 10 km²) were used in combination with a novel probability of exposure metric: the population-weighted number of animals per hexagon. To identify risk factors for STEC O157 infections and their population attributable fraction (PAF), a spatial regression model was fitted using integrated nested Laplace approximation (INLA). Living in hexagonal areas of 25, 50 and 90 km² with twice as much population-weighted small ruminants was associated with an increase of the incidence rate of human STEC O157 infections in summer (RR of 1.09 [95% CI; 1.01-1.17], RR of 1.17 [95% CI; 1.07-1.28] and RR of 1.13 [95% CI; 1.01-1.26]), with a PAF of 49% (95% CI; 8-72%). Results indicate a potential transmission of STEC O157 from small ruminants to humans via the environment. However, the underlying mechanisms warrant further investigation and could offer new targets for control. Furthermore, the newly proposed exposure metric has potential to improve existing spatial modelling studies on infectious diseases related to livestock exposure, especially in densely populated countries like the Netherlands.

Plain Language Summary

I will add this summary during the revision stage

Key words: STEC O157, livestock, small ruminants, population-weighted number of animals, environmental transmission

1 Introduction

Food is generally considered to be the most important route of transmission for Shiga toxin-producing *Escherichia coli* (STEC) O157 (N. J. C. Strachan et al., 2001). However, a growing body of evidence suggests that non-foodborne transmission pathways, such as those mediated by the environment, may be important as well (Berry et al., 2015; Elson et al., 2018; Franz et al., 2018; Friesema et al., 2011; ÓHaiseadha et al., 2017; Norval J. C. Strachan et al., 2006). A recent source attribution modelling study based on STEC serotyping data revealed that domestic ruminants (cattle, sheep and goats) are important sources of human STEC O157 infections, accounting for approximately three-quarters of reported human STEC infections in the Netherlands (Mughini-Gras et al., 2018). This emphasizes the need for both direct and indirect exposure to different types of livestock to be considered as possible transmission routes for STEC O157.

STEC is a bacterial zoonotic agent associated with human disease with varying clinical manifestations, including diarrhea, haemorrhagic colitis and (occasionally fatal) haemolytic uremic syndrome (HUS), a leading cause of acute renal failure among children (Elson et al., 2018; Franz et al., 2018; Mughini-Gras et al., 2018). Human STEC infections is the third most

commonly reported zoonosis in the European Union (EU), with an annual number of laboratory-confirmed STEC infections increasing from 5,901 in 2014 to 8,161 in 2018 (European Food Safety Authority & European Centre for Disease Prevention and Control, 2019). This, in combination with its high virulence and outbreak potential, makes STEC of significant public health concern. Although there are more than a hundred STEC serotypes and their importance is increasingly recognized, STEC O157 is the most important serotype in terms of incidence and clinical significance (Mughini-Gras et al., 2018). In the Netherlands, STEC is a notifiable disease, with an annual incidence between 2 and 7 cases per 100.000 inhabitants (European Centre for Disease Prevention and Control, 2019). The vast majority of cases in the Netherlands are considered sporadic, as outbreaks rarely occur (Franz et al., 2018).

Potential sources of human STEC infection are mainly animals capable of maintaining STEC colonization in absence of continuous exposure to STEC from other sources (i.e. the so-called reservoirs or amplifying hosts, mainly cattle and sheep). But also those that are frequently exposed to STEC from the environment, like birds and other wild animals (Mughini-Gras et al., 2018; Norval J. C. Strachan et al., 2006). According to a recent source attribution study, cattle is the primary source of human STEC O157 infection in the Netherlands, followed by small ruminants (sheep and goats) (Mughini-Gras et al., 2018). These animals can shed high quantities (>105/g) of STEC O157, that subsequently are able to survive for extended periods of time (Chase-Topping et al., 2008; Franz et al., 2014; N. J. C. Strachan et al., 2001). This implies that there is a significant risk of STEC O157 infection linked to environment-mediated transmission to humans (Elson et al., 2018; N. J. C. Strachan et al., 2001).

The Netherlands is one of the world's most densely populated countries, with over 500 inhabitants per km² and a remarkably high concentration of intensive livestock farms as well. The presence of livestock in close proximity to residential areas has arisen questions about the associated public health implications (Smit & Heederik, 2017). Since STEC O157 can potentially be contracted from the soil and water environment, and may be spread through the air after periods of drought in the vicinity of its animal reservoirs, it is conceivable that human STEC O157 incidence in the Netherlands might higher in areas with increased livestock density as well, such as in rural vs. urban areas as shown elsewhere (Berry et al., 2015; Norval J. C. Strachan et al., 2006; N. J. C. Strachan et al., 2001). This could be tested with different methods, of which examples are: (i) spatial regression analysis to determine the probability of exposure (Elson et al., 2018; Friesema et al., 2011; ÓHaiseadha et al., 2017) or (ii) classical case-control studies including relevant spatial variables to determine the importance of particular types of exposure (e.g. number of animals/km²) (de Rooij et al., 2019).

As a spatial regression analyses requires less resources, in terms of data needs and financial support, it can be a preferred way of exploring new ideas. However, only a few studies exist that focus on the spatial association between human STEC O157 infections and the probability of exposure to livestock by means of spatial regression analysis (Elson et al., 2018; Friesema et al., 2011; ÓHaiseadha et al., 2017). Most of those studies only include one domestic ruminant species (cattle or sheep or goat) in the analysis (Friesema et al., 2011; ÓHaiseadha et al., 2017), while ignoring other reservoirs that may affect the outcome of those studies. This is especially important in countries like the Netherlands where high numbers of different types of livestock are present on relatively small geographical scales (Smit & Heederik, 2017). Moreover, the probability of exposure in those studies is strictly defined by the number of animals in a given area, while the probability of exposure on a population level is not only determined by the number of animals in a certain area but also by the number of residents living in that area (Elson

et al., 2018; Friesema et al., 2011; Hallisey et al., 2017; Mulder et al., 2016; ÓHaiseadha et al., 2017).

Therefore, the aim of this study was to assess the spatial association between sporadic human STEC O157 infections and the combined exposures to livestock (cattle, goat, sheep, poultry and pigs) in the Netherlands, using different state-of-the-art methods that include population-weighted numbers of animals in the calculation of the probability of exposure to livestock.

2 Materials and Methods

This study consisted of several parts. First, national surveillance data on notified STEC O157 cases in the Netherlands' general population was gathered together with livestock data (exact locations of registered farms and number of animals therein, per species). Subsequently, the data were transposed into a study-defined spatial division of the Netherlands and we developed a metric for the probability of exposure of the human population to each livestock species that not only includes the number of animals in a certain area, but also the corresponding population number. The last steps involved the spatial regression analysis and calculation of the population attributable fraction (PAF). We used the statistical software environment R (version 3.6.0) (RCT, 2015) and several R packages and functions for data processing and analysis (Arya et al., 2015; Bates et al., 2019; Bivand et al., 2019; De Jonge & Houweling, 2019; Grolemond & Wickham, 2011; Keitt, 2010; Neuwirth, 2015; Pebesma, 2019; Pebesma, Bivand, Racine, et al., 2019; Pebesma, Bivand, Rowlingson, et al., 2019; R-Core, 2017; Rue, 2019; Wickham, 2019; Wickham, Averick, et al., 2019; Wickham, Bryan, et al., 2019; Wickham, Francois, et al., 2019; Wickham, Henry, et al., 2019). An overview is provided in supporting information Table S1. The used R scripts can be found at: <https://github.com/mulderac91/R-STECO157-spatialanalysis>

2.1 Hexagonal grid and population-weighted interpolation

Hexagons are more suitable than rectangular grids in particular applications of ecological modelling, e.g. connectivity and movement paths (Birch et al., 2007). They have the advantage that the nearest neighborhood in a hexagonal grid is simpler and less ambiguous, because each hexagon has exactly six adjacent hexagons which are in a symmetrically equivalent position. Therefore, there is no need for a setting for the relative weighting of diagonal interactions in a nearest neighborhood analysis, as is the case for rectangular grids (Birch et al., 2007; Birch et al., 2000). Furthermore, the grid is fixed over time (Birch et al., 2007). The latter is a solution for the problem of change of, in this case postal code boundaries over time (supporting information Figure S1). Therefore, the Netherlands was divided in a fixed hexagonal grid (Figure 1 - a). To assess consistency of results and reduce the risk of ecological fallacy, we performed the analyses for hexagonal areas with four different sizes: 10 km² (approximately the average area of a four-digit postal code region in the Netherlands), 25 km², 50 km² and 90 km² (approximately the average area of a municipality in the Netherlands) (Shafran-Nathan et al., 2017).

