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Key Points:
- Results indicate that human exposure to small ruminants, and not cattle, is a spatially restricted risk factor for STEC O157 infections
- 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 modeling studies, especially in densely populated countries

Supporting Information:
- Supporting Information S1

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(continued)

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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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 into spatially restricted risk factors that play a role in this transmission, 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 km^2) 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 km^2 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 suggest exposure to small ruminants to be a risk factor, although no evidence on the mode of transmission is provided. Therefore, the underlying mechanisms warrant further investigation and could offer new targets for control. The newly proposed exposure metric has potential to improve existing spatial modeling studies on infectious diseases related to livestock exposure, especially in densely populated countries like the Netherlands.

Plain Language Summary Contaminated food is often the source of infections transmissible between animals and humans. This is typically caused by foodborne pathogens like Shiga toxin-producing *Escherichia coli* (STEC) O157. However, it is increasingly recognized that not all STEC O157 infections are foodborne, as they can also occur via environment-mediated transmission routes. To gain more insights in what can influence the risk for people to acquire a STEC O157 infection, 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 the Netherlands. This was done for the years 2007–2016, with a novel probability of exposure metric: the population-weighted number of animals per hexagon. To identify which livestock exposures increase STEC O157 infection risk and how large the contributions of these exposures are (population attributable fraction (PAF)), a spatial regression analysis was performed. Results suggest that exposure to small ruminants is a spatially restricted risk factor, but no evidence on the mode of transmission is provided. Therefore, the underlying mechanisms warrant further investigation and could offer new targets for control. The newly proposed exposure metric has potential to improve existing spatial modeling studies on infectious diseases related to livestock exposure.

1. Introduction

Food is generally considered to be the most important route of transmission for Shiga toxin-producing *Escherichia coli* (STEC) O157 (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;

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Strachan et al., 2006). A recent source attribution modeling 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; 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; 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; Strachan et al., 2001).

The Netherlands is one of the world's most densely populated countries, with over 500 inhabitants per km^2 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 be higher in areas with increased livestock density as well, such as in rural vs. urban areas as shown elsewhere (Berry et al., 2015; Strachan et al., 2006, 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/km^2) (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; Grolemund & 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 modeling, 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., 2000; Birch et al., 2007). 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 1a). To assess consistency of results and reduce the risk of ecological fallacy, we performed the analyses for hexagonal areas with four different sizes: 10 km^2 (approximately the average area of a four-digit postal code region in the Netherlands), 25 km^2, 50 km^2 and 90 km^2 (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/km^2 to derive the probability of exposure to be able to study the association between STEC O157 infections and livestock densities (Figure 1b) (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 1c, 1d and 1e).

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 1c). Those six-digit postal code points include information about the population numbers at that specific location (Figure 1c). Around these point locations, buffers with a radius of 1 km are constructed (Figure 1d). Farms located within these buffers, also outside the specified hexagon, are included (Figure 1e). The point locations of the farms contain information

