Transmission pathways for sporadic Shiga-Toxin Producing *E. coli* Infections: a systematic review and meta-analysis

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ABSTRACT

Background

Shiga-toxin *E. coli* infections remain a public health concern because of the severity of the gastrointestinal illness and associated complications. Transmission pathways are typically elucidated from outbreaks, with foodborne transmission the primary source. However, most STEC cases are sporadic. This systematic review aimed to identify the most common pathways for sporadic STEC transmission and quantify their importance.

Methods

We systematically reviewed epidemiological studies of sporadic (non-outbreak) STEC cases that investigated potential risk factors. Searches were run in Medline, EMBASE, and Scopus. Included studies needed to confirm STEC infection and investigate ≥20 cases.

Results

31 studies were included, of which 25 were case-control or case-case studies. 62.5% found consumption of undercooked/raw meat associated with STEC infection while 70.4% found contact with animals or their environment a risk factor. Random-effects meta-analysis provided pooled odds ratios and population attributable fraction (PAF). The PAF was 19% for undercooked/raw meat, followed by person to person transmission at 15%. Contact with animals and visiting farm environments had PAFs of 14% and 12% respectively.

Conclusions

Out of potential sources for STEC exposure, undercooked meat and contact with animals and their environment were the most frequently found transmission routes. Decreasing the chances of

acquiring the bacteria by these methods would additionally cut down on the other major transmission route, person-to-person spread.

INTRODUCTION

Shiga-toxin producing *Escherichia coli* (STEC) are a group of Gram-negative bacterial pathogens that exist as normal microbiota in ruminant animals, such as cows and sheep. STEC colonization does not produce symptoms in these animals, but can cause severe disease in humans. Transmission pathways include faecal-oral, food-borne, environmental, and person to person. STEC are characterized by their ability to release shiga-toxin, which kills host cells in the intestine and can enter the bloodstream to affect other organs, such as the kidneys and brain. Most STEC infections are caused by *E. coli* O157:H7, but over 100 different shiga-toxin producing *E. coli* serotypes are associated with human illness [1, 2]. STEC is associated with more severe disease and increased complications compared to other bacterial causes of gastroenteritis [3-5]. Cases typically present with abdominal cramps, vomiting, and/or diarrhea, which may progress to haemorrhagic colitis. About 30% of confirmed cases require hospitalization [6], and about 10% of cases progress to haemolytic uremic syndrome (HUS), characterized by anaemia, kidney failure, and low platelet counts [7].

In outbreaks (groups of linked infections), most cases relate to contaminated food [8, 9]. However, sporadic cases comprise nearly 80% of reported STEC infections [10]. The only previous synthesis of evidence on sporadic cases (Strachan et al.) compared five different case-control studies from the USA and UK between 1998 and 2004 [11]. Since 2004, screening for non-O157 has become more common. A comprehensive and updated review synthesizing sporadic STEC transmission is warranted, including studies since 2004 and enhanced

information about non-O157 infection. In order to gain an understanding about which pathways occur most often for sporadic STEC infections, a systematic review of larger (20+ cases) epidemiological studies investigating exposures and risk factors leading to sporadic STEC infections was performed. Identifying the most common pathways will aid in development of policies and procedures to help reduce the risk of STEC infection.

METHODS

Search Strategy and Inclusion Criteria

Medline, Scopus, and Embase databases were searched through February 19th 2016 with no restrictions on date or language. Search terms included: Bacterial- "STEC, EHEC, VTEC, O157, non-O157, shiga-toxin"; and Participants - "human"; Transmission - "transmission, risk factor, exposure, contamination, outbreak, sporadic, infection" (full search strategy for Medline given in Supplemental Appendix 1). Eight grey literature sources were searched (Supplemental Appendix 2); only the first 100 hits in grey literature were reviewed. Bibliographies of included studies were also checked for further references.

STEC infections in humans needed to be confirmed by an approved laboratory method, including but not limited to directly finding the toxin in stool samples or amplifying either the *stx1* or *stx2* genes from samples via PCR [12]. Any epidemiological study, whether descriptive or analytical, was eligible as long as the focus was on sporadic STEC infections, with a minimum 20 cases to ensure that quantitative results could be extracted. Studies had to present potential transmission data, to identify likely sources of exposure. The protocol for this systematic review is on PROSPERO (registration number CRD42015027593) [13].

Source Selection and Data Extraction

All references were screened by title and abstract independently in duplicate by EK and JB. Full texts of not-excluded articles were read in duplicate to make further exclusions or confirm eligibility. Eligibility disagreements were resolved by discussion (EK and JB). Abstracts without full text, such as conference proceedings, were excluded.

Information extracted from all studies included bibliographic details, study location and time period, criteria used to confirm STEC infection, and ages of participants. For descriptive studies, exposures and the percentage of participants encountering that transmission pathway prior to illness were recorded. For epidemiological studies, the selection of both cases and controls and significant exposures, along with their effect measures and confidence intervals, were extracted. For all studies, elapsed time between infection and interview, interview methods, and transmission pathways covered in the interview or questionnaire were also recorded. Data were extracted by one reviewer into a standardized form and verified by a second reviewer. Articles not in English were extracted by only one reviewer.

Quality Assessment

Quality assessment for the studies was based on the Newcastle-Ottawa scale that was tailored to the potential biases that could exist in these specific study designs [14]. Studies were judged for quality across three categories: study design, comparability of controls, and data collection. Within each category, two to four features that could influence the validity or the generalisability of study results were graded on their risk of bias, as low, high, or unclear. The categories are described in Supplementary Appendix 3. Studies were then labelled as either of "acceptable" or "poor" quality depending on whether 50% or greater of the fields had "unclear" or "high" risk of bias.

