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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)

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Summary

Shiga toxin-producing *Escherichia coli* (STEC) is a zoonotic pathogen of public health concern whose sources and transmission routes are difficult to trace. Using a combined source attribution and case–control analysis, we determined the relative contributions of four putative livestock sources (cattle, small ruminants, pigs, poultry) to human STEC infections and their associated dietary, animal contact, temporal and socio-econo-demographic risk factors in the Netherlands in 2010/2011–2014. Dutch source data were supplemented with those from other European countries with similar STEC epidemiology. Human STEC infections were attributed to sources using both the modified Dutch model (mDM) and the modified Hald model (mHM) supplied with the same O-serotyping data. Cattle accounted for 48.6% (mDM) and 53.1% (mHM) of the 1,183 human cases attributed, followed by small ruminants (mDM: 23.5%; mHM: 25.4%), pigs (mDM: 12.5%; mHM: 5.7%) and poultry (mDM: 2.7%; mHM: 3.1%), whereas the sources of the remaining 12.8% of cases could not be attributed. Of the top five O-serotypes infecting humans, O157, O26, O91 and O103 were mainly attributed to cattle (61%–75%) and O146 to small ruminants (71%–77%). Significant risk factors for human STEC infection as a whole were the consumption of beef, raw/undercooked meat or cured meat/cold cuts. For cattle-attributed STEC infections, specific risk factors were consuming raw meat spreads and beef. Consuming raw/undercooked or minced meat were risk factors for STEC infections attributed to small ruminants. For STEC infections attributed to pigs, only consuming raw/undercooked meat was significant. Consuming minced meat, raw/undercooked meat or cured meat/cold cuts were associated with poultry-attributed STEC infections. Consuming raw vegetables was protective for all STEC infections. We concluded that domestic ruminants account for approximately three-quarters of reported human STEC infections, whereas pigs and poultry play a minor role and that risk factors for human STEC infection vary according to the attributed source.

KEYWORDS

animal reservoirs, *E. coli*, risk factors, shiga toxin-producing *Escherichia coli*, source attribution, transmission routes

1 | INTRODUCTION

Shiga toxin-producing *Escherichia coli* (STEC) is a bacterial zoonotic agent whose infection in humans is associated with varying clinical manifestations, including diarrhoea, haemorrhagic colitis and (occasionally fatal) haemolytic uremic syndrome (HUS) (Karmali, Gannon, & Sargeant, 2010). Shiga toxin-producing *Escherichia coli* is a pathogen of public health concern given its recognized potential to cause large food- and waterborne outbreaks, as well as its association with HUS (Buchholz et al., 2011; Matsell & White, 2009; Nguyen et al., 2015), a leading cause of acute renal failure among children (Keir, 2015).

While most STEC strains associated with human illness belong to serogroup O157, there are more than a hundred of non-O157 serotypes (Gould et al., 2013), some of which have been associated with large outbreaks of severe illness, such as the German O104:H4 outbreak linked to contaminated fenugreek sprouts in 2011 (Buchholz et al., 2011), whereas others are associated with only mild or no illness at all (Coombes, Gilmour, & Goodman, 2011). Although this can be directly related to the virulence profile of the individual STEC strains, it also seems that there is no specific molecular pattern that would unambiguously enable hazard identification for any STEC strain (Franz et al., 2015). The occurrence of certain STEC strains among clinical cases may not only reflect differences in virulence, but also differences in the level of exposure to specific animal sources. However, the relative contributions of different animal reservoirs to the human disease burden for STEC, as well as their transmission routes to humans, have not yet been quantified.

In 2012, 5748 confirmed STEC cases were reported by 28 European Union (EU) and European Economic Area (EEA) countries, resulting in an overall notification rate of 1.5 cases per 100,000 population (European Centre for Disease Prevention and Control, 2014). After Germany and the United Kingdom, the Netherlands had the third highest number of STEC cases reported in the EU/EEA during that year, that is, 1049 cases corresponding to 6.3 cases per 100,000 population (European Centre for Disease Prevention and Control, 2014), although approximately only half of these cases had clinical relevance (i.e., had an acute onset of symptoms or required hospitalization).

Animals capable of maintaining STEC carriage in the absence of continuous exposure to STEC (reservoirs or amplifying hosts), as well as those that are frequently exposed to STEC, from the environment for instance, can serve as potential sources of human STEC infection (Persad & LeJeune, 2014). Although ruminants, and particularly cattle, are regarded as the main reservoir for STEC (Caprioli, Morabito, Brugere, & Oswald, 2005; Karmali et al., 2010), there is evidence for some (wild) birds, pigs, dogs and horses being significant spill-over hosts for STEC (i.e., animals that are susceptible to colonization by STEC but do not maintain such colonization in the absence of continuous exposure (Persad & LeJeune, 2014)). Implicitly, this means that there may be other epidemiologically relevant sources of human STEC infection beyond ruminants.

To identify the main sources of bacterial zoonotic infections and to assess the impact of public health interventions, source attribution using the microbial subtyping approach is often the method of choice (Pires et al., 2009). Given the source specificity of certain subtypes of the pathogen in question and assuming a unidirectional transmission pathway from sources to humans (with humans representing the endpoint), the relative contribution of each source to human cases can be inferred probabilistically by comparing the pathogen subtype distributions in humans and sources. While source attribution studies for pathogens like *Salmonella*, *Campylobacter* and *Listeria* based on the microbial subtyping approach have been performed in many countries worldwide (Barco, Barrucci, Olsen, & Ricci, 2013; Boysen et al., 2014; David et al., 2013; Guo et al., 2011; de Knegt, Pires, & Hald, 2015; Levesque et al., 2013; Little, Pires, Gillespie, Grant, & Nichols, 2010; Mossong et al., 2016; Mughini-Gras & van Pelt, 2014; Mughini-Gras et al., 2012; Mughini-Gras, Barrucci, et al. 2014; Mughini-Gras, Enserink, et al., 2014; Mughini-Gras, Smid, et al., 2014; Mullner, Jones, et al., 2009; Mullner, Spencer, et al., 2009; Pires & Hald, 2010; Pires et al., 2009; Sheppard et al., 2009; Strachan et al., 2009; Wahlstrom, Andersson, Plym-Forshell, & Pires, 2011; Wilson et al., 2008), no comparable study on STEC has been performed so far. Moreover, to understand how STEC strains originating from specific animal sources infect humans (i.e., to uncover the underlying transmission routes), combined (source-assigned) case–control and source attribution analyses have been performed for *Salmonella* (Mughini-Gras, Enserink, et al., 2014) and *Campylobacter* (Mossong et al., 2016; Mughini-Gras et al., 2012) to bridge the gap between attributing human cases at the start of the transmission chain (i.e., reservoir level) and at the point of exposure (i.e., risk factors) to identify source-specific risk factors for human infection.

Focussing on a country like the Netherlands, the aim of this study was to attribute human cases of STEC infection to four putative livestock sources (i.e., cattle, small ruminants, pigs and poultry) and to determine how the STEC strains attributed to each specific source may infect humans.

Impacts

- Shiga toxin-producing *Escherichia coli* (STEC) is a zoonotic pathogen of public health concern whose sources and transmission routes are difficult to trace. We thus performed a combined source attribution and case–control analysis allowing us to determine the fractions of human STEC infections attributable to four putative livestock sources (cattle, small ruminants, pigs, poultry) and their associated risk factors.
- While domestic ruminants accounted for approximately three-quarters of reported human STEC infections, pigs and poultry played a much smaller role.
- Risk factors for human STEC infection varied according to the attributed source, providing an approach for understanding the underlying transmission routes.

