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SUMMARY

Shiga toxin-producing *Escherichia coli* (STEC) infections have been associated with severe illness. Ruminants are seen as the main reservoir and the major transmission route is considered to be foodborne. In The Netherlands, a case-control study was conducted, using data collected during 2008–2012. Patients were interviewed and controls completed a self-administered questionnaire. Patients travelling abroad were excluded from the analyses. STEC O157 and non-O157 were examined separately and differentiated into two age groups (<10 years, ≥ 10 years). We included 130 O157 cases, 78 non-O157 cases and 1563 controls. In both age groups of O157 patients, raw spreadable sausage was the main risk factor for infection. For STEC non-O157 cases aged <10 years, contact with farm animals was the main risk factor and in non-O157 cases aged ≥ 10 years, consumption of beef was the main risk factor. During 2008–2012, risk factors for STEC infections in the Dutch population differed between age groups and serogroup categories, and were related to eating meat and contact with farm animals. Advising the public about the risks of consuming raw or undercooked meat (products) and hygiene habits in case of contact with farm animals, could help in the prevention of STEC infections.

Key words: Analysis of data, Shiga-like toxin-producing *E. coli*, surveillance, zoonotic foodborne diseases.

INTRODUCTION

Since 1982, gastroenteritis from Shiga toxin-producing *Escherichia coli* (STEC) has been identified as a significant health problem, associated with haemorrhagic colitis and haemolytic uremic syndrome (HUS) [1–3]. There are in excess of 100 STEC serogroups associated with human illness, with O157 causing the majority of the infections [4, 5]. HUS develops in 2–15% of STEC patients, which is characterized by acute renal failure, thrombocytopenia and haemolytic anaemia [5]. In Germany in 2011, a large outbreak of STEC O104:H4 occurred with an unprecedented high rate of HUS of 22.3%, increasing the awareness of the potency of non-O157 strains [6]. The main reservoirs for STEC are cattle and sheep, and the major transmission route is considered to be foodborne in which food is contaminated by animal faeces [4, 7]. Most frequently identified risk factors for a STEC infection include consumption of raw and undercooked beef and direct contact with cattle [5, 8–12]. Raw vegetables are increasingly associated with outbreaks [5, 13–17], including the outbreak in Germany in 2011 which was associated with contaminated fenugreek [6, 18].

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In 2011, a study was conducted in The Netherlands that examined a possible spatial association between STEC O157 illness in humans and livestock density [19]. An association was found for STEC O157 infections in children living in areas with higher cattle densities. The present case-control study is a continuation of this earlier work, which aims to overcome the drawbacks of an ecological study and to include non-O157 infections. We examined in more detail potential risk factors for sporadic STEC O157 and non-O157 illness in The Netherlands, and explored the travel destinations of cases and controls who had travelled abroad. We used data that were collected by the National Institute for Public Health and the Environment (RIVM) during 2008–2012.

**METHODS**

**Case notification and confirmation**

Laboratories in The Netherlands that detect an STEC infection must notify the Municipal Health Service (MHS) within 24 h under the terms of the ‘Law of Public Health’ (Wet Publieke Gezondheid: http://wetten.overheid.nl/BWBR0024705) and are requested (but not obligated) to send an isolate to RIVM. The MHS reports the case to RIVM through an online notification system (Osiris). The isolates are examined by RIVM with polymerase chain reaction (PCR) for the presence of the most important virulence genes; Shiga toxin 1 (stx1) and Shiga toxin 2 (stx2). If either of these genes are detected, the STEC case is confirmed. The isolate is also tested for the attaching and effacing gene, the haemolysin gene and is further cultured for O- and H-serotyping [20–22].