In order to perform the spatial regression analyses on the hexagonal grid, the spatial data needed to be transformed from one regional division to the other (Arsenault et al., 2013). For this purpose, we used population-weighted interpolation. This approach has the advantage over areal weighted interpolation that it can more accurately estimate the population demographics in transforming small counts by four-digit postal code regions to aggregated counts for large, non-

standard study zones (hexagons) (Hallisey et al., 2017). A detailed explanation of this approach can be found in supporting information Text S1.

2.2 Population-weighted number of animals

Existing studies have used animals/km² to derive the probability of exposure to be able to study the association between STEC O157 infections and livestock densities (Figure 1 - b) (Elson et al., 2018; Friesema et al., 2011; ÓHaiseadha et al., 2017). Yet, the probability of exposure is not only determined by the number of animals in a certain area, but also by the number and residential addresses of people living in that area and the number of animals in the neighboring areas. For this purpose, we created a new probability of exposure metric: the population-weighted number of animals (Hallisey et al., 2017) (Figure 1-c, d and e).

The metric is constructed as follows. When zooming into one hexagon within the hexagonal grid, the locations of several six-digit postal code points are shown (Figure 1-c). Those six-digit postal code points include information about the population numbers at that specific location (Figure 1-c). Around these point locations, buffers with a radius of 1 km are constructed (Figure 1-d). Farms located within these buffers, also outside the specified hexagon, are included (Figure 1-e). The point locations of the farms contain information about the number of animals (Figure 1-e). See Figure 1-c, d and e as an example. Within the hexagon, we have five six-digit postal code point locations, each with its own population numbers: 100, 1,000, 10, 5 and 1. We have three farms, each with its own number of animals: A, B and C. The 100 and the 10 individuals on the first and second six-digit postal code point locations are exposed to A animals. The 1,000 individuals in the third six-digit postal code point location are not exposed. The 5 individuals in the fourth six-digit postal code point location are exposed to B animals. The only individual in the fifth six-digit postal code point location is exposed to C animals, but from a farm outside the hexagon. The total exposure in this hexagon is then the population-weighted sum of the number of animals, which can be calculated as follows:

$$\text{Population weighted animal number} = \frac{(100 \times A + 10 \times A + 1,000 \times 0 + 5 \times B + 1 \times C)}{(100 + 10 + 1,000 + 5 + 1)}$$

This was done for each hexagon and for each year, taking into account the number of animals and the changing population numbers. In the end, the data were aggregated over the years, resulting in one hexagon-specific exposure metric.

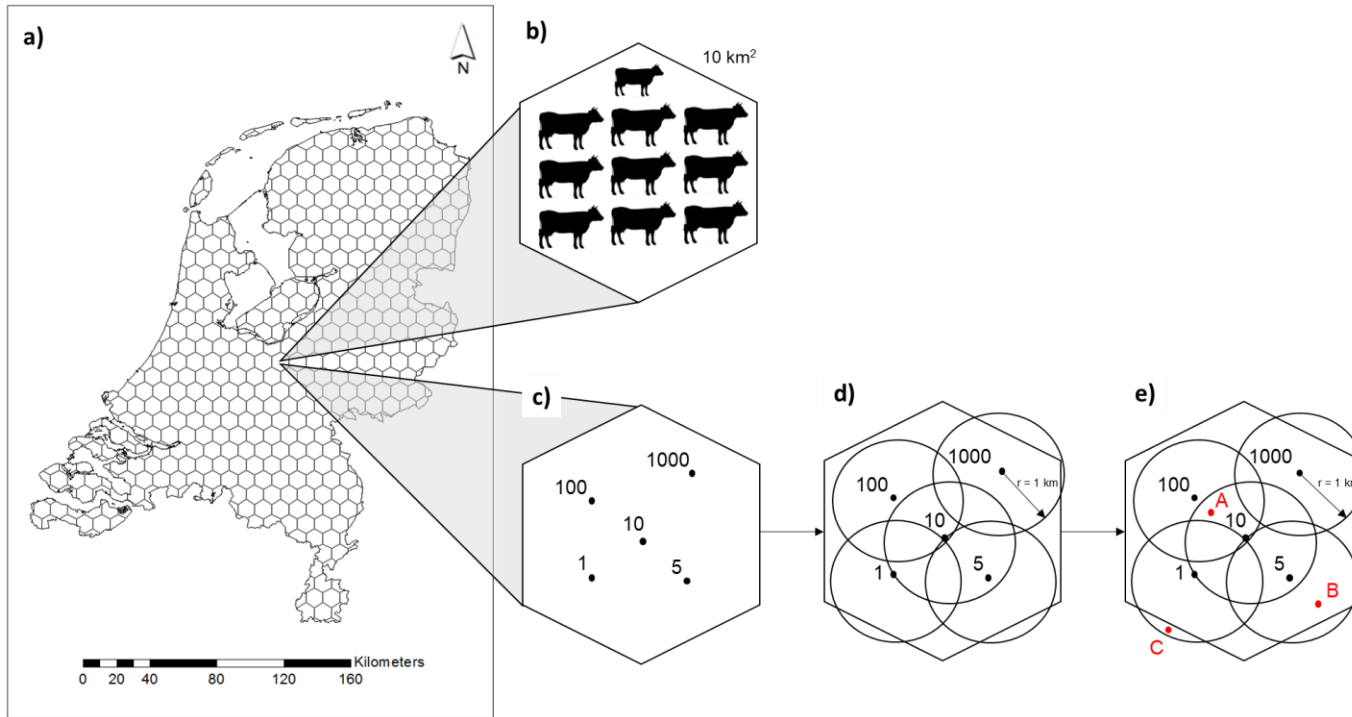


Figure 1.

Explanation of the calculation of the old and the new probability of exposure measures. **a)** Hexagonal grid of the Netherlands. **b)** The old probability of exposure measure in a hexagonal grid cell: the number of animals per km². In this figure: 10 cows per 10 km², thus 1 cow/km². Pictures **c**, **d** and **e** visualize the calculation of the new probability of exposure: the population-weighted animal number. **c)** A hexagonal grid cell, including the six-digit postal code point locations within this cell and their corresponding population numbers. **d)** The buffers with a radius r of 1 km surrounding the six-digit postal code point locations within the hexagonal grid cell. **e)** The hexagonal grid cell including all the information of Figure 1 - d. Here, the point locations of the farms of a certain type of animal are added, which also include information about the specific number of animals. This gives the information that is needed to know which six-digit postal code points (and thus which population numbers) are influenced by which farm(s) and the corresponding animal numbers. With this information and the formula given in Section 2.2, the new probability of exposure can be calculated and aggregated per hexagon.

2.3 Spatial risk factor analysis

A Poisson regression model with log-link function was used to assess the associations between human STEC O157 infections and the population-weighted number of animals for cattle, pigs, poultry, and small ruminants (goats and sheep). The model described the number of human cases as a function of person-years (the population denominator for each hexagon), age category (0-4, 5-9, 10-49 and ≥ 50 years old), gender (male or female), period of infection (spring/summer: May-October, autumn/winter: November-April) and the different population-weighted number of animals (Friesema et al., 2011). Because the population-weighted number of animals x could be zero, we applied a $\log_2(x + 1)$ transformation. Furthermore, several studies have shown a higher risk for human STEC O157 infection in summer (Friesema et al., 2011). Therefore, we performed a stratified analysis based on the period of infection. These variables entered the model as the fixed effect terms.

It is possible that there is additional variation due to unknown spatially varying risk factors. To account for this, two random-effect terms were added to the model. The first random-effect accounted for the spatially structured variation. This variation represented the possible effect of a common unobserved risk factor that led to neighboring hexagons being more alike. This term was modelled by the intrinsic Conditional Autoregressive Model (CAR) (Besag et al., 1991). The second random-effect term represented the unstructured variation. This variation consisted of possible unobserved variation within hexagons, which was modelled by independent and identically distributed (IID) Gaussian noise (Lawson, 2013).