2 | MATERIALS AND METHODS

2.1 | Data collection

We used national surveillance data on human STEC infections that occurred in the general population of the Netherlands and were reported to the Dutch National Institute for Public Health and the Environment (RIVM) between January 2011 and December 2014. A detailed description of this surveillance system has been presented before (Friesema, Schotsborg, Heck, & Van Pelt, 2015). In total, 1183 O-serotyped STEC isolates from human cases (corresponding to 91 different O-serotypes) were obtained during this period. Concurrent (2011–2014) O-serotyped STEC isolates from four potential livestock sources of STEC, that is, cattle (n = 207), sheep and goat (i.e., small ruminants, n = 98), pigs (n = 109) and poultry (n = 30) were collected by the Netherlands' Food and Consumer Product Safety Authority (NVWA) as part of national surveillance activities (i.e., official sampling) of foodborne pathogens on slaughterhouse and retail (carcasses and meat samples). The collection of multiple isolates by herd or retailer cannot be excluded, for example if samples from the same source were sampled at different sites (slaughterhouse and retail) or yielded isolates with different serotypes. Both human and animal isolates were serotyped as described elsewhere (Guinee, Agterberg, & Jansen, 1972; Orskov, Orskov, Jann, & Jann, 1977; Paton & Paton, 1998).

To increase statistical power and serotype diversity, the Dutch livestock isolates were supplemented with others (440 from cattle, 239 from small ruminants, 28 from pigs and 61 from poultry) from 10 European countries by collating available data on reported O-serotyped STEC isolates per livestock source from the European Union Summary Reports on Trends and Sources of Zoonoses, Zoonotic Agents and Food-Borne Outbreaks, as published annually by the European Food Safety Authority (EFSA) from 2011 to 2014 (European Food Safety Authority and European Centre for Disease Prevention and Control, 2013, 2014, 2015a,b). A summary of the isolates used in this study is given in Table 1. The non-Dutch isolates originated from official sampling strategies at the levels of slaughterhouse and retail like the Dutch ones. Selection of the non-Dutch isolates was based on the method proposed by (Smid et al., 2013). This method is based on the assumption that if the subtype frequency distribution of the human isolates of a zoonotic pathogen in one country resembles that of the human isolates in another country, then also those of their respective animal sources can be used interchangeably for the purposes of source attribution (Smid et al., 2013). Using this method, we implicitly assumed that the epidemiology of STEC in the Netherlands, including the food consumption patterns and exposure pathways from livestock to humans, is similar to that in the countries from which the supplementary livestock data were taken and that the diversity of human O-serotype frequency distributions among the different countries mainly reflects differences in the predominant livestock sources. As suggested by Smid et al. (2013), the proportional similarity index (PSI), a straightforward measure of the area of overlap between two frequency distributions of microbial subtypes, was used to measure the similarity between the O-serotype frequency distributions of human STEC infections from the enhanced surveillance in 2012 among 21 European countries. The results of this surveillance are available in the most recent (at the time of analysis) annual epidemiological report of food-and-waterborne diseases and zoonoses published in 2014 by the European Centre for Disease Prevention and Control (2014). The PSI is expressed as:

$$\mathrm{PSI} = 1 - 0.5 \sum_k |P_k - Q_k|$$

where $|P_k - Q_k|$ is the absolute value of the difference in the relative frequency of the serotype k in group P compared to its frequency in group Q. Proportional similarity index values range from 0 to 1, with 0 indicating that the two distributions have no serotypes in common and 1 that they are equal. The countries listed in Table 1, whose livestock isolates were used in the source attribution analysis, were those with the O-serotype frequency distributions among human infections showing the highest PSI values (>0.50) as compared to the O-serotype frequency distribution among human infections in the Netherlands. Thus, they were the countries assumed to have the livestock sources of STEC most similar to those in the Netherlands.

2.2 | Source attribution analysis

In absence of a universally agreed source attribution approach for STEC, two commonly used frequency-matching models for source attribution of zoonotic pathogens were applied in a comparative fashion: the modified Dutch model (mDM) and the modified Hald model (mHM), with some minor adaptations as compared to the numerous applications of these two models described previously for *Salmonella* and *Campylobacter* (Mughini-Gras & van Pelt, 2014; Mughini-Gras, Barrucci, et al., 2014; Mughini-Gras, Enserink, et al., 2014; Mughini-Gras, Smid, et al., 2014; Mullner, Jones, et al., 2009; Mullner, Spencer, et al., 2009). Both models were based on O-serotyping data of the human (the Netherlands only) and livestock STEC isolates summarized in Table 1, and veterinary data were pooled over years and countries to avoid data sparsity and increase certainty in the estimates. Moreover, given the evidenced importance of direct/indirect exposure to livestock, especially cattle, in the occurrence of human STEC infections in the Netherlands (Friesema, Van De Kassteele, de Jager, Heuvelink, & van Pelt 2011), both models did not include food consumption weights to allow also non-foodborne pathways to be reflected in the attributions, as suggested elsewhere for *Campylobacter* (Mullner, Jones, et al., 2009; Mullner, Spencer, et al., 2009). Briefly, in the version of the mDM used here, the expected number of human STEC isolates with O-serotype i originating from source j, denoted as λ_{ij}, is estimated by:

$$\lambda_{ij} = \frac{p_{ij}}{\sum_j p_{ij}} \times e_i$$

where p_{ij} is the prevalence of O-serotype i in source j and e_i is the observed frequency of human isolates of O-serotype i. p_{ij} is given by $\pi_j \times r_{ij}$, where π_j is the overall prevalence of STEC in source j and r_{ij} is the relative frequency of O-serotype i in source j. Details of each parameter estimation are reported in Table 2. The mDM was implemented

TABLE 1 Breakdown of the number of O-serotyped Shiga toxin-producing *Escherichia coli* (STEC) isolates used in the source attribution analysis, including the proportional similarity index (PSI) of the O-serotype frequency distribution of human STEC infections in the Netherlands with that of the human STEC infections in each of the other countries

	Netherlands	Germany	Sweden	Slovenia	France	Belgium	Denmark	Austria	Norway	Ireland	Hungary	Total
2011	270	0	6	1	1	17	1	97	0	75	12	480
Human	255	0	0	0	0	0	0	0	0	0	0	255
Cattle	7	0	2	1	1	11	1	50	0	40	9	122
Pig	3	0	0	0	0	0	0	0	0	5	3	11
Poultry	0	0	0	0	0	0	0	0	0	25	0	25
Sheep & goat	5	0	4	0	0	6	0	47	0	5	0	67
2012	357	39	12	2	1	17	1	96	0	31	6	562
Human	283	0	0	0	0	0	0	0	0	0	0	283
Cattle	32	32	11	2	1	11	1	44	0	13	5	152
Pig	14	4	0	0	0	0	0	0	0	2	0	20
Poultry	4	0	0	0	0	0	0	0	0	16	0	20
Sheep & goat	24	3	1	0	0	6	0	52	0	0	1	87
2013	495	10	6	2	4	13	1	89	1	77	6	704
Human	364	0	0	0	0	0	0	0	0	0	0	364
Cattle	79	8	6	2	4	7	1	43	0	50	5	205
Pig	38	0	0	0	0	0	0	0	0	6	0	44
Poultry	1	0	0	0	0	0	0	0	0	17	0	18
Sheep & goat	13	2	0	0	0	6	0	46	1	4	1	73
2014	505	18	4	0	0	17	1	79	0	22	3	649
Human	281	0	0	0	0	0	0	0	0	0	0	281
Cattle	89	8	4	0	0	15	1	33	0	15	3	168
Pig	54	6	0	0	0	0	0	0	0	2	0	62
Poultry	25	0	0	0	0	0	0	0	0	3	0	28
Sheep & goat	56	4	0	0	0	2	0	46	0	2	0	110
Total	1627	67	28	5	6	64	4	361	1	205	27	2395
PSI*		0.85	0.74	0.73	0.68	0.68	0.68	0.67	0.55	0.53	0.52	

*Proportional similarity index for the O-serotype frequency distribution of human STEC infections in the Netherlands with that of the human STEC infections in each of the other countries (European Centre for Disease Prevention and Control 2014).