**Case questionnaire and case definition**

The surveillance of STEC O157 started in 1999 and of STEC non-O157 in 2007. The MHS interview patients using a RIVM questionnaire as well as the mandatory case report via Osiris. As the RIVM questionnaire is not mandatory, not all patients were interviewed. Questionnaire items include: demographics, signs and symptoms, antibiotic use, evolution of illness. Further items concern the week previous to onset of illness, which include: contact with other persons with diarrhoea before becoming ill, family members becoming ill after the patient, food consumed, contact with animals or manure and travel. A 1-week period was chosen based on the incubation time of STEC infections (usually 3–5 days) [7].

A case was defined as confirmed STEC O157 or non-O157 infection during the period 2008–2012 in a person who was interviewed by the MHS within 1 month after the onset of symptoms, who had not travelled abroad in the week before onset of illness, and exhibited at least one of the following symptoms: diarrhoea, nausea, vomiting, abdominal pain, blood in stool, fever, abdominal cramps, oliguria or anuria. Patients were excluded if they were known secondary cases of a household cluster or if they were part of an (inter)national outbreak.

**Control questionnaire and control definition**

In 2008, the RIVM started a periodic control survey in the general population with a self-administered questionnaire. The design of this survey is described by Friesema et al. [23]. The purpose was to obtain data that can be used to help identify risk factors for several notifiable gastrointestinal and respiratory infections, including STEC. A random sample of the population is contacted by mail three times a year. Questionnaire items were based upon the case questionnaires and include, besides demographics: food consumption, contact with animals, travel and outdoor activities (enquired for the last week and the last 4 weeks). Additional items are: health and underlying diseases, smoking habits, and profession. However, these additional items were not available for the cases. Controls for the current study were selected if they participated between 2008 and 2012 and if they had not been travelled abroad in the week previous to completing the questionnaire.

**Statistical analysis**

Potential risk factors were selected from the variables available from both questionnaires, on the basis of biological plausibility and drawing on risk factors that had been identified in other investigations [8, 10, 13, 19, 24–26]. In the datasets, missing values were addressed by imputing the dataset ten times using the %pmm macro written by Boshuizen et al. [27] in SAS. The %pmm macro uses predictive mean matching making partial use of the SAS MI procedure. Percentages of missing values per variable and case and control group varied between 0% and 7%, except for contact with farm animals and cheese produced from raw milk in the control group aged ≥10 years.
(15% and 27%, respectively). The selected controls were used for both the O157 and non-O157 case-control analyses. Two age groups were created: a younger (<10 years) and an older (≥10 years) age group, because differences in risk factors were expected.

Univariate and multivariable analyses were performed for both age groups on the potential risk factors (Table 1) using logistic regression with O157 or non-O157 illness vs. controls as a dichotomous outcome variable. Risk factors to which <5 patients were exposed, were excluded from analysis. Because the datasets were imputed ten times, analyses were run for each imputation followed by pooling the results with the SAS mianalyze procedure. Age, gender, quarter of the year and level of urbanization (five levels, based upon the number of addresses per square kilometre) were added as potential confounders to all multivariable models. Because Box–Tidwell tests showed a nonlinear relationship between potential confounder age and both outcome variables in the older group, the continuous variable age was transformed into a restricted cubic spline with four knots using the %daspline macro in SAS written by Harrell [28]. Risk factors with \( P < 0.20 \) in the univariate analysis were included in the multivariable model. A final model was determined by stepwise backward elimination of variables. For each step, the least significant variable was removed from the model, until all variables in the model reached significance (\( P < 0.05 \)) and the model was significant. At every step, the estimate of the odds ratio for the remaining exposure(s) were checked for major changes. The risk factors raw and undercooked meat, beef, and raw spreadable sausage were closely related, and could therefore not be included in one model. In those cases, separate multivariable models were tested and the best-fit model (based upon Akaike’s Information Criterion) is described. All analyses were performed with SAS v. 9.3 (SAS Institute Inc., USA).