Synthesis of Results

A table was created containing categories of common exposures, including food, animal contact, water, and other environmental transmission routes across all studies. Whether or not each study asked about a particular exposure was documented (Supplemental Table 1), with any statistically significant results from each study recorded. This let us calculate the percentage of studies finding a particular exposure significant (out of those that assessed that risk factor at all). If studies provided both univariate and adjusted estimates, the results of the adjusted effects were used to fill in the table. We were concerned that whether a risk factor was identified as significant might depend on whether the study was poor or acceptable quality; therefore, Stata was used to perform a t-test comparing the proportions of studies finding a risk factor associated with STEC infection between acceptable and low quality studies [15].

Those categories where over 50% of the studies found that exposure as a risk factor for STEC infection were combined in a random effects meta-analysis using RevMan software [16, 17]. Any available odds ratios were included regardless of significance or method used for analysis (univariate vs. adjusted). If a study provided effect estimates for several similar exposures within a category, the one most similar to those used in the other studies was used. EpiInfo 7 was used to calculate odds ratios when the information was available [18]. The combined odds ratios for these exposures were used to calculate the population attributable fraction (PAF) using the formula $PAF = Pe_{pooled} * [(OR_{pooled} - 1)/OR_{pooled}]$ [19]. $Pe_{pooled,}$ the proportion of exposed cases, was calculated using OpenMeta[Analyst][20]. To assess publication bias, funnel plots were generated in RevMan and a visual assessment made.

RESULTS

From the initial search and after duplicate removal, 5,952 studies were screened on title and abstract (Figure 1). The full texts of 51 studies were obtained and read. 29 studies met all inclusion criteria and were included in the review. Two studies were identified through a review of the bibliographies and a search of the grey literature, raising the total number of included studies to 31 (Table 1).

Included studies were published between 1989-2015. Six were descriptive studies and 21 were case-control studies. The remaining four were classified as case-case studies; three of these compared O157 to non-O157 infections while the final compared STEC infections to diarrheal controls. 13 studies came from North America, 15 from Europe, 2 from Argentina, and 2 studies from Australia or New Zealand. 17 studies investigated just *E. coli* O157 while 14 studies included other STEC serotypes. Four analysed HUS cases as opposed to the STEC + diarrhea case definition used for the other studies.

All studies in this review identified patients from hospitals records or national surveillance schemes. After cases were determined, questionnaires were administered to determine likely routes of STEC infection. Of the 25 analytic studies, a majority (19) matched controls to the cases based on either age, gender or location; only 13 studies used matched analysis in calculating their results. Two studies did not present their results as an odds ratio but instead used χ^2 analysis to determine association. Additionally, 19 of the 25 analytic studies presented results of either adjusted univariate or multivariate analysis, helping to control for potential confounders.

Quality Assessment

Only 7 of the 31 studies received a poor quality rating; 6 of these were the descriptive studies since they received a high risk of bias in all categories concerning controls (Table 1, full analysis given in Supplemental Table 2). 12 of the 25 analytic studies were at low risk of bias for all methodological items, 19 of 25 for comparability of cases and controls, and four of 25 for exposure assessment. Two studies (Slutsker 1998 and Vaillant 2009) were at low risk of bias for all items assessed.

Common Transmission Pathways among all studies

The possible transmission routes were grouped to create several categories of exposure. Before determining the most common transmission pathways, whether or not each study evaluated an exposure route was determined (Supplemental Table 2). All 31 studies assessed some form of beef or other meat in the diet and 27 included questions about farm visits and/or animal contact. All other categories included were investigated in at least two-thirds of the studies.

To determine the most common pathways of transmission, the percentage of studies which assessed that exposure that found it significantly associated with STEC infection was calculated (Table 2; additional results in Supplemental Tables 3A-C). The most common significant exposure was undercooked or raw meat, linked to STEC infection in 62.5% of studies. The next most frequent pathway was person-to-person transmission (12/21 or 57.1% of studies investigating it found it was a transmission route for STEC). The "combined animal contact" category was created to determine the number of studies that found any association with animals or their habitat as a potential source of STEC infection (since it may be difficult to differentiate whether or not the exposure occurred due to contact with the animal, its faeces, or its living environment). Combined thus, the percentage of studies finding animal contact a source

of infection was greater than the percentage of studies finding undercooked or raw meat as a source of infection (70.4% for animal contact vs. 62.5% for undercooked or raw meat).

Sub-group analysis

To determine if study quality affected the results of the most commonly found pathways, the studies were split into their acceptable and low quality rating and the percentage of studies finding a specific risk factor as associated with STEC infection were recalculated for each group (see Table 3). The difference in proportion between the studies of different qualities was significant only for cooked beef and dairy, indicating that study quality does not greatly affect which of the transmission routes was found most often in the included studies.

Twenty-eight of the 31 studies came from one of four regions: USA, Canada, UK, and Europe. The percentage of studies finding a risk factor that was significantly associated with STEC infection was re-calculated for each of these regions to find geographic differences in the STEC transmission routes (Table 4; full break-down by region in Supplemental Table 4). A few trends were apparent. The UK had fewer studies finding undercooked or raw meat as a risk factor for STEC infection while also having the highest percentage of combined animal contact. This suggests that environmental exposures play a larger role in the UK compared to other regions. Furthermore, both European and the UK combined animal contact was high compared to North America, indicating that acquiring STEC from contact with animals or their living environment may be more important for UK/Europe.