TABLE 2 Details of the parameters of the modified Dutch and Hald models for source attribution used in this study

Parameter	Description/Estimation	Source
p_{ij}	Prevalence of O-serotype *i* from source *j*, given by $\pi_j \times r_{ij}$	See below
π_j	Overall prevalence of Shiga toxin-producing *Escherichia coli* (STEC) in source *j*, given by *Beta* ($\alpha_j + 1$, $\beta_j + 1$)	See below
r_{ij}	Relative frequency of O-serotype *i* in source *j*, given by $r_{1j}, r_{2j}, \ldots, 1 - \sum_{i=1}^{I-1} r_{ij} \sim \text{Dirichlet}(X_{1j}, X_{2j}, \ldots, X_{Ij})$ with X_{ij} (i= 1, 2,..., I) being the STEC isolates of O-serotypes *i* from source *j*	Data
α_j	STEC-positive individual sampling units (faeces or food samples) from source *j* in the Netherlands (cattle 346; sheep and goat 102; pig 31) or in the countries listed in Table 1 (poultry 2) during 2011-2014	(European Food Safety Authority and European Centre for Disease Prevention and Control, 2013, 2014, 2015a,b)
β_j	Total number of individual sampling units (faeces or food samples) from source *j* that have been tested for STEC minus α_j in the Netherlands (cattle 6374; sheep and goat 3322; pig 1185) or in the countries listed in Table 1 (poultry 812) during 2011-2014	(European Food Safety Authority and European Centre for Disease Prevention and Control, 2013, 2014, 2015a,b)
e_i	Frequency of human STEC isolates with O-serotype *i*	Data
q_i	Serotype-specific factor, given by log (q_i) ~ *Normal* (0, τ), where τ is given by a fairly diffuse Gamma (0.01, 0.01) distribution, or set to 1 for serotypes unique to a source	(David et al., 2013; Mughini-Gras, Smid, et al., 2014; Mullner, Jones, et al., 2009)
a_j	Source-specific factor, given by an *Exponential* (0.002) distribution	(Mughini-Gras, Smid, et al., 2014; Mullner, Jones, et al., 2009)

in @RISK (Palisade Corp., USA) by setting 10,000 iterations with the Latin hypercube sampling technique and a seed of 1.

The mHM assumed that the expected number of human isolates with O-serotype *i*, denoted as λ_i, is given by:

$$\lambda_i \sim \text{Poisson}\left(\sum_j \lambda_{ij}\right)$$

$$\lambda_{ij} = p_{ij} \times q_i \times a_j$$

where p_{ij} is again the prevalence of O-serotype *i* in source *j* estimated as in the mDM; q_i is the serotype-dependent factor, which putatively accounts for differences in the success of O-serotype *i* to infect humans (e.g., survivability, virulence and pathogenicity); and a_j is the source-dependent factor, which putatively accounts for the ability of sources *j* to act as a vehicle for STEC (e.g., differences in pathogen load, magnitude of exposure, source characteristics influencing pathogen growth, preparation/handling procedures, differences in sensitivity of surveillance programs and randomness of sampling schemes). Further details of the mHM parameters are reported in Table 2. Posterior distributions were obtained by a Markov chain Monte Carlo (MCMC) simulation implemented in WinBUGS 1.4. Five independent Markov chains were run for 50,000 iterations after a burn-in period of 10,000 iterations, which was able to provide convergence as monitored by Kernel density plots and by the method of Gelman and Rubin (1992). From both the mDM and mHM, we extracted the relative posterior probabilities (*Pr*) for each O-serotype to originate from each of the four animal sources.

Of the 1183 human isolates, 151 (12.8%) were discarded because their O-serotypes had no one-to-one matching with any of the isolates from the considered four livestock sources, and therefore, they could not be attributed using the mDM or mHM. These human isolates were then assigned *a priori* to an "unknown" source. Therefore, the final data set comprised 1032 human isolates to be attributed. These isolates comprised 59 different O-serotypes plus a non-typeable (NT) group including those isolates with an undetermined O-serotype. All the 59 O-serotypes included in the analysis were therefore found in humans and in at least one of the considered four livestock sources. Another 29 O-serotypes were found only in the sources, but not in humans (the so-called non-pathogenic types). As suggested by Mullner, Jones, et al. (2009), these types were kept in the models to preserve the whole within-source relative frequency of O-serotypes.

2.3 | Assessment of potential bias

As the livestock isolates were not all from the same country and years as the human isolates and were supplemented with isolates from other European countries within a 4-year time frame, we assessed

potential temporal and geographical biases by quantifying the degree of similarity (as revealed by PSI) of the O-serotype frequency distributions of the Dutch *vs*. non-Dutch isolates, as well as those from the contemporaneous (i.e., same year) *vs*. non-contemporaneous isolates as performed previously (Mughini-Gras, Smid, et al., 2014; Smid et al., 2013). We then calculated PSI values and 95% bootstrap confidence intervals (1000 iterations) between Dutch and non-Dutch source isolates overall and per livestock group, as well as PSI values between Dutch human isolates and isolates from each of the sources of either Dutch or non-Dutch origin. Proportional similarity index values were also calculated between humans and each livestock source over the 4 years, from 2011 to 2014 (i.e., 16 comparisons per human-source-year combination). A linear trend was then fitted over the obtained PSI values to test whether increasing time interval between the human and livestock data sets corresponded to decreasing similarity in their O-serotype distributions.

2.4 | Source-assigned case–control study

We investigated risk factors for human STEC infections caused by O-serotypes attributable to cattle, small ruminants, pigs and poultry. We did so by performing a so-called source-assigned case–control study using the same methodology as previously applied to *Salmonella* (Mughini-Gras, Enserink, et al., 2014). In brief, eight putative risk factors investigated in a previous (2008–2012) Dutch case–control study for human STEC infection in general (Friesema, Schotsborg, et al., 2015) were retested for association with the occurrence of human STEC infections of probable cattle, small ruminant, pig or poultry origin using *Pr*-weighted logistic regression models. A separate model for each source was built, with cases being weighted in each model based on the *Pr*s from each specific source, and two analyses were performed using the *Pr*s from the mDM and mHM, respectively. An equal probability weight of one was assigned to the controls, while the cases were weighted according to the *Pr* values per O-serotype to originate from each of the four livestock sources under study. Consequently, factors associated with STEC infection in the *Pr*-weighted models could be interpreted as source-specific risk factors (Mughini-Gras, Enserink, et al., 2014).

The source-assigned case–control study included 342 STEC cases and 2260 controls, both enrolled in the Netherlands during 2010–2014. Part of these cases and controls was already included in the previous Dutch case–control study for human STEC infection as a whole conducted in 2008–2012 (Friesema, Schotsborg, et al., 2015). The cases also represented a subset of the 1183 O-serotyped STEC cases included in the source attribution analysis (see "Data collection" section) and for which epidemiological information about exposure to potential risk factors was available through the voluntary completion of a standard questionnaire. Questions contained in the questionnaire referred to the week prior to the onset of symptoms. A case was then defined as an O-serotyped STEC infection during the period 2010–2014 in a person who was interviewed within 1 month after the onset of symptoms and who had not travelled abroad in the week before the onset of illness. Patients were excluded if they were known to be secondary cases of a household cluster or if they were part of an outbreak (Friesema, Schotsborg, et al., 2015). The controls were enrolled through a periodic survey of the Dutch population as described in Friesema, van Gageldonk-Lafeber, and van Pelt (2015). Briefly, in 2008, the RIVM started a periodic survey in the Dutch general population based on the thrice-yearly administration of an epidemiological questionnaire to a random, dynamic sample of the open population to obtain data from people to be used as control group for identifying risk factors for several notifiable gastrointestinal infections, and this has already been carried out for STEC (Friesema, Schotsborg, et al., 2015) and *Listeria* (Friesema, Kuiling, et al., 2015). Controls for the current study were selected if they participated to the survey between 2010 and 2014 and if they did not travel abroad in the week before completing the questionnaire.