The spatial regression model was fitted using the integrated nested Laplace approximation technique (INLA) (Rue et al., 2019). For further details we refer to Friesema et al. (2011). Rate ratios (RRs) were calculated from the coefficients of the fixed effects. As the population-weighted animal numbers were transformed, the interpretation of those RRs is as follows: if x increases with a factor two, then the incidence rate increases with a factor $RR = e^{\beta_1}$, provided that x is large enough, approximately >100 . When x is smaller, this factor is less than two for the same RR, but the significance stays the same. Supporting information Text S2 and Figure S2 show a more detailed explanation of this interpretation.

In addition, the population attributable fraction (PAF) and its 95% confidence interval were calculated for the risk factors found (supporting information Text S3). Confidence intervals were obtained by Monte Carlo simulation, using the INLA posterior sampling function with 10,000 samples.

3 Data

3.1 Case data

Since 1999, it is obligatory for diagnostic laboratories in the Netherlands to notify confirmed human STEC infections to the Municipal Health Services (MHSs) (Friesema et al., 2011). The MHSs reports each laboratory-confirmed case to the national surveillance database at the Dutch National Institute for Public Health and the Environment (RIVM) (Friesema et al., 2011). Furthermore, laboratories are asked (but not obliged) to send STEC isolates to the RIVM for confirmation and further typing for national surveillance purposes (Friesema et al., 2017; Friesema et al., 2011).

In this study, a case was defined as an individual with confirmed STEC O157 infection (by the RIVM) during the period 2007-2016. Cases were excluded when they were part of an (inter)national foodborne outbreak, travelled abroad in the week before onset of illness, or when the residential address (postal code) was unknown. A detailed explanation of the different spatial scales (province, municipality and postal code) of the Netherlands and a comparison with the European NUTS classification system is given in supporting information Text S4 and Figure S3 (European Commission - Eurostat, 2019). Those data are protected by Dutch privacy regulations and the Dutch Data Protection Authority (Dutch Data Protection Authority, 2020a, 2020b).

3.2 Livestock data

Livestock data for 2012 was obtained from the Department of Service Arrangements of the Dutch Ministry of Agriculture, Nature and Food Quality. These data are collected yearly, requesting all farmers to report the number of animals reared (CBS, 2019b; RVO, 2019). In our study, we used the total number of goats, sheep, cattle, poultry and pigs per farm. To derive the total number of small ruminants, the total number of goats and sheep per farm were summed together.

3.3 Population data

The population data per four-digit postal code region per year is available through Statistics Netherlands (www.statline.nl) and consists of the number of inhabitants in five-year age categories and gender. The data were downloaded from this website for the years 2007-2016 (CBS, 2019a). Due to privacy regulations (Dutch Data Protection Authority, 2020b), this information was not available per six-digit postal code point location.

3.4 Spatial data

The four-digit postal code region shapefiles of the Netherlands were obtained for each year (2007-2016) from the geodata portal of the RIVM. For the period 2007-2008, there were no postal code region shapefiles available. Therefore, the shapefile of 2009 was used for those years. The six-digit postal code point location shapefile of the Netherlands from 2016 was also obtained from the geodata portal of the RIVM. This file included population numbers per six-digit postal code point location.

4 Results

4.1 Descriptive statistics

Between 2007 and 2016, 599 cases of STEC O157 infection were reported. In this period, two national outbreaks of STEC O157 were registered in the Netherlands, one in 2007 involving 41 cases probably caused by lettuce consumption and linked to an outbreak in Iceland (Friesema et al., 2008) and one in 2009 involving 20 cases caused by contaminated raw meat spread (Greenland et al., 2009). Furthermore, there was a regional outbreak in 2007 involving 7 cases, which reported consumption of raw meat spread and all had bought it at the same regional supermarket chain (Friesema et al., 2011). The cases that were involved in those outbreaks were excluded from the dataset for analysis. Besides, 54 more cases were excluded because information on travel history prior to symptom onset was missing, and 38 cases because there was no data available on geographical location. The remaining 439 cases were included in the

analysis, with a median number of 46 cases per year (range 25-63 cases/year, annual incidence 1.5-3.8/100,000 inhabitants).

Table 1.

Descriptive Statistics of the STEC O157 Cases.

	STEC O157	
	cases	
	N	%
Total	439	100
Gender		
Males	167	38
Females	272	62
Age category		
(years)		
0-4	70	16
5-9	44	10
10-49	200	46
≥ 50	125	28
Period of infection		
Summer	340	77
Winter	99	23

Of all the cases included, 62% (n = 272) were female, 38% (n = 167) were male (Table 1). The highest number of cases (46%) were between 10 and 49 years of age and most were reported in summer (77%). Figure 2 shows that the incidence varies between hexagons and appears to be highest in the northern and eastern regions of the Netherlands. The west and south of the Netherlands show particularly low incidence of STEC O157.

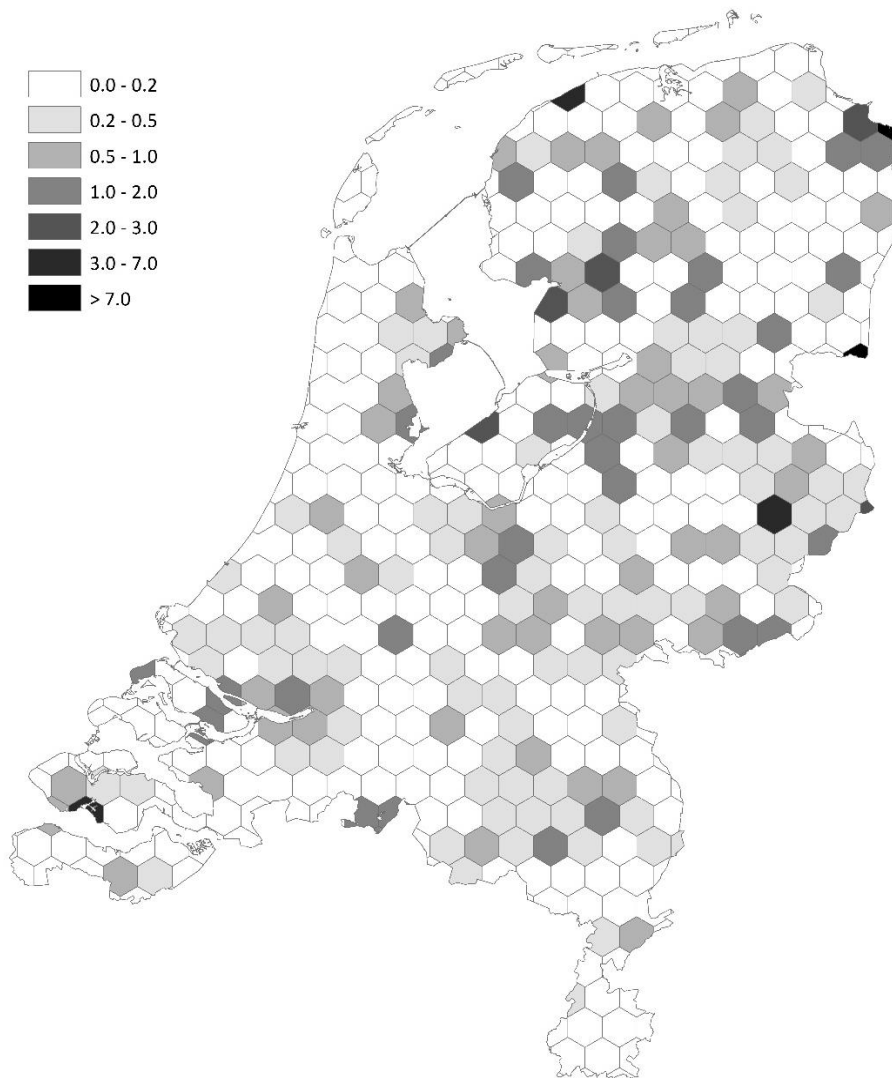


Figure 2.

Cumulative incidence rate (x 100.000 person-years) (2007-2016) of STEC O157 infections in the Netherlands.

Figure 3 shows the population-weighted number of small ruminants, cattle, poultry and pigs in the Netherlands. The population-weighted number of small ruminants appeared to be highest in the central north of the country, central south of the country and the island of Texel. For cattle, it was highest in the center, central north and central south of the country and for poultry it was highest in the center, east and south-east (except the region of South-Limburg). Furthermore, the population-weighted number of pigs was highest in the east and south-east (except the region south-Limburg). Visually, the map for small ruminants in Figure 3 seemed to be most comparable with the one for human STEC O157 infections in Figure 2.