**RESULTS**

**Selection of cases and controls**

During 2008–2012, 2964 STEC patients were reported to RIVM, but only 1711 (57.7%) isolates were sent in. The RIVM confirmed 869 (50.8%) of these isolates of which 309 (35.6%) were typed as O157 and 560 (64.4%) as non-O157. Eleven STEC non-O157 patients and 20 STEC O157 patients were excluded as they were part of the German O104 outbreak due to fenugreek in 2011 [6] or part of the Dutch O157 outbreak due to steak tartare in 2008/2009 [10]. Another five O157 patients were excluded as they were secondary cases within a reported household cluster, and another 350 STEC patients were excluded as they had travelled abroad or had an unknown travel history. Of the remaining 483 STEC patients (208 O157, 275 non-O157) that had been in The Netherlands in the week before becoming ill, a questionnaire was completed by 280 (151 O157, 129 non-O157) of whom 130 O157 and 78 non-O157 patients were interviewed within 1 month after onset of illness. The median age for STEC O157 infections was 21 years (range 0–85 years) and 80 (62.0%) were female. The median age for the two age groups, as used in the analyses, was 4 years (range 0–8 years) and 35 years (range 10–85 years), respectively. The median age for STEC non-O157 infections was 47 years (range 1–92 years) and 41 (52.6%) were female. The median age for the two age groups was 3 years (range 1–9 years) and 56 years (range 10–92 years), respectively.

During 2008–2012, 1786/4929 (36.3%) randomly chosen persons from the general population completed a control questionnaire. The questionnaires of 151 controls were excluded as they had travelled abroad in the week previous to completing the questionnaire or they had omitted this question. Another 68 questionnaires were excluded as they were completed poorly. After these exclusions, 1563 controls

<table>
<thead>
<tr>
<th>Table 1. Potential risk factors included in the case-control analyses during 2008–2012, The Netherlands</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Risk factor</strong></td>
</tr>
<tr>
<td>Raw and undercooked meat</td>
</tr>
<tr>
<td>Beef</td>
</tr>
<tr>
<td>Minced meat</td>
</tr>
<tr>
<td>Raw spreadable sausage</td>
</tr>
<tr>
<td>Cheese produced from raw milk</td>
</tr>
<tr>
<td>Contact with farm animals</td>
</tr>
<tr>
<td>Raw vegetables</td>
</tr>
</tbody>
</table>
with a median age of 47 years (range 0–89 years) of which 54.6% were females remained for analyses. The median age for the two age groups was 4 years (range 0–9 years) and 56 years (range 10–95 years), respectively.

Travel destinations of the excluded patients and controls

The patients and controls who had travelled abroad in the week before illness or completing the questionnaire were excluded from the analyses. Information on travel was available for 633 of the sporadic STEC cases. Of these, 124 had travelled abroad in the week before becoming ill (20%). The destination was within Europe for 40% of the patients (mainly France, Spain and Germany), and 60% had travelled outside Europe: Asia (59%), Africa (32%), North/South America (9%). The most popular travel destinations outside Europe were Turkey (40%) and Egypt (13%). Only minor differences were seen between STEC O157 and non-O157 patients (data not shown). Travel history was known for 1722 controls of whom 91 (5%) had travelled abroad in the week before completing the questionnaire. The majority (87%) had travelled to Europe with Germany, France, Spain and Italy as the most popular destinations. Of the 12 persons who had travelled outside Europe, six had visited Asia, three North/South America, two Africa and one Australia. Four persons had visited Turkey, other countries were mentioned only once.

Table 2. Frequencies of signs and symptoms of patients included in a case-control study to identify risk factors by age group for STEC serogroups O157 and non-O157 in The Netherlands, 2008–2012