The questionnaire for the controls asked similar questions as that for the cases. Thus, potential risk factors were selected from the variables available from both cases and controls based on their biological plausibility and previous investigations (Friesema, Schotsborg, et al., 2015). Besides general demographics like age (≤5, 6–15, 16–45, 46–65, ≥66 years) and gender, urbanization degree of the residence postcode (urban: >2,500 addresses/km^2; intermediate: 500–2,500 addresses/km^2; rural <500 addresses/km^2), socio-economic status (SES, expressed as a normalized score ranging from −4 to +4 based on income, employment and educational level per postcode area, with a higher score indicating lower SES, as provided by Netherlands Statistics), season (winter: December–February; spring: March–May; summer: June–August; autumn: September–November) and year (2010 to 2014), we assessed the association between being an STEC or an STEC-source-assigned case and the following eight variables: (i) consumption of raw and/or undercooked meat; (ii) consumption of beef; (iii) consumption of minced meat; (iv) consumption of cured meat/cold cuts; (v) consumption of raw meat spreads (including also typical Dutch products like "filet americain," a variation of the more popular steak tartare made with raw beef and various seasonings, and "ossenworst" a spreadable raw beef sausage); (vi) consumption of cheese made with raw milk; (vii) consumption of raw vegetables (including also sprouts); and (vii) contact with farm animals. Missing values in these variables were handled using multiple imputation as described in Doorduyn, Van Den Brandhof, Van Duynhoven, Wannet, and Van Pelt (2006).

Logistic regression models were fitted using a backward stepwise variable selection approach. Variables were dropped one by one if they showed a *p*-value ≥.05 and their exclusion from the models did not influence the association of the other covariates; a change of >10% in the regression coefficients of the covariates was seen as a sign of confounding, so the variable in question was retained in the model regardless of its significance. Collinearities between independent variables were explored prior to regression analysis by examining their covariance matrix and selection between collinear variables was based on model fit as revealed by the Akaike information criterion (AIC). Associations were then expressed as adjusted odds ratios (ORs) and corresponding 95% confidence intervals (95% CIs). All final regression models showed an overall statistical significance (likelihood ratio chi-square test, $p < .05$),

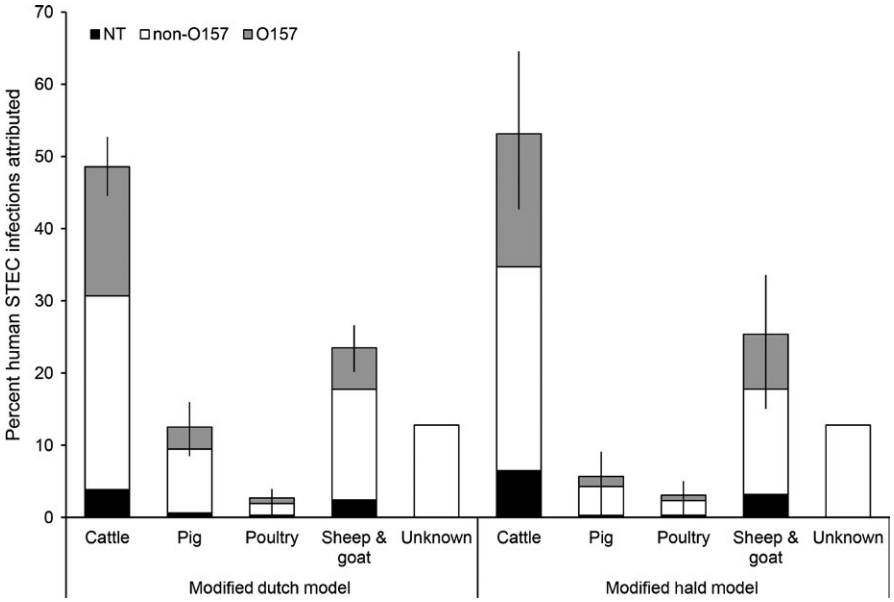

FIGURE 1 Attributions of the human Shiga toxin-producing *Escherichia coli* infections (*n*=1183) in the Netherlands in 2011-2014 to livestock sources, with indication of O157 and non-O157 serotypes, estimated by the modified Dutch and Hald models. Error bars represent 95% confidence intervals. NT = non-typeable isolate

goodness of fit (Hosmer–Lemeshow test, $p > .05$) and negligible impact of influential observations as assessed by Pearson residual, deviance residual and Pregibon leverage statistics. Age, gender, urbanization degree, SES, season and year were always included in the regression models to control for potential confounding effects. Plausible two-way interactions between the aforementioned eight putative risk factors and the variables age, gender, urbanization degree, SES and season were also tested. For comparison purposes, in addition to the *Pr*-weighted models for each source, an overall unweighted model was also developed to identify risk factors for human STEC infection as a whole. Regression models were also fitted with a random intercept at the postal code and/or municipality levels to account for potential clustering of cases, but this did not improve the fit of the models; thus, only the results from the ordinary logistic regression models were reported. Statistical analyses were performed using STATA 14 (StataCorp, USA).

3 | RESULTS

3.1 | Attribution estimates

The attribution estimates from the mDM and from the mHM were very similar (Figure 1). Both models identified cattle as the primary source of human STEC infections, accounting for 48.6% (95% CI: 44.4%–52.6%) of the 1,183 human isolates in the mDM and for 53.1% (95% CI: 41.7%–63.6%) of these same isolates in the mHM. Small ruminants were estimated as the second most important source: the mDM and the mHM attributed 23.5% (95% CI: 20.4%–26.8%) and 25.4% (95% CI: 17.2%–35.7%) of the human isolates to small ruminants, respectively. Pigs were estimated to account for 12.5% (95% CI: 9.0%–16.5%) of human infections by the mDM and for 5.7% (2.2%–11.1%) of them by the mHM. The least important source was estimated to be poultry by both the mDM (2.7%, 95% CI: 1.4%–5.1%) and the mHM (3.1%, 95% CI: 1.1%–6.9%). The source of the remaining 12.8% human isolates was unknown.

The specific attributions of the most frequent O-serotypes found among the 1032 human infections attributed to livestock sources by the two models are reported in Table 3. Serotype O157 was strongly associated with cattle: 64.8% and 68.8% of the 321 human O157 isolates were attributed to cattle by the mDM and mHM, respectively. Also non-O157 serotypes were mainly associated with cattle, as 51.7% (mDM) and 56.1% (mHM) of human non-O157 isolates were attributed to cattle, but the contribution of small ruminants (30.1% and 32.0% in the mDM and mHM, respectively) was also substantial. Of the five most frequent non-O157 serotypes, O26, O91, O103 and O113 were predominantly associated with cattle, whereas O146 was mainly associated with small ruminants (Table 3).

3.2 | Assessment of potential bias

Table 4 shows the PSI values between the O-serotype frequency distributions of the Dutch and non-Dutch isolates from humans and livestock sources. Overall, Dutch human isolates appeared to be more similar to the non-Dutch (PSI 71.2%, 95% CI 63.1%–79.3%) than to the Dutch (PSI 39.8%, 95% CI 18.9%–60.7%) source isolates. The PSI between Dutch and non-Dutch source isolates was 49.5% (95% CI 28.9%–70.2%), so the human cases were attributed based on a much comparable pool of non-local source isolates, indicating that pooling of data over countries was unlikely to introduce a significant bias. Of note, with the exception of cattle isolates, the PSI values between human isolates and the isolates of each source were similar regardless of whether these isolates were of Dutch or non-Dutch origin (Table 4).