Six studies split their analyses to determine risk factors for O157 and non-O157 separately. Out of all the exposure categories previously used in Table 2, only two, undercooked or raw meat and animal contact, had at least three of the 6 studies reporting odds ratios for either

O157 or non-O157 (Table 5). Five out of the 6 studies found that consuming or handling undercooked or raw meat was a risk factor for acquiring O157; none of these studies found this exposure associated with non-O157. Three out of 6 studies found that infection via animal contact was associated with non-O157 strains; only one study found the opposite with more O157 cases reporting contact with animals.

Meta-analyses

Where ≥50% of the studies identified a particular risk factor as significant (Table 2), available data were combined in meta-analysis. Forest plots were created for undercooked or raw meat (Figure 2), farm visits (Figure 3), animal contact (Figure 4), and person-to-person transmission (Figure 5); details on the exposure investigated in each study is given in Supplemental Appendix 4A-D.

20 case-control studies reporting odds ratios asked about the consumption or handling of undercooked or raw meat; information useful for meta-analysis could be extracted from 17 of these studies (Figure 2). The combined odds ratio was 3.08 (95% CI: 1.9, 4.99). Heterogeneity was high with an I^2 score of 86%. To calculate the population attributable fraction (PAF) of STEC infection for undercooked or raw meat, the proportion of exposed cases was calculated for each study; information was not available for two of the 18 included in the meta-analysis. This information was used to generate a pooled proportion of exposed cases; this and the pooled odds ratio were used to calculate a PAF of 19% (95% CI: 13-22%) (Table 6).

14 studies assessed living on or visiting a farm; information for meta-analysis was not available for three of these (Figure 3). The combined odds ratio for visiting a farm was 2.6 (95% CI: 2.11-3.21). Heterogeneity for this risk factor was very low (I^2 = 0%). To calculate the PAF,

information from only one study was not available out of the 11 used to generate the summary odds ratio, providing a combined population attributable factor for farm visits of 12% (95% CI: 10-13%).

18 studies provided odds ratios for contact with ruminant animals; the odds ratio was not available from six of these. The combined odds ratio was 3.02 (95% CI: 2.2-4.16) (Figure 4), with moderate heterogeneity (I^2 = 38%). For animal contact, information on the number of exposed cases was available for all 12 studies used in the meta-analysis; resulting in a combined PAF of 14% (95% CI: 11-15%).

15 studies appropriate for meta-analysis investigated some form of person-to-person transmission; odds ratios were available for 11 of these. The pooled odds ratio was 2.86 (95% CI: 1.69-4.84) (Figure 5), with high heterogeneity (I^2 = 68%). The number of exposed individuals was available from ten of 11 studies, and the summary PAF was 15% (95% CI: 10-19%).

The funnel plots of the studies for all four subgroups was not symmetric around the average value, indicating publication bias in the reported results (bias towards positive correlation: studies that looked for this factor but did not find it significant are underrepresented, see Figure 6) [21, 22]. What is missing in each plot are studies with high standard errors and effect estimates lower than the group average. To determine whether the publication bias affected overall conclusions, a subgroup analysis was performed [23]. The half of the studies with the largest standard errors were dropped since they represent the smaller studies and the meta-analyses run again with only the studies with lower standard errors. For all four risk factors, the odds ratio dropped but remained significantly associated with the exposure (95% CIs above one; see Table 7). Additionally, three of the four funnel plots were more symmetrical

around the pooled odds ratio; only person-to-person transmission still demonstrated evidence of publication bias similar to that which existed before the subgroup analysis was performed (Figure 7).

DISCUSSION

Using data from large case-control or surveillance studies, this review identified and quantified transmission pathways most commonly associated with sporadic STEC infections (about 80% of STEC infections). We included 31 studies from four continents, most of which (24 of 31) had acceptable quality. Two-thirds of the studies included in this systematic review found undercooked ground beef or other meat to be a significant risk factor for acquiring STEC. Where any type of contact with animals, their living environment or their manure were considered together. Animal contact was identified more often than undercooked/raw meat as a potential source of STEC.

Several intriguing results were highlighted by our subgroup analyses. First was the potential difference in the most common STEC transmission pathways between Europe and North America. All the studies from the UK identified some form of animal contact as a source of STEC and had the lowest reported associations with STEC coming from undercooked or raw meat. While continental Europe found undercooked or raw meat significantly associated with STEC as frequently as North America, the European studies also found higher rates of infection from animal contact. The reasons behind these differences are not immediately apparent but suggests different regions may need to focus on different prevention methods to most efficiently reduce the number of STEC cases. Our results also indicate that infections from undercooked or raw meat occur most often because of O157 strains while non-O157 is more often associated with animal contact. Possible hypotheses for this are variations in environmental preferences of

different *E. coli* serotypes or O157 having a lower infectious dose. Little research has been done into the survival or infectious dose of non-O157 strains, but initial studies suggest little difference between O157 and the few non-O157 serotypes tested [24-29]. Still, given the large number of STEC serotypes that can cause infections in humans, more research needs to be performed to help address these issues.

Of the individual risk factors, preventing infections from undercooked or ground beef would cause the greatest single reduction in disease, with a PAF of 19% (although this, and the other PAFs, may have been distorted by publication bias). Our review estimates that 15% of STEC infections could be prevented if transmission no longer occurred via person-to-person contact. PAFs for farm visits and animal contact were 12% and 14%, respectively. It could be argued that because the PAFs from all four risk factors are similar, intervention strategies should target multiple transmission pathways to make major impacts.