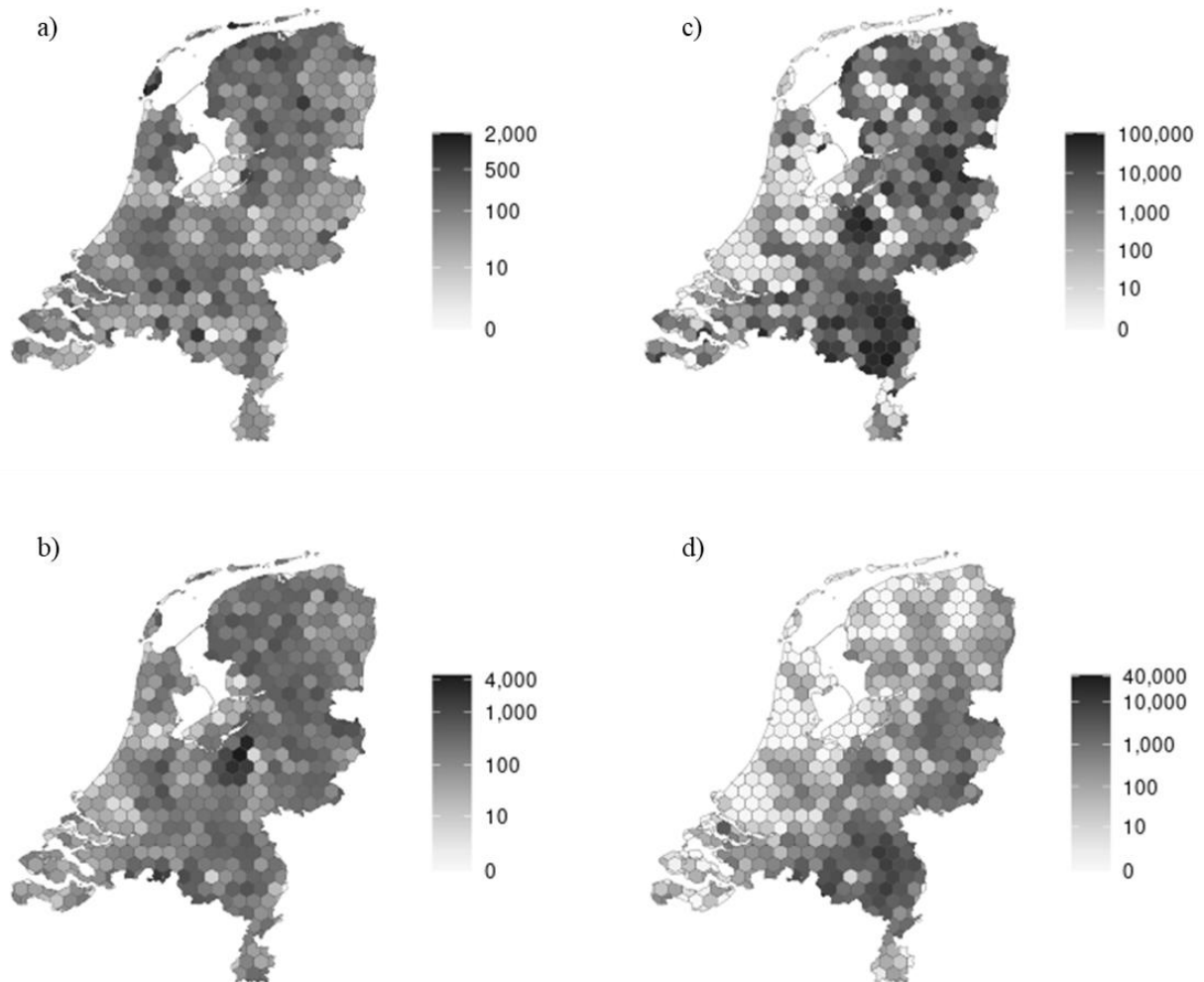


Figure 3.

Maps of the population-weighted number of animals in the Netherlands per hexagon (90 km²) for small ruminants (a), cattle (b), poultry (c) and pigs (d) in 2012.

4.2 Spatial risk factor analysis

Results from the multivariable models for the spatial association between STEC O157 and population-weighted number of animals are presented in Table 2 and Table 3, respectively. For the results of the univariable models, see supporting information Table S2.

311 Table 2.

312 *Results of the Multivariable Spatial Analyses for Summer with Different Hexagonal Areas (90, 50, 25 and 10 km²).*

Period of infection	Variable	Hexagon 90 km ²			Hexagon 50 km ²			Hexagon 25 km ²			Hexagon 10 km ²		
		P-value	RR	95% CI	P-value	RR	95% CI	P-value	RR	95% CI	P-value	RR	95% CI
Summer	Gender												
	Males				<i>Reference category</i>								
	Females	<0.001	1.74	1.40-2.17	<0.001	1.74	1.40-2.18	<0.001	1.74	1.40-2.17	<0.001	1.74	1.40-2.17
	Age category (years)												
	0-4	<0.001	4.05	2.91-5.59	<0.001	4.06	2.91-5.61	<0.001	4.06	2.91-5.60	<0.001	4.06	2.91-5.60
	5-9	<0.001	2.01	1.32-2.97	<0.001	2.01	1.32-2.98	<0.001	2.01	1.32-2.98	<0.001	2.01	1.32-2.97
	10-49	0.27	1.15	0.90-1.49	0.27	1.16	0.90-1.49	0.27	1.15	0.90-1.50	0.27	1.16	0.90-1.49
	≥ 50 (ref)				<i>Reference category</i>								
	Type of animal ^a												
	Small ruminants	0.03	1.13	1.01-1.26	<0.001	1.17	1.07-1.28	0.02	1.09	1.01-1.17	0.14	1.05	0.99-1.11
	Cattle	0.69	0.97	0.86-1.11	0.20	0.94	0.85-1.03	0.38	0.97	0.89-1.04	0.60	0.98	0.92-1.05
	Poultry	0.50	1.01	0.97-1.06	0.76	0.99	0.96-1.03	0.91	1.00	0.97-1.03	0.96	1.00	0.98-1.03
	Pigs	0.83	1.01	0.94-1.07	0.21	1.04	0.98-1.10	0.28	1.03	0.98-1.08	0.03	1.05	1.01-1.09

^a Population-weighted number of animals

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315 Table 3.

316 *Results of the Multivariable Spatial Analyses for Winter with Different Hexagonal Areas (90, 50, 25 and 10 km²).*

Period of infection	Variable	Hexagon 90 km ²			Hexagon 50 km ²			Hexagon 25 km ²			Hexagon 10 km ²		
		P-value	RR	95% CI	P-value	RR	95% CI	P-value	RR	95% CI	P-value	RR	95% CI
Winter	Gender												
	Males				<i>Reference category</i>								
	Females	0.20	1.30	0.87-1.94	0.20	1.30	0.87-1.94	0.20	1.30	0.87-1.94	0.20	1.30	0.87-1.93
	Age category (years)												
	0-4	<0.01	2.80	1.39-5.35	<0.01	2.80	1.39-5.35	<0.01	2.81	1.39-5.36	<0.01	2.80	1.39-5.36
	5-9	<0.01	2.82	1.43-5.30	<0.01	2.82	1.43-5.31	<0.01	2.82	1.43-5.30	<0.01	2.82	1.43-5.31
	10-49	0.67	1.11	0.70-1.78	0.67	1.11	0.70-1.78	0.66	1.11	0.70-1.79	0.66	1.11	0.70-1.78
	≥ 50				<i>Reference category</i>								
	Type of animal ^a												
	Small ruminants	0.11	1.15	0.97-1.37	0.14	1.12	0.96-1.30	0.07	1.12	0.99-1.27	0.58	0.97	0.88-1.08
	Cattle	0.39	0.92	0.75-1.12	0.34	0.93	0.79-1.08	0.66	0.97	0.86-1.10	0.19	1.07	0.97-1.19
	Poultry	0.91	1.00	0.94-1.07	0.93	1.00	0.94-1.06	0.56	1.01	0.97-1.07	0.89	1.00	0.95-1.04
	Pigs	0.51	1.03	0.94-1.13	0.27	1.05	0.96-1.14	0.79	0.99	0.92-1.07	0.65	0.99	0.92-1.05

^a *Population-weighted number of animals*

Living in an hexagonal area of 90 km² with twice as much population-weighted small ruminants increased the incidence rate of reporting STEC O157 infection in summer, with a RR of 1.13 (95% CI 1.01-1.26) (Table 2). Other hexagonal areas have comparable results, except the one of 10 km². Here, small ruminants were not significantly associated with STEC O157 infections. To further explore this, the analyses at this spatial scale was repeated with goats and sheep separately. The results showed that goats are still significant in summer, with a RR of 1.07 (95% CI 1.01-1.3), while sheep no longer pose a risk. In both analyses, pigs are marginally associated with STEC O157 infections, with similar RRs. As other studies showed a clear association with cattle density per municipality in summer, the analyses were repeated with only cattle for hexagonal areas of 90 km². Here, the population-weighted number of cattle only had a marginal significant association with human STEC O157, with a RR of 1.08 (95% CI 1.00-1.17). In winter, none of the animal types were associated with STEC O157 infections (Table 3). Poultry was never associated with STEC O157 infection. As the population-weighted number of small ruminants in an area was the only consistent significant risk factor for different spatial scales within this study, the PAF was calculated for this factor only. The population-weighted number of small ruminants had a PAF of 49% (95% CI of 8%-72%).