In Figure 2, the PSI values for the inter-annual comparisons of O-serotype distributions between humans and each of the livestock sources are plotted. A linear function was fitted over these PSI values. No source had O-serotype distributions that appeared to be significantly less similar to the human ones as the time difference between the years of collection increased (cattle: slope −0.008, $p = .483$; small ruminants: slope 0.011, $p = .516$; pig: slope 0.009, $p = .605$; poultry:

TABLE 3 Attributions (%) with 95% confidence intervals of the most frequent O-serotypes among human infections attributed to livestock sources by the modified Dutch model (mDM) and by modified Hald model (mHM)

Serotype	Human cases attributed (*n*)	Cattle		Sheep & goat		Pig		Poultry	
		mDM	mHM	mDM	mHM	mDM	mHM	mDM	mHM
O157	321	64.8 (57.2–72.2)	68.8 (44.8–88.9)	19.5 (19.5–25.6)	21.4 (7.2–43.0)	13.1 (7.5–20.1)	5.0 (0.9–14.3)	2.6 (0.3–7.2)	4.7 (0.3–15.9)
Non-O157	604	51.7 (46.9–56.4)	56.1 (47.3–73.6)	30.1 (26.4–34.1)	32.0 (18.5–41.9)	14.4 (10.4–19.4)	7.1 (2.1–12.2)	3.7 (2.6–6.0)	4.8 (1.2–9.7)
O26	105	68.8 (56.9–79.6)	74.3 (47.4–99.3)	12.1 (6.1–20.0)	13.5 (2.6–34.0)	15.8 (7.5–20.1)	6.2 (0.8–19.0)	3.3 (0.3–7.2)	6.0 (0.3–21.2)
O91	87	61.0 (44.1–76.7)	67.3 (33.2–97.8)	22.3 (10.9–37.0)	24.8 (4.8–58.1)	15.7 (4.5–32.2)	6.3 (0.5–21.7)	1.0 (0.0–3.9)	1.6 (0.0–7.1)
O103	52	66.7 (46.4–83.8)	74.8 (38.4–100)	17.2 (5.9–33.0)	19.0 (2.4–51.8)	16.1 (3.7–35.6)	6.2 (0.4–23.2)	0.0 (0.0–0.0)	0.0 (0.0–0.0)
O146	50	15.7 (6.0–28.9)	16.9 (2.4–46.0)	71.2 (53.7–85.4)	76.8 (43.2–100)	12.1 (2.6–27.9)	4.6 (0.3–17.5)	1.0 (0.0–3.9)	1.7 (0.0–7.4)
O113	36	58.0 (45.4–70.0)	62.1 (30.0–98.7)	28.3 (18.1–40.0)	31.4 (9.0–64.8)	12.9 (4.9–24.3)	5.1 (0.5–17.1)	0.8 (0.1–2.8)	1.4 (0.0–5.8)
Others	274	45.6 (41.7–49.5)	48.9 (15.1–66.3)	34.4 (31.1–37.7)	35.5 (11.6–80.3)	13.9 (11.0–17.1)	8.5 (1.3–26.2)	6.1 (5.3–7.8)	7.1 (1.0–11.4)
Non-typeable	89	50.3 (39.2–61.1)	56.5 (27.4–86.2)	31.1 (21.8–41.7)	35.2 (12.2–65.8)	18.0 (9.3–29.1)	7.3 (1.1–21.9)	0.6 (0.0–2.0)	1.0 (0.0–4.1)

slope −0.007, $p = .610$). Proportional similarity index values for inter-annual comparisons of O-serotype distributions within sources and within humans were also computed, but none showed significant trends, thereby justifying pooling of data over the years.

3.3 | Source-specific risk factors

Significant risk factors for human STEC infection as a whole were the consumption of beef, raw/undercooked meat, and cured meat/cold cuts (Tables 5 and 6), whereas consuming raw vegetables was associated with decreased STEC risk. Other significant risk factors for STEC infection in general were male gender, low SES, living in rural *vs*. urban areas, and summer and autumn seasons *vs*. the winter, as well as summer, autumn and winter seasons *vs*. the spring. The odds of being a case were significantly greater in 2011, 2013 and 2014 as compared to 2010, and by including year as a continuous variable, there was a significant ($p < .001$) linear trend towards increased STEC risk over the study years (OR 1.21, 95% CI 1.09–1.34). Moreover, compared to children below 5 years of age, any of the other age groups above 15 years had a decreased risk of STEC infection (Tables 5 and 6).

Looking at the *Pr*-weighted models revealing the specific factors associated with infection with O-serotypes originating from each source, both those based on the *Prs* from the mDM and those from the mHM gave essentially the same results (Tables 5 and 6). For the cattle-associated STEC infections, significant risk factors were the consumption of raw meat spreads and the consumption of beef. Consuming raw/undercooked meat or consuming minced meat were significant risk factors for infection with O-serotypes attributable to small ruminants. For STEC infections of probable pig origin, only the consumption of raw/undercooked meat was a significant risk factor. Consuming minced meat, raw/undercooked meat, or cured meat/cold cuts, and living in an intermediate *vs*. urban area, were associated with increased risk of acquiring a STEC infection of probable poultry origin. Conversely, consuming raw vegetables was a protective factor for infection with O-serotypes attributable to any source (Tables 5 and 6). In the *Pr*-weighted models, only a few deviations from the overall (unweighted) model were observed as regard to age and season, with small ruminant- and poultry-associated infections being significantly less likely to occur from 45 years of age onwards and no significant increase observed in the risk of poultry-associated STEC infections during summer or autumn as compared to winter, at least when using the *Prs* from the mDM. Male gender remained a significant risk factor only for pig-associated STEC infections, low SES for cattle- and pig-associated infections, and living in rural areas for cattle- and poultry-associated infections. The increasing inter-annual trend in STEC infection risk could be observed for all sources (Tables 5 and 6). In all models, no significant interactions were found.

4 | DISCUSSION

The aim of this study was to quantify the relative contributions of cattle, small ruminants, pigs and poultry to the human disease burden of

TABLE 4 Proportional similarity index values (%) and corresponding 95% bootstrap confidence intervals between the O-serotype frequency distributions of the Dutch and non-Dutch isolates from humans and from each livestock source

		Non–Dutch livestock isolates			
Dutch isolates	**Human**	**Cattle**	**Small ruminants**	**Pigs**	**Poultry**
Human	–	65.5 (55.2–75.8)	48.9 (30.5–67.1)	31.5 (3.0–60.0)	39.6 (8.5–70.9)
Cattle	32.8 (7.0–58.5)	39.3 (8.5–70.1)	–	–	–
Small ruminants	45.6 (61.6–29.7)	–	63.5 (49.7–76.8)	–	–
Pigs	30.1 (7.0–53.0)	–	–	14.6 (0.0–59.5)	–
Poultry	38.6 (14.2–63.2)	–	–	–	16.7 (0.0–65.5)