Many attributes of the primary research data may limit our results. Exclusion criteria (such as history of diarrhea in cases or controls) were applied inconsistently between studies. Furthermore, each study asked about a slightly different exposure duration. Most studies asked about 1-2 weeks prior to the onset of symptoms, but the full relevant exposure period range is 5-30 days prior to infection. Shorter timeframes may have missed potential sources of infection while longer ones possibly recorded many exposures that were not relevant. While the geographical subgroup analyses revealed interesting trends, there were few studies (6 to 8) in each group. Only a small number of studies (n=6) included exposure to both O157 and non-O157. Some studies could not be included in meta-analyses as information was missing, possibly because calculated odds ratios were not statistically significant and therefore not reported. This, along with the likely publication bias, suggests that our summary odds ratios, and

the PAFs based on them, are overestimated. However, the odds ratios obtained after our sensitivity analysis indicate that these four transmission routes are definitely associated with sporadic STEC infections.

In summary, by combining the results from 31 studies, this systematic review identified the most common transmission pathways for sporadic STEC infections. These included consuming undercooked meat, contact with animals or their environment, and person-to-person transmission after contact with someone with diarrhea. One caveat to the reported odds ratios and PAF values is combining the data from all available published studies. Our subgroup analysis by region suggests that different pathways play more predominant roles in different areas. This, combined with the fact that STEC incidence rates vary by country, indicates that case-control studies need to be performed to identify the best prevention strategies for each country.

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Conflict of Interest

The authors declare no conflicts of interest in completing this work.

References

1. Organization WH. Report of the WHO Working Group Meeting on Shiga-Like Toxin Producing Escherichia Coli (SLTEC) with Emphasis on Zoonotic Aspects, Bergamo, Italy, 1 July 1994. **1995**.
2. Caprioli A, Morabito S, Brugère H, Oswald E. Enterohaemorrhagic Escherichia coli: emerging issues on virulence and modes of transmission. Vet Res **2005**; 36(3): 289-311.
3. Hall G, Yohannes K, Raupach J, Becker N, Kirk M. Estimating community incidence of Salmonella, Campylobacter, and Shiga toxin-producing Escherichia coli infections, Australia. Emerging infectious diseases **2008**; 14(10): 1601-9.
4. Tam CC, Rodrigues LC, Viviani L, et al. Longitudinal study of infectious intestinal disease in the UK (IID2 study): incidence in the community and presenting to general practice. Gut **2012**; 61(1): 69-77.
5. Scallan E, Hoekstra RM, Angulo FJ, et al. Foodborne illness acquired in the United States--major pathogens. Emerging infectious diseases **2011**; 17(1): 7-15.
6. Byrne L, Jenkins C, Launders N, Elson R, Adak GK. The epidemiology, microbiology and clinical impact of Shiga toxin-producing Escherichia coli in England, 2009-2012. Epidemiology and infection **2015**: 1-13.
7. Gould LH, Demma L, Jones TF, et al. Hemolytic uremic syndrome and death in persons with Escherichia coli O157:H7 infection, foodborne diseases active surveillance network sites, 2000-2006. Clinical Infectious Diseases **2009**; 49(10): 1480-5.
8. Heiman KE, Mody RK, Johnson SD, Griffin PM, Gould LH. Escherichia coli O157 Outbreaks in the United States, 2003-2012. Emerging infectious diseases **2015**; 21(8): 1293-301.
9. Adams NL, Byrne L, Smith GA, et al. Shiga Toxin-Producing Escherichia coli O157, England and Wales, 1983-2012. Emerging infectious diseases **2016**; 22(4): 590-7.
10. Thomas A, Cheasty T, Frost JA, Chart H, Smith HR, Rowe B. Vero cytotoxin-producing Escherichia coli, particularly serogroup O157, associated with human infections in England and Wales: 1992-4. Epidemiology and infection **1996**; 117(1): 1-10.
11. Strachan NJ, Dunn GM, Locking ME, Reid TM, Ogden ID. Escherichia coli O157: burger bug or environmental pathogen? International Journal of Food Microbiology **2006**; 112(2): 129-37.
12. Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB. Recent advances in understanding enteric pathogenic Escherichia coli. Clin Microbiol Rev **2013**; 26(4): 822-80.
13. Kintz E BJ, Hooper L, Hunter P. Identification of risk factors for Shiga-toxin producing E. coli infections: a systematic review. PROSPERO: International prospective register of systematic reviews **2015.** CRD42015027593 (Available from: http://www.crd.york.ac.uk/prospero/display_record.asp?ID=CRD42015027593).