<table>
<thead>
<tr>
<th>Symptom</th>
<th>&lt;10 years</th>
<th>≥10 years</th>
<th>&lt;10 years</th>
<th>≥10 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhoea</td>
<td>38 97%</td>
<td>99 94%</td>
<td>13 87%</td>
<td>59 97%</td>
</tr>
<tr>
<td>Abdominal cramps</td>
<td>32 82%</td>
<td>91 71%</td>
<td>9 64%</td>
<td>45 71%</td>
</tr>
<tr>
<td>Blood in stool</td>
<td>31 79%</td>
<td>93 49%</td>
<td>5 36%</td>
<td>31 49%</td>
</tr>
<tr>
<td>Nausea</td>
<td>18 46%</td>
<td>69 49%</td>
<td>4 29%</td>
<td>31 49%</td>
</tr>
<tr>
<td>Vomiting</td>
<td>24 62%</td>
<td>42 24%</td>
<td>2 14%</td>
<td>15 24%</td>
</tr>
<tr>
<td>Reduced urine volume</td>
<td>18 46%</td>
<td>31 22%</td>
<td>2 15%</td>
<td>14 22%</td>
</tr>
<tr>
<td>Fever</td>
<td>13 33%</td>
<td>19 19%</td>
<td>3 21%</td>
<td>12 19%</td>
</tr>
</tbody>
</table>

Clinical and microbiological information

Hospitalization was required for 63/130 STEC O157 patients (49%), and for 23/78 STEC non-O157 patients (30%). The median age of hospitalized STEC O157 patients was 18 years (range 0–85 years) and of hospitalized non-O157 patients 59 years (range 2–91 years). Median duration of the hospitalization was 3 days (range 1–24 days) and 6 days (range 2–19 days), respectively. HUS was diagnosed in 13 (11%) STEC O157 patients and in four (5%) STEC non-O157 patients. Non-O157 serogroups with HUS were: O26 (n = 2), O83 (n = 1), and O non-typable (ONT) (n = 1). The median age of STEC O157 patients with HUS was 3 years (range 0–60 years) and of non-O157 patients 29 years (range 2–81 years). The most frequent (self-reported) signs and symptoms (Table 2) were, in descending order: diarrhoea, abdominal cramps, and blood in stool.

The most frequent non-O157 serogroup (Table 3) was O26 (n = 19, 9%). Of the 130 STEC O157 isolates, 49 (38%) were only positive for stx2 and 80 (62%) were positive for stx1 and stx2, for one isolate it was unknown. Of the 78 STEC non-O157 isolates, 35 (45%) were only positive for stx1, 34 (44%) were only positive for stx2, and nine (11%) were positive for stx1 and stx2.

Univariate and multivariable analyses

In the younger group of patients with STEC O157 illness, significant and near significant risk factors were
found in the univariate analyses for meat-related risk factors and contact with husbandry animals (Table 4). The best-fitting final model consisted of raw spreadable sausage as risk factor \( \text{OR} 10.0, 95\% \text{ CI} 2.3\text{–}43.5 \). In the older STEC O157 patients, raw spreadable sausage had the strongest association with symptomatic infection in both the univariate and multivariable analysis \( \text{OR} 2.1, 95\% \text{ CI} 1.3\text{–}3.6 \). Consumption of raw vegetables was significant in the multivariable analysis, suggesting it as being protective.

By univariate analysis, a significant risk factor was contact with farm animals for the younger group of STEC non-O157 patients which remained significant after adjusting for age, gender, quarter of the year and level of urbanization \( \text{OR} 5.8, 95\% \text{ CI} 1.1\text{–}30.4 \; \text{Table 5} \). In the older group of STEC non-O157 patients, the meat-related risk factors were most important. In the final model, beef \( \text{OR} 4.0, 95\% \text{ CI} 1.3\text{–}12.2 \) appeared to be the strongest risk factor with STEC non-O157 illness. Raw vegetables was a putative protective factor in the univariate analysis, but not in the multivariable analysis.

