

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

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23 **ABSTRACT**

24 ***Background***

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

30 ***Methods***

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

34 ***Results***

35 31 studies were included, of which 25 were case-control or case-case studies. 62.5% found
36 consumption of undercooked/raw meat associated with STEC infection while 70.4% found
37 contact with animals or their environment a risk factor. Random-effects meta-analysis provided
38 pooled odds ratios and population attributable fraction (PAF). The PAF was 19% for
39 undercooked/raw meat, followed by person to person transmission at 15%. Contact with animals
40 and visiting farm environments had PAFs of 14% and 12% respectively.

41 ***Conclusions***

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

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

46

47 **INTRODUCTION**

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

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

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

72

73 **METHODS**

74 **Search Strategy and Inclusion Criteria**

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

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

89 **Source Selection and Data Extraction**

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

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

103 **Quality Assessment**

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

112 **Synthesis of Results**

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

123 Those categories where over 50% of the studies found that exposure as a risk factor for
124 STEC infection were combined in a random effects meta-analysis using RevMan software [16,
125 17]. Any available odds ratios were included regardless of significance or method used for
126 analysis (univariate vs. adjusted). If a study provided effect estimates for several similar
127 exposures within a category, the one most similar to those used in the other studies was used.
128 EpiInfo 7 was used to calculate odds ratios when the information was available [18]. The
129 combined odds ratios for these exposures were used to calculate the population attributable
130 fraction (PAF) using the formula $PAF = P_{e_{pooled}} * [(OR_{pooled} - 1)/OR_{pooled}]$ [19]. $P_{e_{pooled}}$, the
131 proportion of exposed cases, was calculated using OpenMeta[Analyst][20]. To assess
132 publication bias, funnel plots were generated in RevMan and a visual assessment made.

133 **RESULTS**

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

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

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

154 **Quality Assessment**

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

161 **Common Transmission Pathways among all studies**

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

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

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

180 **Sub-group analysis**

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

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

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

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

205 **Meta-analyses**

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

211 20 case-control studies reporting odds ratios asked about the consumption or handling of
212 undercooked or raw meat; information useful for meta-analysis could be extracted from 17 of
213 these studies (Figure 2). The combined odds ratio was 3.08 (95% CI: 1.9, 4.99). Heterogeneity
214 was high with an I^2 score of 86%. To calculate the population attributable fraction (PAF) of
215 STEC infection for undercooked or raw meat, the proportion of exposed cases was calculated for
216 each study; information was not available for two of the 18 included in the meta-analysis. This
217 information was used to generate a pooled proportion of exposed cases; this and the pooled odds
218 ratio were used to calculate a PAF of 19% (95% CI: 13-22%) (Table 6).

219 14 studies assessed living on or visiting a farm; information for meta-analysis was not
220 available for three of these (Figure 3). The combined odds ratio for visiting a farm was 2.6 (95%
221 CI: 2.11-3.21). Heterogeneity for this risk factor was very low ($I^2 = 0\%$). To calculate the PAF,

222 information from only one study was not available out of the 11 used to generate the summary
223 odds ratio, providing a combined population attributable factor for farm visits of 12% (95% CI:
224 10-13%).

225 18 studies provided odds ratios for contact with ruminant animals; the odds ratio was not
226 available from six of these. The combined odds ratio was 3.02 (95% CI: 2.2-4.16) (Figure 4),
227 with moderate heterogeneity ($I^2 = 38\%$). For animal contact, information on the number of
228 exposed cases was available for all 12 studies used in the meta-analysis; resulting in a combined
229 PAF of 14% (95% CI: 11-15%).

230 15 studies appropriate for meta-analysis investigated some form of person-to-person
231 transmission; odds ratios were available for 11 of these. The pooled odds ratio was 2.86 (95%
232 CI: 1.69-4.84) (Figure 5), with high heterogeneity ($I^2 = 68\%$). The number of exposed
233 individuals was available from ten of 11 studies, and the summary PAF was 15% (95% CI: 10-
234 19%).

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

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

248 **DISCUSSION**

249 Using data from large case-control or surveillance studies, this review identified and
250 quantified transmission pathways most commonly associated with sporadic STEC infections
251 (about 80% of STEC infections). We included 31 studies from four continents, most of which
252 (24 of 31) had acceptable quality. Two-thirds of the studies included in this systematic review
253 found undercooked ground beef or other meat to be a significant risk factor for acquiring STEC.
254 Where any type of contact with animals, their living environment or their manure were
255 considered together. Animal contact was identified more often than undercooked/raw meat as a
256 potential source of STEC.

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

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

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

280 Many attributes of the primary research data may limit our results. Exclusion criteria
281 (such as history of diarrhea in cases or controls) were applied inconsistently between studies.
282 Furthermore, each study asked about a slightly different exposure duration. Most studies asked
283 about 1-2 weeks prior to the onset of symptoms, but the full relevant exposure period range is 5-
284 30 days prior to infection. Shorter timeframes may have missed potential sources of infection
285 while longer ones possibly recorded many exposures that were not relevant. While the
286 geographical subgroup analyses revealed interesting trends, there were few studies (6 to 8) in
287 each group. Only a small number of studies (n=6) included exposure to both O157 and non-
288 O157. Some studies could not be included in meta-analyses as information was missing,
289 possibly because calculated odds ratios were not statistically significant and therefore not
290 reported. This, along with the likely publication bias, suggests that our summary odds ratios, and

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

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

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315 **Conflict of Interest**

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

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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

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

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

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

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506 **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%

507 a: RF = Risk Factor

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521 **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

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536 **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%

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554 **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

555 a: odds ratio given

556 b: no associated risk factor found

557 c: 95% confidence interval not provided

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572 **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]

573 a: 95% confidence interval in brackets

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584 **Table 7: Odds ratios after subgroup 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]

585 a: 95% confidence interval in brackets

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592 **Figure Legends**

593 **Figure 1: PRISMA flow diagram of included studies.**

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

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

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602 **Figure 3: Meta-analysis of farm visits.**

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

607

608 **Figure 4: Meta-analysis for animal contact.**

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

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614 **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.

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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.

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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.

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