14. Wells GA SB, O'Connell D, Peterson J, Welch V, Losos M, et al. The Newcastle-Ottawa Scale (NOS) for assessing the quality if nonrandomized studies in meta-analyses.
15. StataCorp. Stata Statisical Software: Release 13. College Station, TX: StataCorp LP **2013**.
16. Hedges LV, Vevea JL. Fixed- and random-effects models in meta-analysis. Psychol Methods **1998**; 3(4): 486-504.
17. Review Manager (RevMan) [Computer program]. Version 5.3. Copenhagen: The Nordic Cochrane Centre TCC, 2014.
18. Dean AG AT, Sunki GG, Friedman R,, Lantinga M SS, Zubieta JC, Sullivan KM, Brendel KA, Gao Z,, Fontaine N SM, Fuller G, Smith DC, Nitschke DA, and Fagan RF. . Epi Info™, a database and statistics program for public health professionals. CDC **2011**.
19. Rockhill B, Newman B, Weinberg C. Use and misuse of population attributable fractions. Am J Public Health **1998**; 88(1): 15-9.
20. Wallace BC, Issa J. Dahabreh, Thomas A. Trikalinos, Joseph Lau, Paul Trow, and Christopher H. Schmid. Closing the Gap between Methodologists and End-Users: R as a Computational Back-End. Journal of Statistical Software **2012**.
21. Sterne JA, Sutton AJ, Ioannidis JP, et al. Recommendations for examining and interpreting funnel plot asymmetry in meta-analyses of randomised controlled trials. BMJ **2011**; 343: d4002.
22. Song F, Parekh S, Hooper L, et al. Dissemination and publication of research findings: an updated review of related biases. Health technology assessment **2010**; 14(8): iii, ix-xi, 1-193.
23. Higgins JPT GSe. Cochrane Handbook for Systematic Reviews of Interventions. **2011**; Version 5.1.0 [updated March 2011].
24. Berry ED, Barkocy-Gallagher GA, Siragusa GR. Stationary-phase acid resistance and injury of recent bovine Escherichia coli O157 and non-O157 biotype I Escherichia coli isolatest. Journal of food protection **2004**; 67(3): 583-90.
25. Buvens G, Posse B, De Schrijver K, De Zutter L, Lauwers S, Pierard D. Virulence profiling and quantification of verocytotoxin-producing Escherichia coli O145:H28 and O26:H11 isolated during an ice cream-related hemolytic uremic syndrome outbreak. Foodborne pathogens and disease **2011**; 8(3): 421-6.
26. Duffy G, Walsh C, Blair IS, McDowell DA. Survival of antibiotic resistant and antibiotic sensitive strains of E. coli O157 and E. coli O26 in food matrices. Int J Food Microbiol **2006**; 109(3): 179-86.
27. Large TM, Walk ST, Whittam TS. Variation in acid resistance among shiga toxin-producing clones of pathogenic Escherichia coli. Applied and environmental microbiology **2005**; 71(5): 2493-500.
28. Luchansky JB, Porto-Fett AC, Shoyer BA, et al. Fate of Shiga toxin-producing O157:H7 and non-O157:H7 Escherichia coli cells within blade-tenderized beef steaks after cooking on a commercial open-flame gas grill. Journal of food protection **2012**; 75(1): 62-70.
29. Paton AW, Ratcliff RM, Doyle RM, et al. Molecular microbiological investigation of an outbreak of hemolytic-uremic syndrome caused by dry fermented sausage contaminated with Shiga-like toxin-producing Escherichia coli. Journal of clinical microbiology **1996**; 34(7): 1622-7.
30. Bryant HE, Athar MA, Pai CH. Risk factors for Escherichia coli O157:H7 infection in an urban community. The Journal of infectious diseases **1989**; 160(5): 858-64.
31. Byrne L, Vanstone GL, Perry NT, et al. Epidemiology and microbiology of Shiga toxin-producing Escherichia coli other than serogroup O157 in England, 2009-2013. Journal of medical microbiology **2014**; 63(Pt 9): 1181-8.
32. Byrne L, Jenkins C, Launders N, Elson R, Adak GK. The epidemiology, microbiology and clinical impact of Shiga toxin-producing Escherichia coli in England, 2009-2012. Epidemiology and infection **2015**; 143(16): 3475-87.