The variation in the spatially structured residual risks of the main model showed some dependence on region and period of infection (Figure 4), with a slightly increased residual risk for STEC O157 infection in the northern, mid-eastern and south-western regions of the Netherlands in winter and in the mid-eastern region in summer. A lower residual risk was found in the mid-west to north-west and the south-east region for both periods of infection.

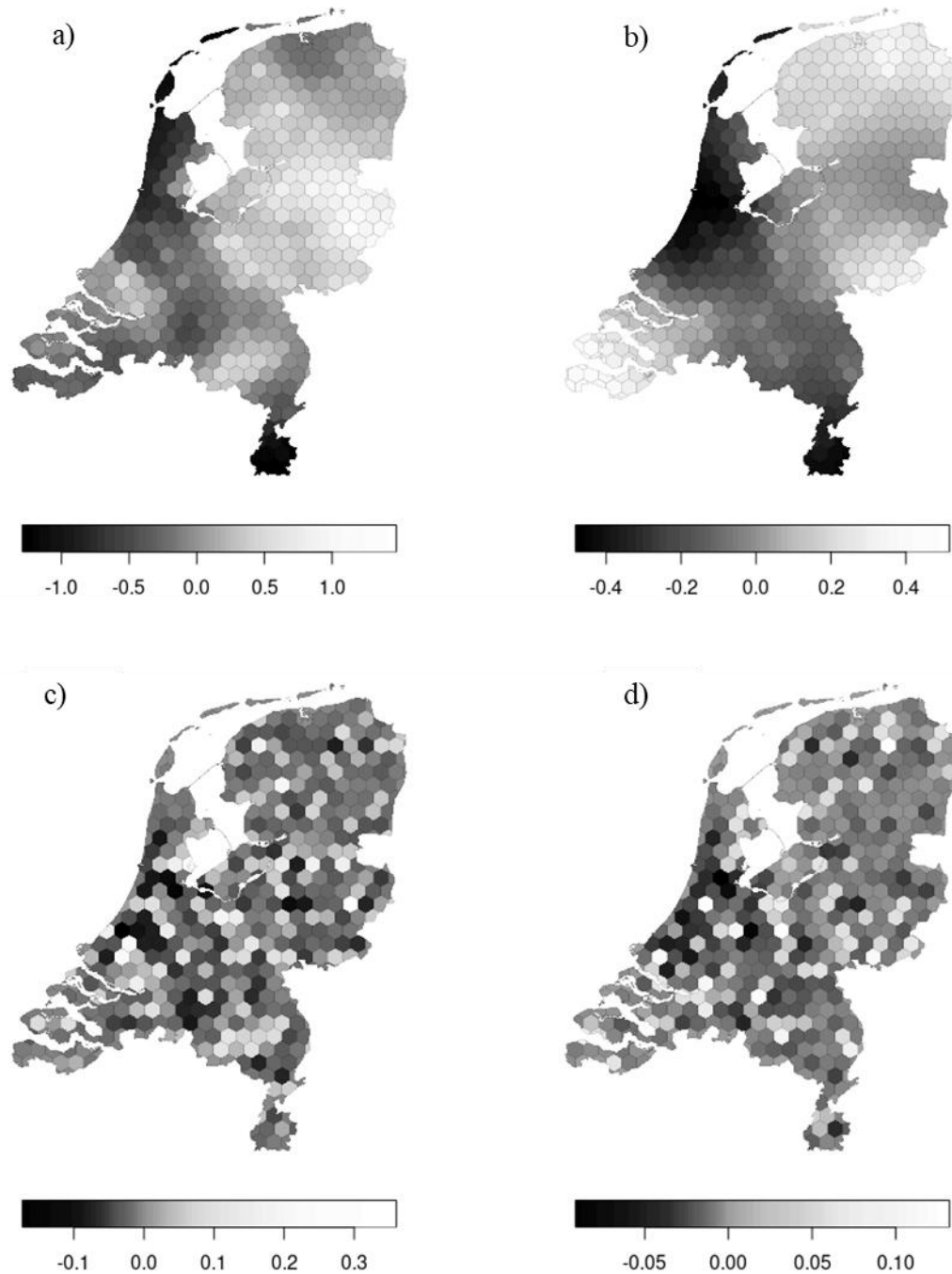


Figure 4.

Maps of the spatially structured variation modelled by the Conditional Autoregressive Model (CAR) in summer (a) and winter (b) and maps of the spatially unstructured variation modelled by independent and identically distributed (IID) Gaussian noise in summer (a) and winter (b) for hexagonal areas of 90 km².

5 Discussion

The aim of this study was to explore the spatial association between sporadic human STEC O157 infections and the exposure to livestock (small ruminants, cattle, poultry, and pigs) in the Netherlands, a country with high densities of humans and livestock animals, for the years 2007-2016. This was done using a state-of-the-art spatial analysis method, in which hexagonal areas were used in combination with a novel metric that was applied to define the probability of exposure: the population-weighted number of animals per hexagon.

Results showed that there is a consistent significant association between the population-weighted number of small ruminants and the incidence of reported human STEC O157 infections in summer with a PAF of 49%. This means that in the absence of environmental exposure to small ruminants, the number of sporadic STEC O157 infections due to environmental transmission should be expected to decrease by 49%, although the uncertainty ranges between 8% and 72%. Since we only accounted for livestock density as a risk factor for infection with O157, we were not able to quantify the relative importance of environmental transmission within the broader context of all possible transmission routes, such as food. The risk of ecological biases could also not be quantified in this regard. Although we corrected for age and gender at an individual level, areas can still differ regarding confounders that are not included in our analyses, as is also suggested by the maps of the spatially structured variation (CAR).

The finding that small ruminants are important contributors to human STEC O157 infections is supported by a Dutch source attribution study (Mughini-Gras et al., 2018), which shows that while domestic ruminants (cattle, sheep, and goats) are responsible for approximately three-quarters of reported human STEC (all serotypes) infections, small ruminants in particular accounted for 25% of all STEC infections. In the Netherlands, STEC O157 has been isolated from sheep and goats (Heuvelink et al., 1998; Heuvelink et al., 2002). Additionally, STEC was detected at almost all dairy goat and sheep farms in the Netherlands that were included in the Dutch surveillance of zoonoses in 2016, although STEC O157 was only detected at one farm (Opsteegh 2018). This reflects a common paradox regarding the results of animal sampling, in which small ruminants are generally considered as a primary reservoir for STEC O157, but their occurrence is infrequently demonstrated. This may reflect the sporadic and/or intermittent nature of STEC O157 carriage and low numbers of bacteria residing in colonized animals, or insufficiently sensitive sampling and culturing approaches (Ferens & Hovde, 2011).