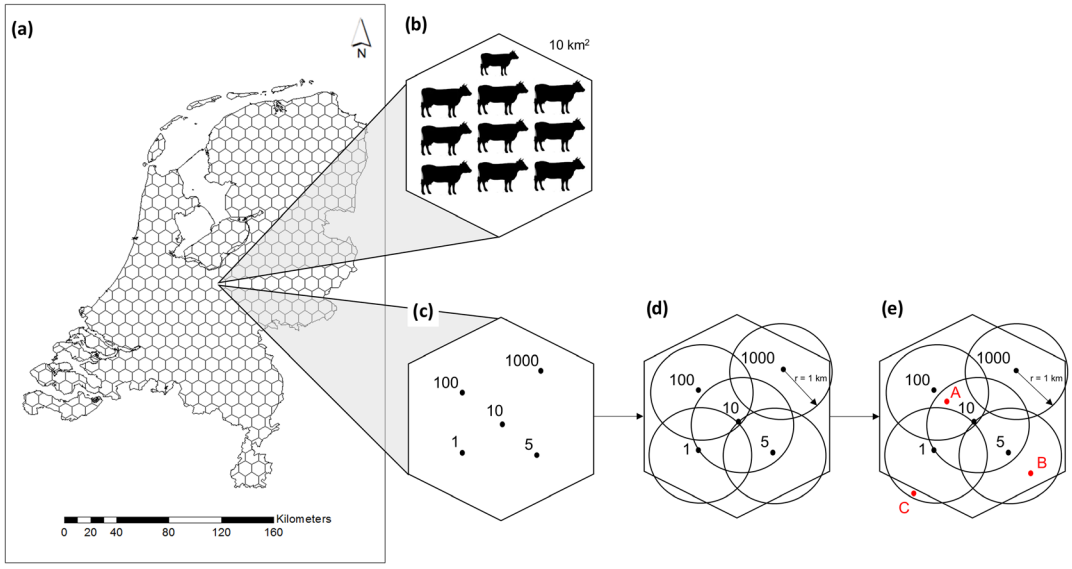

Figure 1. Explanation of the calculation of the old and the new probability of exposure measures. **a)** Hexagonal grid of the Netherlands. **b)** the old probability of exposure measure in a hexagonal grid cell: The number of animals per km^2. In this figure: 10 cows per 10 km^2, thus 1 cow/km^2. Pictures **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 1d. 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.

about the number of animals (Figure 1e). See Figures 1c, 1d and 1e 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:

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

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.

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). As the dependent variable in the model was the case count, i.e. the number of human STEC O157 cases (redistributed with the population weighted interpolation technique) within a hexagon, the assumption was that those case counts followed a Poisson distribution. Person-years were used as the offset of the model (the population denominator for each hexagon), and the confounders included were: age category (0–4, 5–9, 10–49 and ≥50 years old), gender (male or female), and period of infection (spring/summer: May–October, autumn/winter: November–April). The different population-weighted

Table 1
Total number of food-producing animals, total number of farms and the mean number of animals per farm per type of food-producing animal (goat, sheep, cattle, poultry, pigs) in the Netherlands

Type of animal	Total number of animals	Total number of farms	Mean number of animals per farm
	N	N	N
Goats	398,508	3,954	101
Sheep	1,049,517	13,962	75
Cattle	3,895,657	33,908	115
Poultry	96,802,429	2,889	33,507
Pigs	12,138,896	6,961	1,744

number of animals were included as covariates in the model (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. To be able to perform those analyses, it was assumed that residents acquired the infection at or in close proximity to their homes.

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 modeled by the intrinsic Conditional Autoregressive Model (CAR) (Besag et al., 1991). The second random-effect term represented the unstructured variation, which was used to correct for possible overdispersion of the data. This variation consisted of possible unobserved variation within hexagons, which was modeled 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., 2011, 2017).

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, traveled 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 of food-producing animals 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 food-producing 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 (Table 1). To derive the total number of small ruminants, the total number of goats and sheep per farm were summed together.

Table 2
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

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).

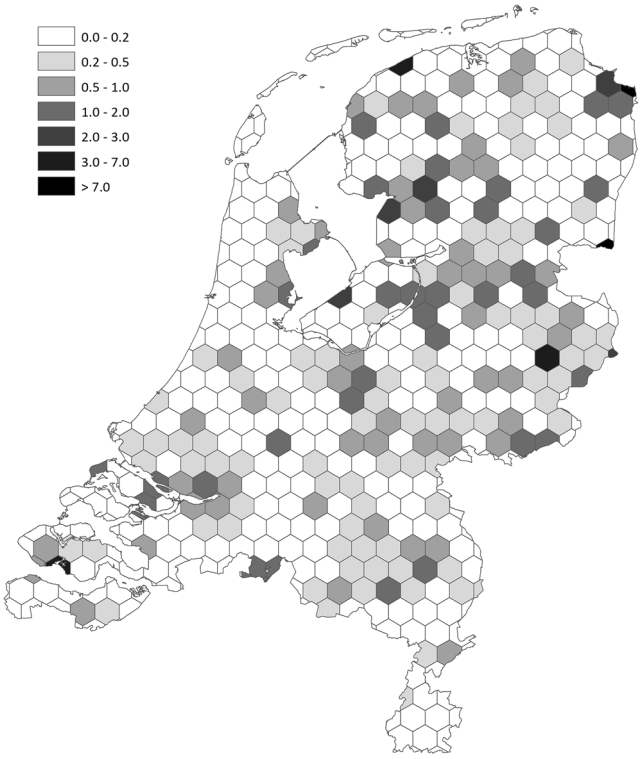

Figure 2. Cumulative incidence rate (x 100,000 person-years) (2007–2016) of STEC O157 infections in the Netherlands.