33. Coia JE, Sharp JCM, Campbell DM, Curnow J, Ramsay CN. Environmental risk factors for sporadic Escherichia coli O157 infection in Scotland: Results of a descriptive epidemiology study. J Infection **1998**; 36(3): 317-21.
34. Denno DM, Keene WE, Hutter CM, et al. Tri-county comprehensive assessment of risk factors for sporadic reportable bacterial enteric infection in children. The Journal of infectious diseases **2009**; 199(4): 467-76.
35. Eklund M, Nuorti JP, Ruutu P, Siitonen A. Shigatoxigenic Escherichia coli (STEC) infections in Finland during 1998-2002: a population-based surveillance study. Epidemiology and infection **2005**; 133(5): 845-52.
36. Friesema IH, Schotsborg M, Heck ME, Van Pelt W. Risk factors for sporadic Shiga toxin-producing Escherichia coli O157 and non-O157 illness in The Netherlands, 2008-2012, using periodically surveyed controls. Epidemiology and infection **2015**; 143(7): 1360-7.
37. Gianviti A, Rosmini F, Caprioli A, et al. Haemolytic-uraemic syndrome in childhood: surveillance and case-control studies in Italy. Italian HUS Study Group. Pediatric nephrology (Berlin, Germany) **1994**; 8(6): 705-9.
38. Holton D, Wilson J, Ellis A, et al. A Canadian multicentre case-control study of sporadic Escherichia coli 0157:H7 infection. Can J Infect Dis **1999**; 10(2): 117-21.
39. Huber HC, Kugler R, Liebl B. [Infections with enterohemorrhagic Escherichia coli (EHEC)--results of an epidemiologic survey in Bavaria for the April 1996 to May 1997 time frame]. Gesundheitswesen (Bundesverband der Arzte des Offentlichen Gesundheitsdienstes (Germany)) **1998**; 60(3): 159-65.
40. Jaros P, Cookson AL, Campbell DM, et al. A prospective case-control and molecular epidemiological study of human cases of Shiga toxin-producing Escherichia coli in New Zealand. BMC infectious diseases **2013**; 13: 450.
41. Kassenborg HD, Hedberg CW, Hoekstra M, et al. Farm visits and undercooked hamburgers as major risk factors for sporadic Escherichia coli O157:H7 infection: data from a case-control study in 5 FoodNet sites. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **2004**; 38 Suppl 3: S271-8.
42. Le Saux N, Spika JS, Friesen B, et al. Ground beef consumption in noncommercial settings is a risk factor for sporadic Escherichia coli O157:H7 infection in Canada. The Journal of infectious diseases **1993**; 167(2): 500-2.
43. Locking ME, O'Brien SJ, Reilly WJ, et al. Risk factors for sporadic cases of Escherichia coli O157 infection: the importance of contact with animal excreta. Epidemiology and infection **2001**; 127(2): 215-20.
44. MacDonald KL, O'Leary MJ, Cohen ML, et al. Escherichia coli O157:H7, an emerging gastrointestinal pathogen. Results of a one-year, prospective, population-based study. Jama **1988**; 259(24): 3567-70.
45. McPherson M, Lalor K, Combs B, Raupach J, Stafford R, Kirk MD. Serogroup-specific risk factors for Shiga toxin-producing Escherichia coli infection in Australia. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **2009**; 49(2): 249-56.
46. Mead PS, Finelli L, Lambert-Fair MA, et al. Risk factors for sporadic infection with Escherichia coli O157:H7. Archives of internal medicine **1997**; 157(2): 204-8.
47. O'Brien SJ, Adak GK, Gilham C. Contact with farming environment as a major risk factor for Shiga toxin (Vero cytotoxin)-producing Escherichia coli O157 infection in humans. Emerging infectious diseases **2001**; 7(6): 1049-51.
48. Parry SM, Salmon RL, Willshaw GA, Cheasty T. Risk factors for and prevention of sporadic infections with vero cytotoxin (shiga toxin) producing Escherichia coli O157. Lancet **1998**; 351(9108): 1019-22.

49. Pierard D, Crowcroft N, De Bock S, et al. A case-control study of sporadic infection with O157 and non-O157 verocytotoxin-producing Escherichia coli. Epidemiology and infection **1999**; 122(3): 359-65.
50. Proctor ME, Davis JP. Escherichia coli O157:H7 infections in Wisconsin, 1992-1999. WMJ **2000**; 99(5): 32-7.
51. Rivas M, Sosa-Estani S, Rangel J, et al. Risk factors for sporadic Shiga toxin-producing Escherichia coli infections in children, Argentina. Emerging infectious diseases **2008**; 14(5): 763-71.
52. Rivero MA, Passucci JA, Rodriguez EM, Signorini ML, Tarabla HD, Parma AE. Factors associated with sporadic verotoxigenic Escherichia coli infection in children with diarrhea from the Central Eastern Area of Argentina. Foodborne pathogens and disease **2011**; 8(8): 901-6.
53. Rowe PC, Orrbine E, Lior H, Wells GA, McLaine PN. Diarrhoea in close contacts as a risk factor for childhood haemolytic uraemic syndrome. The CPKDRC co-investigators. Epidemiology and infection **1993**; 110(1): 9-16.
54. Slutsker L, Ries AA, Maloney K, Wells JG, Greene KD, Griffin PM. A nationwide case-control study of Escherichia coli O157:H7 infection in the United States. The Journal of infectious diseases **1998**; 177(4): 962-6.
55. Vaillant V, Espie E, de Valk H, et al. Undercooked ground beef and person-to-person transmission as major risk factors for sporadic hemolytic uremic syndrome related to Shiga-toxin producing Escherchia coli infections in children in France. The Pediatric infectious disease journal **2009**; 28(7): 650-3.
56. Van Duynhoven YT, De Jager CM, Heuvelink AE, et al. Enhanced laboratory-based surveillance of Shiga-toxin-producing Escherichia coli O157 in The Netherlands. European journal of clinical microbiology & infectious diseases : official publication of the European Society of Clinical Microbiology **2002**; 21(7): 513-22.
57. Voetsch AC, Kennedy MH, Keene WE, et al. Risk factors for sporadic Shiga toxin-producing Escherichia coli O157 infections in FoodNet sites, 1999-2000. Epidemiology and infection **2007**; 135(6): 993-1000.
58. Wang X, Taylor M, Hoang L, et al. Comparison of clinical and epidemiological features of Shiga toxin-producing Escherichia coli O157 and non-O157 infections in British Columbia, 2009 to 2011. The Canadian journal of infectious diseases & medical microbiology = Journal canadien des maladies infectieuses et de la microbiologie medicale / AMMI Canada **2013**; 24(4): e102-6.
59. Waters JR, Sharp JC, Dev VJ. Infection caused by Escherichia coli O157:H7 in Alberta, Canada, and in Scotland: a five-year review, 1987-1991. Clinical infectious diseases : an official publication of the Infectious Diseases Society of America **1994**; 19(5): 834-43.
60. Werber D, Behnke SC, Fruth A, et al. Shiga toxin-producing Escherichia coli infection in Germany: different risk factors for different age groups. American journal of epidemiology **2007**; 165(4): 425-34.