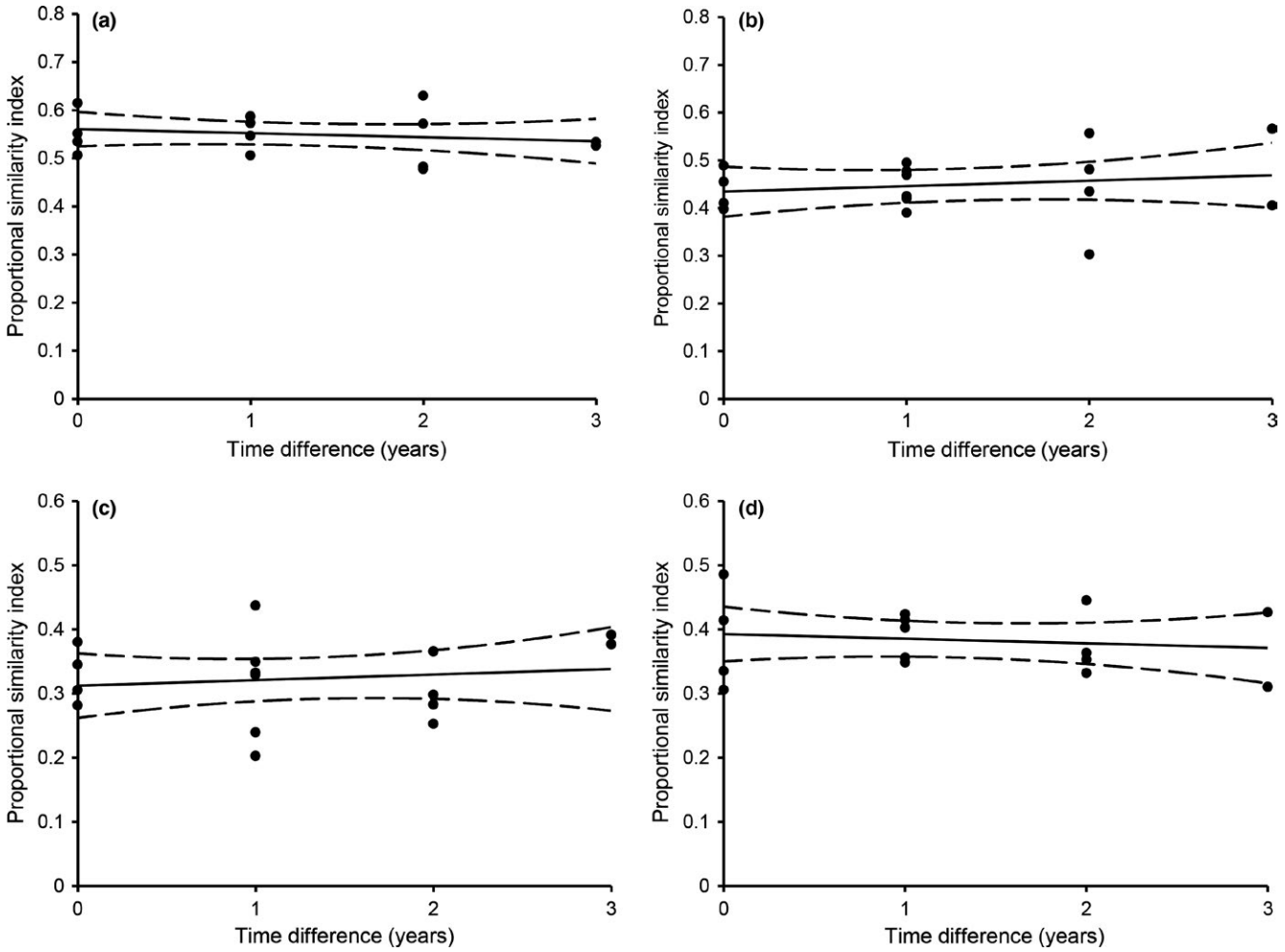

FIGURE 2 Similarity of O-serotype frequency distributions between human and cattle (a), small ruminant (b), pig (c) and poultry (d) isolates collected in different years (2011 to 2014)

STEC infection and to determine risk factors for infection with STEC strains originating from these livestock sources in the Netherlands. Previous studies with this same aim focussed on *Campylobacter* (Mossong et al., 2016; Mughini-Gras et al., 2012) and *Salmonella* (Mughini-Gras, Enserink, et al., 2014). We then used quantitative risk modelling to attribute stochastically human STEC infections to sources based on O-serotyping data. Subsequently, a source-assigned case–control study allowed for the identification of source-specific risk factors, which also provided an indication of the underlying transmission pathways.

Cattle was estimated to be the most important source of human STEC infections in the Netherlands, accounting for 49%–53% of human cases, followed by small ruminants, pigs and poultry. This is in line with the recognized role of cattle as the main reservoir for STEC strains that are highly virulent to humans, such as those within the serogroup O157 (Caprioli et al., 2005), which was indeed strongly

TABLE 5 Adjusted odds ratios and 95% confidence intervals of the factors associated with human Shiga toxin-producing *Escherichia coli* infections attributable to each of the four animal reservoirs (according to the *Prs* from the modified Dutch model) and overall as given by the final multivariable logistic regression model

	Overall (unweighted)	Cattle	Small ruminants	Pigs	Poultry
Age (years)					
≤5	Reference	Reference	Reference	Reference	Reference
6–15	0.93 (0.56–1.55)	1.00 (0.58–1.71)	1.14 (0.62–2.10)	0.96 (0.53–1.74)	0.64 (0.28–1.44)
16–45	0.56 (0.36–0.87)	0.56 (0.35–0.89)	0.85 (0.49–1.47)	0.57 (0.34–0.95)	0.58 (0.30–1.10)
46–65	0.12 (0.08–0.20)	0.10 (0.06–0.18)	0.24 (0.12–0.48)	0.12 (0.07–0.22)	0.16 (0.06–0.42)
≥66	0.20 (0.12–0.31)	0.18 (0.11–0.29)	0.31 (0.17–0.55)	0.24 (0.14–0.43)	0.25 (0.08–0.81)
Gender (♂ *vs.* ♀)	1.31 (1.01–1.70)	1.28 (0.97–1.69)	1.28 (0.92–1.77)	1.39 (1.02–1.88)	1.59 (0.76–3.35)
Year					
2010	Reference	Reference	Reference	Reference	Reference
2011	1.86 (1.19–2.90)	2.04 (1.23–3.38)	2.27 (1.26–4.11)	2.11 (1.19–3.72)	1.42 (0.75–2.69)
2012	1.37 (0.87–2.16)	1.45 (0.87–2.42)	1.22 (0.68–2.18)	1.48 (0.87–2.53)	2.48 (0.68–9.03)
2013	2.19 (1.41–3.42)	2.29 (1.36–3.86)	3.65 (1.88–7.08)	2.86 (1.56–5.23)	9.94 (0.87–114.03)
2014	2.46 (1.57–3.86)	2.96 (1.75–5.01)	3.63 (1.85–7.10)	3.39 (1.81–6.32)	9.23 (1.15–74.00)
Season					
Winter	Reference*	Reference	Reference	Reference	Reference
Spring	0.55 (0.35–0.89)	0.65 (0.39–1.08)	0.70 (0.40–1.23)	0.77 (0.44–1.35)	0.27 (0.06–1.20)
Summer	5.71 (3.77–8.64)	7.20 (4.48–11.55)	8.54 (4.72–15.46)	8.28 (4.77–14.37)	13.82 (0.66–291.36)
Autumn	2.15 (1.46–3.16)	2.57 (1.66–3.99)	2.41 (1.44–4.04)	2.26 (1.40–3.66)	1.02 (0.29–3.58)
SES	1.17 (1.02–1.34)	1.19 (1.02–1.40)	1.10 (0.92–1.31)	1.25 (1.03–1.50)	1.29 (0.86–1.95)
Urbanization					
Urban	Reference	Reference	Reference	Reference	Reference
Intermediate	1.37 (0.92–2.04)	1.34 (0.89–2.02)	0.93 (0.58–1.49)	1.30 (0.83–2.04)	2.71 (1.29–5.68)
Rural	1.62 (1.04–2.54)	1.61 (1.01–2.57)	1.03 (0.60–1.75)	1.41 (0.86–2.33)	3.75 (1.27–11.04)
Raw meat spreads	n.s.	1.67 (1.24–2.25)	n.s.	n.s.	n.s.
Beef	1.39 (1.15–1.92)	1.60 (1.15–2.23)	n.s.	n.s.	n.s.
Raw vegetables	0.27 (0.19–0.40)	0.27 (0.18–0.42)	0.22 (0.14–0.36)	0.32 (0.20–0.52)	0.43 (0.19–0.96)
Minced meat	n.s.	n.s.	1.57 (1.03–2.40)	n.s.	1.90 (1.08–3.34)
Undercooked meat	1.67 (1.24–2.24)	n.s.	1.81 (1.26–2.58)	2.03 (1.49–2.78)	2.12 (1.06–4.26)
Cured meat	1.35 (1.03–1.77)	n.s.	n.s.	n.s.	2.84 (1.46–5.53)

Dietary factors refer to the week prior to the onset of symptoms (cases) or questionnaire completion (controls). n.s., not significant; SES, socio-economic status, continuous variable expressed as a normalized score ranging from −4 to +4 based on income, employment and educational level per postcode area, with a higher score indicating lower SES.