Of all the cases included, 62% (n = 272) were female, 38% (n = 167) were male (Table 2). 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 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 3a seemed to be most comparable with the one for human STEC O157 infections in Figure 2.

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

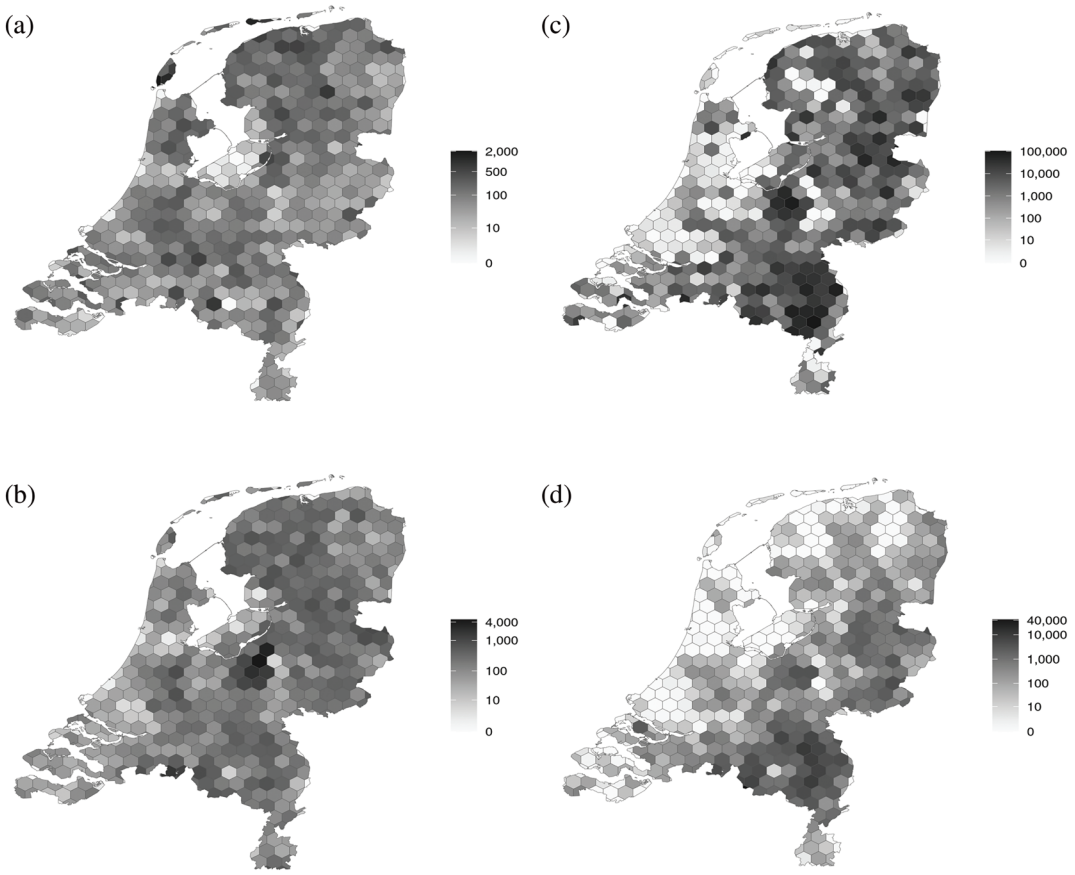

Figure 3. Maps of the population-weighted number of animals in the Netherlands per hexagon (90 km^2) for small ruminants (a), cattle (b), poultry (c) and pigs (d) in 2012.

Table 3 and Table 4, respectively. For the results of the univariable models, see supporting information Table S2.