485 **Table 1: Characteristics of Included Studies**

Reference	Study Dates	Country	Design	Outcome/STEC	# cases/controls	Quality[a]
Bryant et al. 1989 [30]	Summers 1986 & 1987	Canada	Case-control	Diarrhea/O157	Diarrhea control: 52 per group Community control: 49 per group	acceptable
Byrne et al. 2014 [31]	2009-2013	England	Matched case-case	Diarrhea/O157 and non	2300 O157 / 67 Non O 157	acceptable
Byrne et al. 2015 [32]	2009-2012	England	Other[b]	Diarrhea/all	1772 cases	acceptable
Coia et al. 1998 [33]	July 1992-December 1993	Scotland	Descriptive	Diarrhea/O157	138 cases	poor
Denno et al. 2009 [34]	November 2003-2005	United States	Case-control	Diarrhea/O157	39 cases/ 78 controls	acceptable
Eklund et al. 2005 [35]	1998-2002	Finland	Descriptive	Diarrhea/all	26 O157/27 non	poor
Friesema et al. 2015 [36]	2008-2012	Netherlands	Case-control	Diarrhea/all	130 O157/ 78 non O157/ 1563 controls	poor
Gianviti et al. 1994 [37]	May 1988 – April 1992	Italy	Matched Case-control	HUS/all	43 cases/ 43 controls	acceptable
Holton et al. 1999 [38]	June-September 1991	Canada	Matched Case-control	Diarrhea/O157	100 cases/ 200 controls	acceptable
Huber et al. 1998 [39]	April 1996 – March 1997	Germany	Descriptive	Diarrhea/all	300 cases	acceptable
Jaros et al. 2013 and Jaros 2014[c] [40]	July 2011-2012	New Zealand	Case-control	Diarrhea/all	113 cases/ 506 controls	acceptable
Kassenborg et al. 2004 [41]	March 1996 – April 1997	United States	Matched Case-control	Diarrhea/O157	196 cases/ 372 controls	acceptable
Le Saux et al. 1993 [42]	June-September 1990	Canada	Matched Case-control	Diarrhea/O157	110 cases/ 220 controls	acceptable

Locking et al. 2001 [43]	October 1996-March 1999	Scotland	Matched Case-control	Diarrhea/O157	183 cases/ 545 controls	acceptable
MacDonald et al. 1988 [44]	May 1985 – April 1986	United States	Case-control	Diarrhea/O157	24 cases/ 48 controls	acceptable
McPherson et al. 2009 [45]	July 2003 – April 2007	Australia	Case-control	Diarrhea/all	113 cases/ 304 controls	acceptable
Mead et al. 1997 [46]	July 1994	United States	Matched Case-control	Diarrhea/O157	23 cases/ 46 controls	poor
O'Brien et al. 2001 [47]	October 1996-December 1997	England	Case-control	Diarrhea/O157	369 cases/ 511 controls	acceptable
Parry et al. 1998 [48]	March 1994-February 1996	England and Wales	Matched Case-control	Diarrhea/O157	85 cases/ 142 controls	acceptable
Pierard et al. 1999 [49]	Unclear	Belgium	Matched Case-control	Diarrhea/all	37 cases/ 69 controls	acceptable
Proctor et al. 2000 [50]	1992-1999	United States	Descriptive	Diarrhea/O157	994 cases	poor
Rivas et al. 2008 [51]	2001-2002	Argentina	Matched Case-control	Diarrhea/all	150 cases/ 300 controls	acceptable
Rivero et al. 2011 [52]	December 2002 – April 2009	Argentina	Case-case	Diarrhea/all	63 cases/ 374 controls	acceptable
Rowe et al. 1993 [53]	May-August 1990	Canada	Case-control	HUS/O157	34 cases/ 102 controls	acceptable
Slutsker et al. 1998 [54]	October 1990-1992	United States	Matched Case-control	Diarrhea/O157	73 cases/ 142 controls	acceptable
Vaillant et al. 2009 [55]	2000-2001	France	Matched Case-control	HUS/all	105 cases/ 196 controls	acceptable
Van Dunhoven et al. 2002 [56]	January 1999 – June 2001	Netherlands	Descriptive	Diarrhea/O157	82 cases	poor
Voestch et al. 2006 [57]	1999-2000	United States	Case-Control	Diarrhea/O157	283 cases/ 534 controls	acceptable
Wang et al. 2013 [58]	2009-2011	Canada	Case-case	Diarrhea/all	154 O157/ 63 non O157	acceptable
Waters et al. 1994 [59]	1987-1991	Canada	Descriptive	Diarrhea/O157	1484 cases	poor

	1987-1991	Scotland	Descriptive	Diarrhea/O157	505 cases	
Werber et al. 2007 [60]	April 2001-March 2003	Germany	Matched Case control	Diarrhea/all	29 O157/ 173 non O157/ 202 controls	acceptable

a: refer to text and Supplemental Appendix 3 for determination of quality

b: categorical χ^2 analysis based on national surveillance data

d: Dissertation thesis "Epidemiological investigations of STEC O157 and O26 in New Zealand slaughter cattle, and the source attribution of human illness" containing additional information to Jaros et al. 2013.