*When spring is set as reference: winter 1.81 (1.13–2.89), autumn 3.88 (2.56–5.8), summer 10.30 (6.63–15.99).

associated with cattle in this study (65%–69%), as also supported by numerous investigations conducted in several countries during recent years (Friesema, Schotsborg, et al., 2015; Kassenborg et al., 2004; McPherson et al., 2009; Werber et al., 2007). Yet, the fraction of human STEC infections attributable to cattle, while believed to be the largest, has never been quantified as carried out in this study, and this also applies to the fractions for the other sources. Shiga toxin-producing *Escherichia coli*, including O157 and other serogroups associated with human infections, are frequently isolated from sheep (Heuvelink et al., 1998; Urdahl, Beutin, Skjerve, Zimmermann, & Wasteson, 2003), goats (Pritchard, Willshaw, Bailey, Carson, & Cheasty, 2000) and foods of animal origin from these species, including meat (Chapman, Siddons, Cerdan Malo, & Harkin, 2000) and milk (Rubini et al., 1999). Moreover, small ruminants may also have a relevant role in spreading STEC in the environment (Howie, Mukerjee, Cowden, Leith, & Reid, 2003). Therefore, small ruminants too are considered important reservoirs for human STEC infection, and this is supported by our results showing small ruminants to be the second most important source of human STEC infection in the Netherlands, accounting for 24%–26% of all cases. Of note is the relatively large attribution of non-O157 serotypes (30%–32%), particularly serotype O146 (71%–77%), to small ruminants, as this serotype is indeed predominantly found in sheep

TABLE 6 Adjusted odds ratios and 95% confidence intervals of the factors associated with human Shiga toxin-producing *Escherichia coli* infections attributable to each of the four animal reservoirs (according to the *Prs* from the modified Hald model) and overall as given by the final multivariable logistic regression model

	Overall (unweighted)	Cattle	Small ruminants	Pigs	Poultry
Age (years)					
≤5	Reference	Reference	Reference	Reference	Reference
6–15	0.93 (0.56–1.55)	1.00 (0.59–1.72)	1.14 (0.63–2.08)	0.96 (0.51–1.81)	0.73 (0.37–1.45)
16–45	0.56 (0.36–0.87)	0.56 (0.36–0.89)	0.83 (0.49–1.43)	0.56 (0.32–0.97)	0.59 (0.33–1.04)
46–65	0.12 (0.08–0.20)	0.10 (0.06–0.18)	0.23 (0.12–0.45)	0.13 (0.07–0.23)	0.14 (0.06–0.33)
≥66	0.20 (0.12–0.31)	0.18 (0.11–0.29)	0.31 (0.17–0.55)	0.27 (0.14–0.53)	0.23 (0.10–0.57)
Gender (♂ *vs.* ♀)	1.31 (1.01–1.70)	1.29 (0.97–1.70)	1.28 (0.93–1.77)	1.44 (1.03–2.02)	1.49 (0.86–2.59)
Year					
2010	Reference	Reference	Reference	Reference	Reference
2011	1.86 (1.19–2.90)	2.03 (1.23–3.35)	2.25 (1.26–4.01)	2.29 (1.19–4.40)	1.60 (0.87–2.95)
2012	1.37 (0.87–2.16)	1.46 (0.88–2.43)	1.23 (0.70–2.18)	1.48 (0.85–2.60)	2.12 (0.82–5.45)
2013	2.19 (1.41–3.42)	2.28 (1.36–3.83)	3.53 (1.84–6.75)	3.01 (1.56–5.79)	6.25 (1.29–30.31)
2014	2.46 (1.57–3.86)	2.93 (1.74–4.94)	3.55 (1.83–6.86)	3.63 (1.83–7.22)	6.23 (1.48–26.20)
Season					
Winter	Reference*	Reference	Reference	Reference	Reference
Spring	0.55 (0.35–0.89)	0.66 (0.39–1.09)	0.71 (0.40–1.24)	0.77 (0.41–1.42)	0.39 (0.12–1.23)
Summer	5.71 (3.77–8.64)	7.16 (4.48–11.47)	8.29 (4.61–14.89)	8.71 (4.71–16.11)	11.65 (1.56–87.00)
Autumn	2.15 (1.46–3.16)	2.54 (1.64–3.94)	2.39 (1.43–3.97)	2.18 (1.30–3.68)	1.48 (0.55–3.97)
SES	1.17 (1.02–1.34)	1.20 (1.03–1.40)	1.10 (0.92–1.31)	1.32 (1.03–1.69)	1.26 (0.92–1.72)
Urbanization					
Urban	Reference	Reference	Reference	Reference	Reference
Intermediate	1.37 (0.92–2.04)	1.34 (0.89–2.01)	0.95 (0.60–1.50)	1.33 (0.82–2.16)	2.17 (1.18–3.98)
Rural	1.62 (1.04–2.54)	1.61 (1.01–2.55)	1.04 (0.62–1.77)	1.38 (0.81–2.33)	2.77 (1.17–6.54)
Raw meat spreads	n.s.	1.65 (1.23–2.22)	n.s.	n.s.	n.s.
Beef	1.39 (1.15–1.92)	1.61 (1.16–2.23)	n.s.	n.s.	n.s.
Raw vegetables	0.27 (0.19–0.40)	0.28 (0.18–0.42)	0.23 (0.14–0.36)	0.35 (0.20–0.61)	0.38 (0.19–0.75)
Minced meat	n.s.	n.s.	1.57 (1.04–2.37)	n.s.	1.69 (1.02–2.78)
Undercooked meat	1.67 (1.24–2.24)	n.s.	1.82 (1.28–2.58)	2.02 (1.43–2.85)	1.96 (1.16–3.30)
Cured meat	1.35 (1.03–1.77)	n.s.	n.s.	n.s.	2.16 (1.28–3.64)

Dietary factors refer to the week prior to the onset of symptoms (cases) or completion of the questionnaire (controls). n.s., not significant; SES, socio-economic status, continuous variable expressed as a normalized score ranging from −4 to +4 based on income, employment and educational level per postcode area, with a higher score indicating lower SES.

*When spring is set as reference: winter 1.81 (1.13–2.89), autumn 3.88 (2.56–5.8), summer 10.30 (6.63–15.99).

(Beutin et al., 1997; Blanco et al., 2003; Frohlicher, Krause, Zweifel, Beutin, & Stephan, 2008).

Shiga toxin-producing *Escherichia coli* has been isolated only sporadically from animals other than ruminants, and in most cases, it is unclear whether these animals can be deemed to be actual hosts for STEC or merely as spill-over hosts momentarily colonized by STEC after exposure to ruminant manure (Caprioli et al., 2005). This seems to be particularly relevant for poultry, as its estimated contribution to human STEC infections was minimal in this study (around 3%), and no STEC is usually to be found in live chickens (Beutin, Geier, Steinruck, Zimmermann, & Scheutz, 1993; Heuvelink, Zwartkruis-Nahuis, van den Biggelaar, van Leeuwen, & de Boer, 1999), even though O157 strains have been isolated from retail poultry products (Leclercq & Mahillon, 2003). Interestingly, pigs accounted for a 6%–13% of human STEC infections, indicating that they may be a non-negligible source of STEC associated with human illness. However, as pointed out before (Caprioli et al., 2005), the generally low faecal carriage of strains like O157 (<2%) in pigs in industrialized countries could be the result of accidental exposure of pigs to ruminant manure. Although the prevalence of STEC in pigs can be as high as 68%, the relatively low attributions to pigs are likely due to most STEC strains from pigs carrying the stx2e gene, which might limit disease potential for humans as other virulence genes might more be important for human disease than

stx2e (Fratamico, Bagi, Bush, & Solow, 2004; Tseng, Fratamico, Bagi, Manzinger, & Funk, 2015).