Given the presence of STEC in small ruminants' feces and farms, it is plausible that human infections occur via environmental transmission. In the Netherlands, small ruminants are usually kept in deep litter houses, with partially open walls or roofs (Schimmer et al., 2011). An initial layer of litter (usually straw or sawdust material) is spread for the animals to use for bedding material and to defecate on. As soon as this layer is soiled, new layers are added, which can build up to a depth of 1-2 meters. This process generates a lot of dust, which is easily spread into the environment through the often (partially) open housing system. As a result, the transport of STEC O157 in dust through the air can be one of the possible environmental transmission routes if infected animals are present on the farm (Chase-Topping et al., 2008; Schimmer et al., 2011). The plausibility of air-borne transmission is supported by a study focusing on microbial air pollution from livestock farms in the Netherlands, where a higher concentration of commensals, amongst which *Escherichia coli*, in dust particles was found in rural areas with higher farm density (de Rooij et al., 2019). Although no significant associations with the number of goats and sheep were found, the presence of livestock-related microbial markers, such as *Escherichia coli*, indicates that microbial air pollution with *Escherichia coli* is reasonable. The same phenomenon

was observed for *Campylobacter*, which coincides with a higher *Campylobacter* incidence in poultry-dense areas, the main reservoir of *Campylobacter* (de Rooij et al., 2019; Poulsen et al., 2018). Furthermore, transmission of STEC O157 to humans may occur via soil or water, since dust precipitates and the stable litter that is stored outside the stable comes into contact with soil and possibly fresh water systems through washout after heavy rainfall (Elson et al., 2018).

Whilst several studies, including a Dutch one, showed a significant spatial association between cattle and STEC O157 infections (Friesema et al., 2011; ÓHaiseadha et al., 2017; Norval J. C. Strachan et al., 2006; Widgren et al., 2018), we did not. This could have several possible explanations.

First, a major difference is the inclusion of small ruminants in this study, next to cattle. Cattle farms are widely distributed in the Netherlands, while small ruminants have a more profound environmental spread. To study whether this could lead to different results, the analyses were performed for a model with only cattle. The results showed that the population-weighted number of cattle had a marginal significant association with human STEC O157, while this effect is not significant anymore after the inclusion of pigs, poultry and small ruminants. This might indicate that the spatial association observed for cattle could be due to its spatial relatedness with small ruminants, the latter which may play a more important role in environmental STEC O157 transmission. This proves that it is meaningful to look at the combined effects of all possible reservoirs for STEC O157. Such a combined analysis is especially important in a country like the Netherlands, which has a peculiar situation in terms of livestock and population density as compared to other countries (Smit & Heederik, 2017). Indeed, it is one of the most densely populated countries in the world in combination with a high density of intensive livestock farms (Smit & Heederik, 2017). An example of such a situation is the Q-fever epidemic in the Netherlands (Schimmer et al., 2011), which became an epidemic because most goat farms were located very close to locations with a high population density (Schimmer et al., 2011). As all the different types of livestock farms in the Netherlands are intertwined and mixed throughout the landscape, spatial inter-relatedness with other animal species does play a role (de Rooij et al., 2019). This makes it complicated to disentangle the effects and to look at each type of livestock separately, emphasizing that a more complete model in terms of possible reservoirs of STEC O157 is necessary for a proper analysis (de Rooij et al., 2019).

Second, livestock farming in the Netherlands underwent several changes in the past few years that could explain the different findings as well (Bos et al., 2013). There was a reduction in the number of farms over the years, which was paralleled by an increase in the number of animals per farm, with cows being increasingly kept inside throughout the year (Bos et al., 2013; Groot & van't Hooft, 2016; Smit & Heederik, 2017). As cattle is more often kept inside and their housing is closed, it is possible that aerial spread of STEC from cattle is reduced over the years and that small ruminants play a more important role nowadays.

Third, this study used a different spatial metric as response variable in order to do the spatial regression analyses on the hexagonal grid. Here, the population-weighted number of animals was used instead of animal density as exposure measure to transform the spatial data from one regional division to the other (Elson et al., 2018; Friesema et al., 2011; Hallsley et al., 2017; ÓHaiseadha et al., 2017). However, our approach has the advantage over areal weighted interpolation that it can more accurately estimate the population demographics in transforming small counts by four-digit postal code regions to aggregated counts for large, non-standard study zones (hexagons) (Hallsley et al., 2017). Moreover, because the probability of exposure on a population level is not only determined by the number of animals in a certain area, but also by

the number of residents in a certain area and where they live inside an area, this study is more likely to have captured true environmental exposure, as exposure is less likely to occur when nobody lives in the vicinity of these animals (Mulder et al., 2016). Furthermore, in contrast to other studies, we took into account potential exposure to animals in neighboring hexagons, because pathogen spread is not hold back by “invisible” hexagonal boundaries.

In this study, no associations were found between poultry, pigs and STEC O157 infections in the multivariable model. This supports the finding that STEC has been isolated only sporadically from animals other than ruminants and these animals can merely be seen as spill-over hosts (Caprioli et al., 2005; Mughini-Gras et al., 2018). Also, a low estimated contribution to human STEC infections has previously been found for poultry and pigs in the Netherlands (Mughini-Gras et al., 2018). However, pigs did show a positive association with human STEC infections at a hexagonal size of 10 km² in the multivariable model. This could be due to several reasons, such as limitations of power and more limited exposure metric contrasts at this smaller spatial scale (de Rooij et al., 2019).

The association between small ruminants and human STEC O157 infections was only present in the summer. This is in agreement with the incidence of human STEC O157 infections being highest in summer, as well as the seasonality of fecal excretion of STEC in farm animals (Friesema et al., 2011; Heuvelink et al., 1998). Furthermore, humans are more likely to have direct or indirect contact with animals in summer as they probably spend more time outside (Friesema et al., 2011). Similar to what is described globally, women had a higher risk than men to acquire a STEC O157 infection in summer and the incidence of STEC O157 was highest in children <10 years and strongest in children <5 years (Elson et al., 2018; Friesema et al., 2011).

Compared to a previous Dutch study (Friesema et al., 2011), underreporting of the human STEC O157 infections and the geographical laboratory bias did not change. Human STEC O157 cases included in this study likely represent the more severe cases, as mild cases often go unnoticed, because they may not always seek medical attention or do not get laboratory tested and hence, do not end up in the surveillance records (Friesema et al., 2011; van den Brandhof et al., 2006). The laboratory surveillance is based on a voluntary system, but despite the fact that the notification is mandatory, it is not guaranteed that all laboratories send in their isolates on a regular basis.

6 Conclusions

Results of this study indicate that living in proximity of small ruminants, is a risk factor for acquiring STEC O157 infection, possibly due to environmental transmission via the air by microbial contamination of dust particles with STEC O157, soil or water. However, the exact underlying mechanisms warrant further investigation, and could offer new targets for control. The finding that small ruminants, and not cattle, are significantly associated with human STEC O157 infection is in contradiction with earlier studies. It could be explained by the inclusion of small ruminants in the analysis, a changing farming landscape over the years, and the newly developed exposure metric, the population-weighted number of animals per hexagon, which showed potential to improve existing spatial modelling studies on infectious diseases related to livestock exposure, especially in densely populated regions.

Abbreviations

STEC O157 = Shiga Toxin-producing *Escherichia coli*
PAF = Population attributable fraction
INLA = Integrated nested Laplace approximation
EU = European Union
CAR = Conditional Autoregressive Model
IID = Independent and identically distributed
RR = Rate ratio
MHS = Municipal Health Service
RIVM = Dutch National Institute for Public Health and the Environment
PCR = Polymerase chain reaction
Stx₁ = Shiga Toxin 1
Stx₂ = Shiga Toxin 2
CI = Confidence interval

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Conflict of interest

None declared

Data availability

Links and citations to most of the data used in this study can be found in the main text. The four-digit postal code region shapefiles and the six-digit postal code point locations of the Netherlands that were used within this study were obtained by the RIVM from the company: Iris International. Those shapefiles can only be given to those for whom permission has been granted by this company. They can be reached at this address: Gr.v. Prinstererlaan 20, 2271 EN, Voorburg, the Netherlands. Tel: +31(0)70-3863891, fax: +31(0)70-3873625, e-mail: info@iris-int.nl.

The STEC O157 case data are available within OSIRIS, the Dutch surveillance system and only researchers within the RIVM with access to this database can use those data as it contains privacy sensitive information of cases and therefore are not accessible to the public or research community following the legislation of the Dutch law and the Dutch Data Protection Authority (Dutch Data Protection Authority, 2020a, 2020b).

Authors' contributions

Conceptualization – ACM, JvdK, RP, LMG, EF; Data curation – ACM, JvdK; Formal analysis: ACM, Funding acquisition: EF, LMG; Investigation – ACM, JvdK, DH, RP, LMG, EF; Methodology – ACM, JvdK; Project administration – ACM, EF; Resources – JvdK, EF, LMG;

Software – JvdK, ACM; Supervision – EF, LMG, DH; Validation – JvdK; Visualization – ACM;
Writing – original draft – ACM; Writing – review & editing – ACM, JvdK, DH, RP, LMG, EF.