Table 2: Results of Systematic Review with exposures split into general categories

	Food					Animal Contact			Animal Contact: Combined	Water		Other Environmental	
	Pink or Raw Meat	Cooked Beef	Other Meat	Dairy	Produce	Farm Visits	Contact with Ruminants	Contact with manure		Drinking	Recreational	Travel	Person-to-person
# studies finding RF[a] significant	20	7	8	8	2	10	13	6	19	8	8	6	12
# asking about RF	32	31	29	24	24	19	24	15	27	21	20	21	21
Percentage	**62.5%**	**22.6%**	**27.6%**	**33.3%**	**8.3%**	**52.6%**	**54.2%**	**40%**	**70.4%**	**38.1%**	**40%**	**28.6%**	**57.1%**

a: RF = Risk Factor

Table 3: Study quality does not affect the proportion of studies finding different risk factors as associated with STEC infections

	Food					Animal Contact			Animal Contact: Combined	Water		Other Environmental	
	Pink or Raw Meat	Cooked Beef	Other Meat	Dairy	Produce	Farm Visits	Contact with Ruminants	Contact with manure		Drinking	Recreational	Travel	Person-to-person
acceptable quality studies	63.6%	12.5%	31.8%	22.2%	12.5%	50%	45%	45.5%	66.7%	29.4%	40%	23.5%	53.3%
low quality studies	75%	57.1%	14.3%	66.7%	0%	60%	75%	25%	83.3%	75%	40%	23.5%	66.7%
p-value	**0.558**	**0.013**	**0.367**	**0.045**	**0.296**	**0.701**	**0.273**	**0.474**	**0.432**	**0.091**	**1**	**0.291**	**0.575**

Table 4: Percentage of studies from different regions finding different risk factors significant

	Undercooked or Raw Meat	Animal Contact: Combined	Person-to-person
USA	71.43%	42.876%	66.67%
Canada	66.67%	33.33%	100%
UK	50%	100%	50%
Europe	75%	75%	50%

Table 5: Odds ratios separated by STEC serogroup

Study	Pink or Raw Meat[a]	Animal Contact
Byrne 2014	O157 **8.05** [1.11, 58.30]	NON **3.3** [1.69, 6.40]
Friesema 2015 (< 10 yrs)	O157 **9.97** [2.29, 43.38]	NON **5.8** [1.10, 30.75]
Friesema 2015 (> 10 yrs)	O157 **2.10** [1.26, 3.50]	-[b]
McPherson 2009	O157 **4.57** [1.42, 14.70]	NON **5.0** [2.09, 11.99]
Rivas 2008	O157 **17.64** [3.08, 100.92]	O157 **6.6**[c]
Wang 2013	-[b]	-[b]

a: odds ratio given

b: no associated risk factor found

c: 95% confidence interval not provided

Table 6: Population attributable fractions for risk factors included in meta-analysis

Exposure	Pe_{pooled}	OR_{pooled}[a]	PAF[a]
Pink or Raw Meat	0.279	3.08 [1.9, 4.99]	0.19 [0.13, 0.22]
Farm Visits	0.19	2.6 [2.11, 2.31]	0.12 [0.10, 0.13]
Animal Contact	0.204	3.02 [2.2, 4.16]	0.14 [0.11, 0.15]
Person-to-person	0.236	2.86 [1.69, 4.84]	0.15 [0.10, 0.19]

a: 95% confidence interval in brackets

Table 7: Odds ratios after sugroup analysis

Exposure	OR_{pooled}[a]
Pink or Raw Meat	2.07 [1.22, 3.51]
Farm Visits	2.48 [1.99, 3.09]
Animal Contact	2.5 [1.72, 3.62]
Person-to-person	2.0 [1.14, 3.5]

a: 95% confidence interval in brackets

Figure Legends

Figure 1: PRISMA flow diagram of included studies.

Figure 2: Meta-analysis of undercooked or raw meat.

For Werber, exposure to undercooked or raw meat was only significant in age groups over 10 years old. For Friesema, those under 10 had an OR of 10 (2.3-43.5), but this was not included in the meta-analysis to prevent over-representation of this study in the results. * OR was adjusted for possible confounders. "Not estimable" means no data relevant to this risk-factor could be extracted.

Figure 3: Meta-analysis of farm visits.

This risk factor was only significant in the Kassenborg study for children under 6 years old. The Werber study values were calculated using EpiInfo from data provided in the manuscript. * OR was adjusted for possible confounders. "Not estimable" means no data relevant to this risk-factor could be extracted.

Figure 4: Meta-analysis for animal contact.

For Friesema, animal contact was only significant for non-O157 and cases under 10 years old. Similarly, Weber found this risk factor significant for those under three years old. Kassenborg found it significant for those over 6 years of age. * OR was adjusted for possible confounders. "Not estimable" means no data relevant to this risk-factor could be extracted.

Figure 5: Meta-analysis for person-to-person transmission.

615 The OR for Werber was calculated by combining data, given in the paper, from all age groups
616 using EpiInfo. * OR was adjusted for possible confounders. "Not estimable" means no data
617 relevant to this risk-factor could be extracted.
618
619 **Figure 6: Funnel plots of studies included in meta-analysis.**
620 A. Funnel plot of studies investigating undercooked or raw meat, with OR plotted against SE. B.
621 Funnel plot of studies investigating farm visits, with OR plotted against SE. C. Funnel plot of
622 studies investigating animal contact, with OR plotted against SE. D. Funnel plot of studies
623 investigating person-to-person transmission, with OR plotted against SE.
624
625 **Figure 7: Funnel plots of studies after subgroup analysis.**
626 A. Funnel plot of studies investigating undercooked or raw meat, with OR plotted against SE. B.
627 Funnel plot of studies investigating farm visits, with OR plotted against SE. C. Funnel plot of
628 studies investigating animal contact, with OR plotted against SE. D. Funnel plot of studies
629 investigating person-to-person transmission, with OR plotted against SE.
630
631