Living in an hexagonal area of 90 km^2 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 3). Other hexagonal areas have comparable results, except the one of 10 km^2. Here, small ruminants

Table 3
Results of the Multivariable Spatial Analyses for Summer with Different Hexagonal Areas (90, 50, 25 and 10 km^2)

		Hexagon 90 km^2			Hexagon 50 km^2			Hexagon 25 km^2			Hexagon 10 km^2		
Period of infection	Variable	P-value	RR	95% CI	P-value	RR	95% CI	P-value	RR	95% CI	P-value	RR	95% CI
Summer	Gender												
	Males						*Reference category*						
	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)						*Reference category*						
	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

Table 4
Results of the Multivariable Spatial Analyses for Winter with Different Hexagonal Areas (90, 50, 25 and 10 km^2)

		Hexagon 90 km^2			Hexagon 50 km^2			Hexagon 25 km^2			Hexagon 10 km^2		
Period of infection	Variable	P-value	RR	95% CI	P-value	RR	95% CI	P-value	RR	95% CI	P-value	RR	95% CI
Winter	Gender												
	Males						*Reference category*						
	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						*Reference category*						
	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

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 km^2. 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 4). 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.

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 exposure to small ruminants, the number of sporadic STEC O157 infections 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 STEC O157, we were not able to quantify the relative importance of this spatially restricted risk factor within the broader context of all possible risk factors. Another limitation of only using one risk factor in the analyses is that the risk of ecological biases could not be quantified. 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).