References

- Arsenault, J., Michel, P., Berke, O., Ravel, A., & Gosselin, P. (2013). How to choose geographical units in ecological studies: proposal and application to campylobacteriosis. *Spatial and spatio-temporal epidemiology*, 7, 11-24. doi:<https://doi.org/10.1016/j.sste.2013.04.004>
- Arya, S., Mount, D., Kemp, S. E., & Jefferis, G. (2015). RANN: Fast Nearest Neighbour Search (Wraps Arya and Mount's ANN Library). Retrieved from <http://cran.rapporter.net/bin/macosx/contrib/3.2/PACKAGES>
- Bates, D., Maechler, M., Davis, T. A., Oehlschlägel, J., & Riedy, J. (2019). Matrix: Sparse and Dense Matrix Classes and Methods. Retrieved from <https://cran.r-project.org/web/packages/Matrix/index.html>
- Berry, E. D., Wells, J. E., Bono, J. L., Woodbury, B. L., Kalchayanand, N., Norman, K. N., et al. (2015). Effect of Proximity to a Cattle Feedlot on O157:H7 Contamination of Leafy Greens and Evaluation of the Potential for Airborne Transmission. *Applied and Environmental Microbiology*, 81(3), 1101-1110. doi:<https://doi.org/10.1128/AEM.02998-14>
- Besag, J., York, J., & Mollié, A. (1991). Bayesian image restoration, with two applications in spatial statistics. *Annals of the institute of statistical mathematics*, 43(1), 1-20. doi:<https://doi.org/10.1007/BF00116466>
- Birch, C. P. D., Oom, S. P., & Beecham, J. A. (2007). Rectangular and hexagonal grids used for observation, experiment and simulation in ecology. *Ecological Modelling*, 206(3), 347-359. doi:<https://doi.org/10.1016/j.ecolmodel.2007.03.041>
- Birch, C. P. D., Vuichard, N., & Werkman, B. R. (2000). Modelling the effects of patch size on vegetation dynamics: Bracken [*Pteridium aquilinum* (L.) Kuhn] under grazing. *Annals of Botany*, 85, 63-76. doi:<https://doi.org/10.1006/anbo.1999.1081>
- Bivand, R., Altman, M., Anselin, L., Assunção, R., Berke, O., Bernat, A., et al. (2019). spdep: Spatial Dependence: Weighting Schemes, Statistics. Retrieved from <https://cran.r-project.org/web/packages/spdep/index.html>
- Bos, J. F., Smit, A. B. L., & Schröder, J. J. (2013). Is agricultural intensification in The Netherlands running up to its limits? *NJAS-Wageningen Journal of Life Sciences*, 66, 65-73. doi:<https://doi.org/10.1016/j.njas.2013.06.001>
- Caprioli, A., Morabito, S., Brugère, H., & Oswald, E. (2005). Enterohaemorrhagic *Escherichia coli*: emerging issues on virulence and modes of transmission. *Veterinary research*, 36(3), 289-311. doi:<https://doi.org/10.1051/vetres:2005002>
- CBS. (2019a). Bevolking; geslacht, leeftijd en viercijferige postcode, 1 januari. Retrieved from <https://opendata.cbs.nl/statline/#/CBS/nl/dataset/83502NED/table?ts=1575556895525>
- CBS. (2019b). Landbouwtelling. Retrieved from <https://www.cbs.nl/nl-nl/onze-diensten/methoden/onderzoeksomschrijvingen/korte-onderzoeksbeschrijvingen/landbouwtelling>
- Chase-Topping, M., Gally, D., Low, C., Matthews, L., & Woolhouse, M. (2008). Super-shedding and the link between human infection and livestock carriage of *Escherichia coli* O157. *Nature Reviews Microbiology*, 6(12), 904. doi:<https://doi.org/10.1038/nrmicro2029>
- De Jonge, E., & Houweling, S. (2019). cbsodataR: Statistics Netherlands (CBS) Open Data API Client. Retrieved from <https://rdr.io/cran/cbsodataR/>
- de Rooij, M. M. T., Hoek, G., Schmitt, H., Janse, I., Swart, A., Maassen, C. B. M., et al. (2019). Insights into Livestock-Related Microbial Concentrations in Air at Residential Level in a Livestock Dense Area. *Environmental Science & Technology*, 53(13), 7746-7758. doi:<https://doi.org/10.1021/acs.est.8b07029>
- Dutch Data Protection Authority. (2020a). Retrieved from <https://www.autoriteitpersoonsgegevens.nl/en>
- Dutch Data Protection Authority. (2020b). Retrieved from <https://www.autoriteitpersoonsgegevens.nl/en/about-dutch-dpa/tasks-and-powers-dutch-dpa>
- Elson, R., Grace, K., Vivancos, R., Jenkins, C., Adak, G. K., O'Brien, S. J., & Lake, I. R. (2018). A spatial and temporal analysis of risk factors associated with sporadic Shiga toxin-producing *Escherichia coli* O157 infection in England between 2009 and 2015. *Epidemiol Infect*, 146(15), 1928-1939. doi:<https://doi.org/10.1017/S095026881800256X>
- European Centre for Disease Prevention and Control. (2019). *Annual Epidemiological report for 2017 - Shiga toxin/verocytotoxin-producing Escherichia coli (STEC/VTEC) infection - Annual Epidemiological Report for 2017*. Retrieved from Stockholm: <https://www.ecdc.europa.eu/en/publications-data/shiga-toxinverocytotoxin-producing-escherichia-coli-stecvtec-infection-annual>