The source-specific factors identified in our study were epidemiologically plausible according to the source in question. For instance, consumption of beef and raw meat spreads (which in the Netherlands are mostly produced from beef) was specific risk factors for infection with STEC strains attributed to cattle. Raw/undercooked meat (of any origin) was a risk factor for infections attributed to small ruminants, pigs and poultry, whose meats are usually consumed well cooked, so the consumption of, for instance, undercooked mutton, pork or chicken may be assumed to be a generally accidental event posing a risk for infection with STEC strains from these animals. Other associations, such as the consumption of minced meat as a risk factor for small ruminant- and poultry-attributed STEC infections, as well as the consumption of cured meat/cold cuts for poultry-attributed infections, are quite unclear. Yet, they certainly deserve further investigation, as in the Netherlands there are a number of specific products that can reflect such risks, for instance medium–rare hamburgers of lamb meat (often called "Greek hamburgers") or poultry-based deli meats like smoked chicken or roasted turkey ham.

In general, the risk factor analysis showed that risk factors for STEC infection in the Netherlands vary according to the attributable reservoir, entailing that STEC may infect humans through different transmission routes depending on their original reservoirs. This is of importance since in the past years, remarkable changes in the epidemiology of human STEC infections have occurred. For instance, in addition to relatively well-known foods of bovine origin like hamburgers, several outbreaks have been associated with low pH products, such as fermented salami, mayonnaise and yoghurt (Caprioli et al., 2005). In addition, outbreaks associated with environmental exposures are also reported (Howie et al., 2003), with spatial analyses indicating an increased risk for STEC associated with the magnitude of cattle farming (Friesema et al., 2011), possibly due to dispersion of manure in the environment that can cause the contamination of different items, including vegetables. Indeed, vegetables fertilized with manure or contaminated during harvesting or processing are a cause for concern and have been involved in several outbreaks (Franz & van Bruggen, 2008). An example is the outbreak of STEC O157 in the Netherlands and Iceland in 2007 linked to contaminated lettuce (Friesema et al., 2008) or again the outbreak of STEC O104:H4 in Germany in 2011 linked to fenugreek sprouts (Buchholz et al., 2011). We found consumption of raw vegetables to be a protective factor. Several studies on risk factors for STEC infection have found consuming raw vegetables to have a protective effect (Friesema, Schotsborg, et al., 2015; Locking et al., 2011; McPherson et al., 2009; Werber et al., 2007), and in the present study, raw vegetables were protective for infection with STEC strains of any origin. This finding is not limited to STEC, but the same has been found for other bacteria, including *Salmonella* (Mughini-Gras, Enserink, et al., 2014) and *Campylobacter* (Mughini-Gras et al., 2012). A diet rich in vegetables may have genuinely beneficial effects on general health, meaning that the benefits of eating raw vegetables would somehow exceed the risk of acquiring STEC or other bacterial infections. However, it is also true that the controls returning the questionnaire may just have been particularly motivated people with a generally healthier lifestyle than the cases (selection bias), providing an alternative explanation as to why raw vegetables had such a protective effect in this study.

This study has several limitations. Although STEC cases over a period of 5 years were included in the source attribution analysis, only a subsample of them could be included in the case–control study as well. This is due to the lower number of cases that also agreed to be interviewed, which entails a tendency towards some overrepresentation of more severe illness. Moreover, as the cases were generally identified by passive surveillance (so they were already likely to represent the more serious cases occurring in the population) and the controls were not tested for STEC carriage, the risk factors studied here especially represent risk factors for serious STEC infection. Due to the study design and questionnaire limitations, analysis of more detailed risk factors was not possible. Moreover, the controls were known to slightly deviate from the general population of the Netherlands, with a small underrepresentation of men, young people and people living in large cities (Friesema, Gageldonk-Lafeber, et al., 2015), thereby supporting the choice of always adjusting the multivariable analyses for gender, age and urbanization degree. With regard to potential misclassification of the exposure, we did not investigate private behaviours that can generate shame or stigma (e.g., sexual orientation, drug abuse, personal hygiene), and recall period was the same for both cases and controls, so we can reasonably assume that if misclassification occurred, it was mainly non-differential, so biasing the ORs towards the null hypothesis. While cases were acquired from a (passive) laboratory surveillance system, controls were acquired at random from the general population. Many case–control studies use similar sources of controls, often relying on random or sequential digit dialling from telephone directories in addition to general population registries. However, it is well recognized that acquiring cases and controls from different sources may lead to some selection bias, as even when controls are selected from the same catchment area of the surveillance system, there is no guarantee that, had the controls become ill, they would have sought medical attention and been reported as were the cases (Fullerton et al., 2012). Our study was based on countrywide laboratory surveillance data for STEC, and controls came from the same population and geographical area (i.e., catchment area of the surveillance system) as the cases, so we can assume that they had the same chance to be recognized as cases if they had developed the disease. Yet, as Fullerton et al. (2012) pointed out, there are factors affecting the identification process of the cases that have the potential to make the cases ascertained by surveillance systems substantially different from population-based controls.

With a limited number of isolates and diversity of serotypes therein, not only our sample would have been less likely to be representative, but the source attribution models would have performed poorly (i.e., large uncertainty in the estimates, large fraction of non-attributable cases, failure of the models to converge). We have therefore used both local and non-local livestock data within a 4-year timeframe for the source attribution analysis. Inherent to this

approach was the assumption that the non-local livestock data were also representative of the STEC strains circulating in the Netherlands and that there were no major differences in the occurrence of these strains over the study period. Although this may be considered a major limitation, the application of the method of Smid et al. (2013) provided a way to minimize potential biases. We also performed additional analyses to assess the degree of (dis)similarity of livestock data over time and between local and non-local sources. Altogether, these analyses confirmed that the non-local livestock data were more similar than they were different to the local sources, thereby acting as a good surrogate. Moreover, no significant temporal trends were found in the similarity of livestock data, indicating that temporal variation in their O-serotype distribution was unlikely to have introduced a significant bias in the analysis. We also found that human isolates were more similar to the non-Dutch cattle isolates than the Dutch cattle isolates. In general, foods and animals can be moved freely throughout the EU, provided that they meet EU's standards for animal health and welfare, as well as safety, certification and proper use of foods thereof. According to Netherlands Statistics (www.cbs.nl), in the years 2010–2014, about 49%–52% of beef available for consumption in the Netherlands was imported (88%–91% of which from other EU countries), providing a possible explanation for the high similarity between human and non-Dutch cattle isolates we found. Other limitations of this study concern the potential clustering of source isolates by herd or retailer, although including only different serotypes from a cluster did prevent sample inflation. In addition, there were no data available concerning other potentially relevant sources, including companion animals and wildlife, although the non-attributable fraction of 12.8% of cases suggests these other sources may be important to investigate in future studies.

In conclusion, this is the first combined source attribution and case–control analysis for STEC allowing for both the quantification of the fractions of human STEC infections attributable to four putative livestock sources and their associated risk factors. We showed that domestic ruminants account altogether for approximately three-quarters of reported human STEC infections in the Netherlands, whereas pigs and poultry play a minor role as sources of STEC. We also showed that risk factors for STEC infection may vary according to the attributable source, providing an approach for generating hypotheses on the transmission pathways for STEC, as its epidemiology has changed over the past years and a growing number of unusual vehicles are being associated with human infection.

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