**DISCUSSION**

The aim of this study was to identify risk factors for sporadic symptomatic STEC infections in The Netherlands. Consumption of beef and/or raw or undercooked meat appeared to be the most important, with raw spreadable sausage (STEC O157 patients in both age groups) and beef (STEC non-O157 patients, \( \geq 10 \) years) remaining in the final model. Consumption of steak tartare, a raw spreadable sausage, was also implicated in the STEC O157 outbreaks of 2005 [9] and 2008–2009 [10] in The Netherlands. For the STEC non-O157 patients aged <10 years, the most important risk factor was contact with farm animals. In a geographical analysis, performed in 2011, an association was found for STEC O157 infections in children living in areas with higher cattle densities, but STEC non-O157 infections were not included in that study [19]. The results of the current study are supported by the fact that cattle are considered the main reservoir for STEC [5, 29] and are in line with results from investigations in several other countries in recent years [8, 24, 25]. Main risk factors for sporadic STEC O157 and non-O157 illness that were identified in these studies were: consuming sliced corned beef, raw spreadable sausage, lamb, occupational exposure to raw red meat, touching a ruminant, and occupational contact with animals. All the mentioned risk factors indicate ruminants, especially cattle, as a (direct or indirect) source. Occupational exposure and consumption of lamb or corned beef was not analysed in our study.

Raw vegetables, which was also examined in our study, has played a key role in several outbreaks of STEC illness in recent years: lettuce was identified as the vehicle in an outbreak of STEC O157 in 2007 in

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**Table 4. Results of univariate and multivariable analyses by age group for STEC O157 in The Netherlands, 2008–2012**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>&lt;10 years</th>
<th>Univariate OR (95% CI)</th>
<th>Multivariable* OR (95% CI)</th>
<th>≥10 years</th>
<th>Univariate OR (95% CI)</th>
<th>Multivariable* OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td></td>
<td>Cases</td>
<td>Controls</td>
<td></td>
</tr>
<tr>
<td>Raw and undercooked meat</td>
<td>26%</td>
<td>12%</td>
<td>2.5 (1.0–6.1)</td>
<td>52%</td>
<td>43%</td>
<td>1.4 (0.9–2.1)</td>
</tr>
<tr>
<td>Beef</td>
<td>95%</td>
<td>83%</td>
<td>3.9 (0.9–17.5)</td>
<td>87%</td>
<td>82%</td>
<td>1.4 (0.8–2.7)</td>
</tr>
<tr>
<td>Minced meat</td>
<td>87%</td>
<td>78%</td>
<td>2.0 (0.7–5.5)</td>
<td>74%</td>
<td>67%</td>
<td>1.3 (0.8–2.2)</td>
</tr>
<tr>
<td>Raw spreadable sausage</td>
<td>21%</td>
<td>8%</td>
<td>3.1 (1.1–8.6)</td>
<td>36%</td>
<td>25%</td>
<td>1.7 (1.1–2.6)</td>
</tr>
<tr>
<td>Cheese produced from raw milk</td>
<td>5%</td>
<td>0%</td>
<td>†</td>
<td>5%</td>
<td>10%</td>
<td>0.5 (0.2–1.3)</td>
</tr>
<tr>
<td>Contact with farm animals</td>
<td>44%</td>
<td>28%</td>
<td>2.1 (1.0–4.4)</td>
<td>18%</td>
<td>18%</td>
<td>1.0 (0.6–1.7)</td>
</tr>
<tr>
<td>Raw vegetables</td>
<td>72%</td>
<td>65%</td>
<td>1.4 (0.6–3.1)</td>
<td>75%</td>
<td>82%</td>
<td>0.7 (0.4–1.1)</td>
</tr>
</tbody>
</table>

OR, Odds ratio; CI, confidence interval.
* Adjusted for age, gender, quarter of the year and level of urbanization.
† Not determined because of low number of cases \( \text{n} < 5 \).
Bold values indicate results with \( \text{P} < 0.05 \).
The Netherlands and Iceland [13]; fenugreek was determined as the probable cause of outbreaks of STEC O104:H4 in 2011 in Germany [6] and France [18]; and several outbreaks in the USA [15, 17, 30]. By contrast, the results of several recent studies of risk factors for STEC illness found a putative protective effect of consuming raw vegetables in relation to STEC illness (O157 and non-O157) [8, 24, 26]. In the present study, raw vegetables were a putative protective factor in both older groups in the univariate models, but remained only significant in the multivariable model of O157 patients. The preventive effect in sporadic cases could be explained by certain substances found in vegetables such as antioxidants and carotenoids in vegetables which lead to a stronger immune system [24], but which is undone in case of a (large) contamination of vegetables with STEC in an outbreak.