- European Commission - Eurostat. (2019). Nuts - Nnuts - Nomenclature of territorial units for statistics. Retrieved from <https://ec.europa.eu/eurostat/web/nuts/background>
- European Food Safety Authority, & European Centre for Disease Prevention and Control. (2019). The European Union One Health 2018 Zoonoses Report. *EFSA Journal*, 17(12), e05926. doi:<https://doi.org/10.2903/j.efsa.2019.5926>
- Ferens, W. A., & Hovde, C. J. (2011). Escherichia coli O157: H7: animal reservoir and sources of human infection. *Foodborne pathogens and disease*, 8(4), 465-487. doi:<https://doi.org/10.1089/fpd.2010.0673>
- Franz, E., Rotariu, O., Lopes, B. S., MacRae, M., Bono, J. L., Laing, C., et al. (2018). Phylogeographic analysis reveals multiple international transmission events have driven the global emergence of Escherichia coli O157: H7. *Clinical Infectious Diseases*, 69(3), 428-437. doi:<https://doi.org/10.1093/cid/ciy919>
- Franz, E., Schijven, J., de Roda Husman, A. M., & Blaak, H. (2014). Meta-Regression Analysis of Commensal and Pathogenic Escherichia coli Survival in Soil and Water. *Environmental Science & Technology*, 48(12), 6763-6771. doi:<https://doi.org/10.1021/es501677c>
- Friesema, I., Kuiling, S., van der Voort, M., in 't Veld, P. H., Heck, M. E. O. C., & Franz, E. (2017). Surveillance van shigatoxineproducerende Escherichia coli (STEC) in Nederland, 2016. Retrieved from <https://www.rivm.nl/surveillance-van-shigatoxineproducerende-escherichia-coli-stec-in-nederland-2016>
- Friesema, I., Sigmundsdottir, G., Van Der Zwaluw, K., Heuvelink, A., Schimmer, B., De Jager, C., et al. (2008). An international outbreak of Shiga toxin-producing Escherichia coli O157 infection due to lettuce, September–October 2007. *Euro Surveill.*, 13(50), 19065. doi:<https://doi.org/10.2807/ese.13.50.19065-en>
- Friesema, I., Van De Kasstele, J., De Jager, C. M., Heuvelink, A. E., & Van Pelt, W. (2011). Geographical association between livestock density and human Shiga toxin-producing Escherichia coli O157 infections. *Epidemiol. Infect.*, 139(7), 1081-1087. doi:<https://doi.org/10.1017/S0950268810002050>
- Greenland, K., De Jager, C., Heuvelink, A., Van Der Zwaluw, K., Heck, M., Notermans, D., et al. (2009). Nationwide outbreak of STEC O157 infection in the Netherlands, December 2008-January 2009: continuous risk of consuming raw beef products. *Euro Surveill.*, 14(8). Retrieved from <http://researchonline.lshtm.ac.uk/id/eprint/748775/>
- Grolemund, G., & Wickham, H. (2011). Dates and times made easy with lubridate. *Journal of Statistical Software*, 40(3), 1-25.
- Groot, M. J., & van't Hooft, K. E. (2016). The Hidden Effects of Dairy Farming on Public and Environmental Health in the Netherlands, India, Ethiopia, and Uganda, Considering the Use of Antibiotics and Other Agro-chemicals. *Frontiers in Public Health*, 4(12). doi:<https://doi.org/10.3389/fpubh.2016.00012>
- Hallisey, E., Tai, E., Berens, A., Wilt, G., Peipins, L., Lewis, B., et al. (2017). Transforming geographic scale: a comparison of combined population and areal weighting to other interpolation methods. *International Journal of Health Geographics*, 16(1), 29. doi:<https://doi.org/10.1186/s12942-017-0102-z>
- Heuvelink, A., Van Den Biggelaar, F., De Boer, E., Herbes, R., Melchers, W., Huis, J., & Monnens, L. (1998). Isolation and characterization of verocytotoxin-producing Escherichia coli O157 strains from Dutch cattle and sheep. *Journal of Clinical Microbiology*, 36(4), 878-882. doi:<https://doi.org/10.1128/JCM.36.4.878-882>
- Heuvelink, A., Van Heerwaarden, C., Zwartkruis-Nahuis, J., Van Oosterom, R., Edink, K., Van Duynhoven, Y., & De Boer, E. (2002). Escherichia coli O157 infection associated with a petting zoo. *Epidemiology & Infection*, 129(2), 295-302. doi:<https://doi.org/10.1017/S095026880200732X>
- Keitt, T. H. (2010). rgdal: Bindings for the Geospatial Data Abstraction Library, R package version 0.6-28. <http://cran.r-project.org/package=rgdal>.
- Lawson, A. B. (2013). *Bayesian disease mapping: hierarchical modeling in spatial epidemiology*: Chapman and Hall/CRC.
- Mughini-Gras, L., van Pelt, W., van der Voort, M., Heck, M., Friesema, I., & Franz, E. (2018). Attribution of human infections with Shiga toxin-producing Escherichia coli (STEC) to livestock sources and identification of source-specific risk factors, The Netherlands (2010–2014). *Zoonoses and Public Health*, 65(1), e8-e22. doi:<https://doi.org/10.1111/zph.12403>
- Mulder, A. C., Snablie, M., & Braks, M. A. (2016). From guessing to GIS-ing: empowering land managers. In *Ecology and prevention of Lyme borreliosis* (Vol. 4, pp. 373-387): Wageningen Academic Publishers.
- Neuwirth, E. (2015). Package 'RColorBrewer'. Retrieved from <https://cran.r-project.org/web/packages/RColorBrewer/RColorBrewer.pdf>
- ÓHaiseadha, C., Hynds, P. D., Fallon, U. B., & O'Dwyer, J. (2017). A geostatistical investigation of agricultural and infrastructural risk factors associated with primary verotoxigenic E. coli (VTEC) infection in the Republic

- of Ireland, 2008–2013. *Epidemiology and Infection*, 145(1), 95–105.
doi:<https://doi.org/10.1017/S095026881600193X>
- Pebesma, E. (2019). `st_make_grid`. Retrieved from https://www.rdocumentation.org/packages/sf/versions/0.8-0/topics/st_make_grid
- Pebesma, E., Bivand, R., Racine, E., Sumner, M., Cook, I., Keitt, T., et al. (2019). `sf`: Simple Features for R. Retrieved from <https://cran.r-project.org/web/packages/sf/index.html>
- Pebesma, E., Bivand, R., Rowlingson, B., Gomez-Rubio, V., Hijmans, R., Sumner, M., et al. (2019). `sp`: Classes and Methods for Spatial Data. Retrieved from <https://cran.r-project.org/web/packages/sp/index.html>
- Poulsen, M. N., Pollak, J., Sills, D. L., Casey, J. A., Rasmussen, S. G., Nachman, K. E., et al. (2018). Residential proximity to high-density poultry operations associated with campylobacteriosis and infectious diarrhea. *International Journal of Hygiene and Environmental Health*, 221(2), 323–333.
doi:<https://doi.org/10.1016/j.ijheh.2017.12.005>
- R-Core. (2017). `parallel` v3.6.0. Retrieved from <https://www.rdocumentation.org/packages/parallel/versions/3.6.0>
- RCT. (2015). R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, Austria. <https://www.R-project.org>
- Rue, H. (2019). The R-INLA project. Retrieved from <http://www.r-inla.org/home>
- Rue, H., Lindgren, F., Simpson, D., Martino, S., Teixeira Krainski, E., Bakka, H., et al. (2019). INLA: Full Bayesian Analysis of Latent Gaussian Models using Integrated Nested Laplace Approximations. *R package version*, 19(03).
- RVO. (2019). Gecombineerde opgave. Retrieved from <https://mijn.rvo.nl/gecombineerde-opgave>
- Schimmer, B., Luttikholt, S., Hautvast, J. L., Graat, E. A., Vellema, P., & van Duynhoven, Y. T. (2011). Seroprevalence and risk factors of Q fever in goats on commercial dairy goat farms in the Netherlands, 2009–2010. *BMC Veterinary Research*, 7(1), 81. doi:<https://doi.org/10.1186/1746-6148-7-81>
- Shafran-Nathan, R., Levy, I., Levin, N., & Broday, D. M. (2017). Ecological bias in environmental health studies: the problem of aggregation of multiple data sources. *Air Quality, Atmosphere & Health*, 10(4), 411–420. doi:<https://doi.org/10.1007/s11869-016-0436-x>
- Smit, L. A. M., & Heederik, D. (2017). Impacts of Intensive Livestock Production on Human Health in Densely Populated Regions. *GeoHealth*, 1(7), 272–277. doi:<https://doi.org/10.1002/2017GH000103>
- Strachan, N. J. C., Dunn, G. M., Locking, M. E., Reid, T. M. S., & Ogden, I. D. (2006). *Escherichia coli* O157: Burger bug or environmental pathogen? *International Journal of Food Microbiology*, 112(2), 129–137. doi:<https://doi.org/10.1016/j.ijfoodmicro.2006.06.021>
- Strachan, N. J. C., Fenlon, D. R., & Ogden, I. D. (2001). Modelling the vector pathway and infection of humans in an environmental outbreak of *Escherichia coli* O157. *FEMS Microbiology Letters*, 203(1), 69–73. doi:<https://doi.org/10.1111/j.1574-6968.2001.tb10822.x>
- van den Brandhof, W. E., Bartelds, A. I., Koopmans, M. P., & van Duynhoven, Y. T. (2006). General practitioner practices in requesting laboratory tests for patients with gastroenteritis in the Netherlands, 2001–2002. *BMC Family Practice*, 7(1), 56. doi:<https://doi.org/10.1186/1471-2296-7-56>
- Wickham, H. (2019). `stringr` v1.4.0. Retrieved from <https://www.rdocumentation.org/packages/stringr/versions/1.4.0>
- Wickham, H., Averick, M., Bryan, J., Chang, W., McGowan, L., François, R., et al. (2019). Welcome to the Tidyverse. *Journal of Open Source Software*, 4(43), 1686.
- Wickham, H., Bryan, J., Rstudio, Kalicinski, M., Valery, K., Leittenne, C., et al. (2019). Package ‘`readxl`’. Retrieved from <https://cran.r-project.org/web/packages/readxl/readxl.pdf>
- Wickham, H., François, R., Henry, L., & Müller, K. (2019). Package ‘`dplyr`’. Retrieved from <https://cran.r-project.org/web/packages/dplyr/dplyr.pdf>
- Wickham, H., Henry, L., & Rstudio. (2019). `tidyr`: Tidy Messy Data. Retrieved from <https://cran.r-project.org/web/packages/tidyr/index.html>
- Widgren, S., Engblom, S., Emanuelson, U., & Lindberg, A. (2018). Spatio-temporal modelling of verotoxigenic *Escherichia coli* O157 in cattle in Sweden: exploring options for control. *Veterinary research*, 49(1), 78. doi:<https://doi.org/10.1186/s13567-018-0574-2>