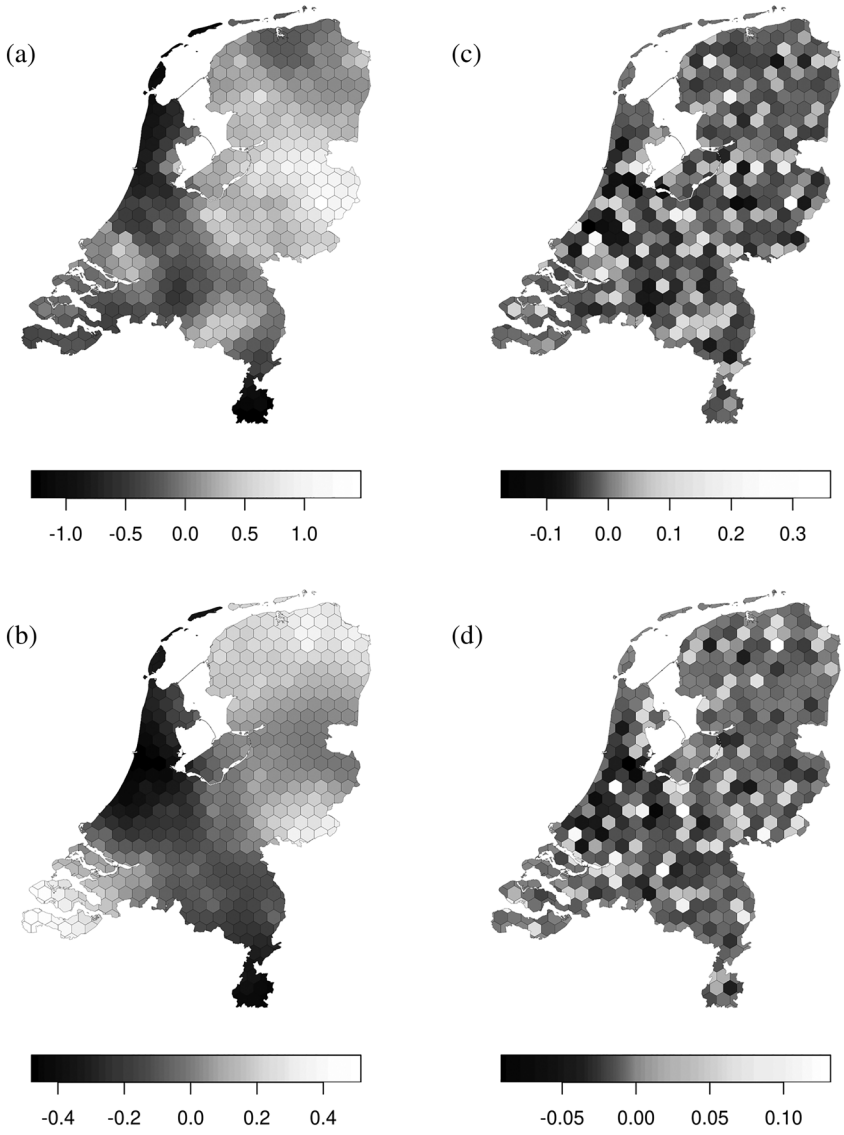

Figure 4. Maps of the spatially structured variation modeled by the conditional autoregressive model (CAR) in summer (a) and winter (b) and maps of the spatially unstructured variation modeled by independent and identically distributed (IID) Gaussian noise in summer (c) and winter (d) for hexagonal areas of 90 km^2.

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 et al., 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, among 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).

Despite the above reasoning, the results did not provide evidence for a particular mode of transmission (e.g. through food, the environment or direct contact with (small) ruminants through petting or feeding animals at 'children farms'). There were no data available on individually reported exposures of the cases. Furthermore, the focus of this study was on food-producing animal farms. However, it has been shown before that visiting a petting farm can be a potential source of STEC O157 infection (Heuvelink et al., 2002; Valkenburgh & Heuvelink, 2006). As these petting farms often host small ruminants as well, it is recommended to dive deeper into the combined effects of petting farms and food-producing animal farms on STEC O157 infection risk in future studies. This could be done, for instance, using a case–control study design with individually reported exposures, which includes risk factors related to direct contact with the animals (e.g. visiting 'children farms'), as well as spatial risk factors (e.g. distance to farms, number of animals in the neighborhood or a combination of those two) to investigate the effect of potentially spatially restricted risk factors. This way, it is also possible to include the consumption of particular food items in the analysis, as transmission through food is considered as the most important risk factor of acquiring a STEC O157 infection (Strachan et al., 2006).

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; 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; Hallisey 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) (Hallisey 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 km^2 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).

A buffer radius of 1 km was used in the analyses. However, the question remained whether different buffer sizes would influence our results. Therefore, the analyses were repeated for other buffer sizes. Buffers with a radius of 0.10 km, 0.25 km, 0.50 km, 0.75 km, 1.25 km, 1.50 km, 1.75 km and 2 km were used, but they did not show significant changes in the RRs and the 95% CIs were comparable to the results of the analyses with a buffer radius of 1 km. This means that the results of our analyses were not sensitive to the buffer radius size and that the analytical approach used was not suitable for assessing possible dose–response relationships.

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. Furthermore, the assumption was made that STEC O157 infections were acquired at or in close proximity to the home. However, people travel and it is possible that residents of urban areas went and acquired the infection in the countryside, or *vice versa*. This could lead to an underestimation of the spatial association between small ruminants and human STEC O157 cases and warrants further research in the future.

6. Conclusions

Results of this study indicate that living in proximity of small ruminants, is a spatially restricted risk factor for acquiring STEC O157 infection. As this study did not have individually reported exposures available, it could not provide evidence on the specific mode of transmission. Therefore, 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 modeling studies on infectious diseases related to livestock exposure, especially in densely populated regions.

Abbreviations

CAR	Conditional Autoregressive Model
CI	Confidence interval
EU	European Union
IID	Independent and identically distributed
INLA	Integrated nested Laplace approximation
MHS	Municipal Health Service
PAF	Population attributable fraction
PCR	Polymerase chain reaction
RIVM	Dutch National Institute for Public Health and the Environment
RR	Rate ratio
STEC O157	Shiga Toxin-producing *Escherichia coli*
Stx_1	Shiga Toxin 1
Stx_2	Shiga Toxin 2

Conflict of Interest

The authors declare no conflicts of interest relevant to this study.

Data Availability Statement

The used R scripts for data analyses can be found at: https://github.com/mulderac91/R-STECO157-spatialanalysis. Livestock data of food-producing animals for 2012 was obtained from the Department of Service Arrangements of the Dutch Ministry of Agriculture, Nature and Food Quality (CBS, 2019b; RVO, 2019). The population data per four-digit postal code region per year is available through Statistics Netherlands (www.statline.nl). The data were downloaded for the years 2007–2016 (CBS, 2019a).

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, 2,271 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).

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