Consumption of raw milk or associated dairy products can be a source of STEC, as the product is not heated to clear a contamination [31–33]. In the current study, cheese produced from raw milk did not appear to be a risk factor. Consumption of raw milk was not included in the analyses, as only one STEC O157 case aged <10 years and two STEC O157 patients aged ≥10 years reported its consumption in the week before becoming ill.

Although STEC patients over a period of 5 years were included in the study, a low number of confirmed cases could be included, especially in the younger group of STEC non-O157 patients. This was mainly caused by the low percentage of RIVM-confirmed cases that were interviewed by the MHS, and also interviewed within 1 month after onset of symptoms. It is expected that these cases are representative, although there may be a tendency towards more severe illness. Nevertheless, the data used in these analyses are less likely to be affected by recall bias than if less strict inclusion criteria are used. Due to the study size and questionnaire limitations, analysis of more detailed risk factors was not appropriate. The controls slightly deviated from the general population with a small underrepresentation of men, young people, people living in large cities, and persons with both parents born outside The Netherlands [23]. In the current study, the multivariable analyses were adjusted for gender, age, quarter of the year and urbanization level; there was insufficient information regarding ethnicity available for the STEC patients to adjust for it. Only a small portion of STEC patients were born outside The Netherlands, and controls were less prone to respond to the questionnaire when both parents were born outside The Netherlands. Therefore, caution is warranted when extrapolating to other ethnicities.

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Table 5. Results of univariate and multivariable analyses by age group for STEC non-O157 in The Netherlands, 2008–2012

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>&lt;10 years</th>
<th>≥10 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>Raw and undercooked meat</td>
<td>13%</td>
<td>12%</td>
</tr>
<tr>
<td>Beef</td>
<td>93%</td>
<td>83%</td>
</tr>
<tr>
<td>Minced meat</td>
<td>93%</td>
<td>78%</td>
</tr>
<tr>
<td>Raw spreadable sausage</td>
<td>13%</td>
<td>8%</td>
</tr>
<tr>
<td>Cheese produced from raw milk</td>
<td>0%</td>
<td>0%</td>
</tr>
<tr>
<td>Contact with farm animals</td>
<td>73%</td>
<td>28%</td>
</tr>
<tr>
<td>Raw vegetables</td>
<td>60%</td>
<td>65%</td>
</tr>
</tbody>
</table>

OR, Odds ratio; CI, confidence interval.
* Adjusted for age, gender, quarter of the year and level of urbanization.
† Not determined because of low number of cases (n < 5).
Bold values indicate results with P < 0·05.
The questions were kept as simple and short as possible to enable completion of the questionnaire without support. Therefore, the effect of the difference in administration is expected to be low.

In conclusion, this study shows that during 2008–2012, STEC risk factors for the Dutch population differed between age groups and serogroups, and were related to eating (raw and undercooked) meat and contact with farm animals. Prevention efforts should, therefore, focus on educating and reminding the general public and specifically parents of young children, pregnant women, older people, and the immunocompromised, about the risks of consuming raw or undercooked meat (products). In case of contact with farm animals, whether occasionally or regularly, emphasis should be placed on teaching and reinforcing adequate (hand) hygiene habits, especially in small children. Although public health recommendations could be effective in reducing the risk of STEC infections, they still rely heavily on education and compliance [25]. Thus, it is unlikely that they will be completely effective. Therefore, measures to control STEC should start at the farm level to prevent STEC entering the food chain [7].

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DECLARATION OF INTEREST

None.

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