Running title: STEC and salad outbreaks

Review Paper

Outbreaks of Shiga toxin-producing *Escherichia coli* linked to sprouted seeds, salad, and leafy greens: a systematic review

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Abstract

Shiga toxin-producing *E. coli* (STEC) outbreaks involving ready-to-eat salad products have been described in the scientific literature since 1995. These products typically do not undergo a definitive control step such as cooking to eliminate pathogens. To reduce the number of STEC infections from salad products, efforts will need to focus on preventing and reducing contamination throughout the food chain. We performed a systematic review of STEC outbreaks involving sprouted seeds, salad, or leafy green products to determine if there were recurrent features, such as availability of microbiological evidence or identification of the contamination event, which may inform future investigations and prevention and control strategies. Thirty-five STEC outbreaks linked to contaminated leafy greens were identified for inclusion. The outbreaks occurred from 1995-2018 and ranged in size from 8 cases to over 8,500. Detection of STEC in the food product was rare (4/35). For the remaining outbreaks, the determination of leafy greens as the source of the outbreak mainly relied on analytical epidemiology (20/35) or descriptive evidence (11/35). The trace back investigation in 21/32 outbreaks was not able to identify possible routes of where the STEC bacteria came from or how the leaves were contaminated. Investigations in eight outbreaks found poor practice during processing that may have contributed to the outbreak, such as insufficient post-harvest disinfection of the product. Six outbreak investigations were able to identify the outbreak strain in animal feces near the growing fields; two of these were also able to find it in irrigation water on the farms, providing a likely route of contamination. These results highlight the limitations of relying on microbiological confirmation as a basis to initiate investigations of upstream production to understand the source of contamination. This review also demonstrates the importance of, and difficulties associated with, food-chain trace back studies to inform control measures and future prevention.
Highlights

- Systematic review identified 35 STEC outbreaks linked to contaminated leafy greens
- Most (20/35) outbreaks relied on epidemiological evidence to identify leafy greens
- 21/35 studies found no evidence for how original contamination occurred
- 11 studies identified water as the probable vector in contaminating product
- Only 2 studies were able to identify the likely source and route of contamination

Shiga toxin-producing *Escherichia coli* (STEC) cause gastrointestinal infections characterised by bloody diarrhea. Shiga-toxins which enter the bloodstream can lead to haemolytic uremic syndrome (HUS), a serious complication of STEC infection which damages the kidneys and can lead to longer term sequelae (11). HUS most often commonly affects children and is the leading cause of acute renal failure in children in the UK and elsewhere (46). Given the severity of STEC infection and the potential for complications, STEC remains a pathogen of public health research in an effort to reduce the number of infections.

The first documented outbreak of STEC O157:H7 occurred in 1982 and was due to contaminated hamburgers from an American fast food restaurant (48), leading to the nickname “burger bug” (76). Over the last 20 years, control measures that have been implemented to reduce infections from contaminated meat products include adequate cooking, ground beef irradiation, and industry practices to reduce contamination both at slaughter and at retail (2, 60, 73, 74).

Meanwhile, other food vehicles, including raw dairy products, leafy greens and raw vegetables have been implicated in outbreaks of STEC (9, 10, 37, 45). In the United States, vegetable produce is the most frequent cause of STEC O157:H7 outbreaks after meat (34, 59). In England and Wales, produce has been increasingly reported as the vehicle in STEC outbreaks (2), motivating research to identify the basis for effective control measures.

Several factors may contribute to leafy greens serving as a food vehicle for STEC outbreaks (43). Contamination during production could result from direct spread of animal feces via wildlife or
rain water run-off, particularly if animal reservoirs of STEC, such as cattle, sheep or deer, are in close proximity to where produce is grown (37, 44, 50). During cultivation, produce may also be contaminated by irrigation water sourced from rivers and/or ponds (Jenkins et al. 2015). Due to the non-homogenous distribution of STEC in the environment, cross contamination during processing is possible if a batch of product from a contaminated field on the farm is mixed with other batches of product. This can increase the extent of consumer exposure to the affected product. Furthermore, low sanitizer concentration in flume and/or wash water can contribute to dissemination of pathogens during processing. These factors, combined with the low infectious dose for STEC infection, less than 100 bacteria (75, 80), increase the chance of an individual ingesting enough bacteria to cause illness. Since these products are often eaten raw and washing may not provide a definitive control measure (65), there is no critical control point for prevention of infection. The lack of control measures at the point of consumption means that effective control measures further up the food chain are important. Understanding the mechanisms of contamination involved and collating this data are required to inform better control measures. We performed a systematic review to determine if there are identifiable recurrent features across STEC outbreaks caused by salad leaves that may inform investigation and control.

**Materials and Methods**

**Search strategy.** Medline Ovid and Scopus databases were searched with no restriction on year or language of publication up to April 7th, 2019. The search strategy was limited to title/abstract/keyword using the following MeSH terms/keywords: ((stec OR ehec OR vtec OR O157 OR shiga-toxin) AND (outbreak) AND (salad OR leaf OR leaves OR produce OR sprout OR seed)). Reference lists from relevant papers were screened for additional eligible articles. For grey literature, “stec salad outbreak” was searched in Google Scholar and the first 100 hits reviewed. The websites of the Centres for Disease Control and Prevention, the Canadian Public Health Association,
the European Centre for Disease Prevention and Control, and the Australian Department of Health were also searched for STEC outbreaks.

**Inclusion criteria and data extraction.** All references were screened by title and abstract by EK. To be considered for inclusion, papers must describe an outbreak of STEC linked to salad, sprouts, or leaves. Full-texts of potential studies were then acquired and screened for eligibility. Outbreaks were excluded if STEC was not a confirmed cause of the illnesses, if no food item was identified as the likely source, if a majority of the outbreak’s epidemiological information (number of cases, onset of symptoms, etc) was not available or if no details of the outbreak investigation were provided. For each article, the following information was extracted: location and date of the outbreak, duration of outbreak, identified food item causing the outbreak, strain of STEC isolated, the number of cases and associated hospitalizations, HUS cases, and deaths, age range and gender ratio of cases. If analytical epidemiology studies were performed, then the type of study performed, number of controls (if applicable), and odds ratios (or relative risk or hazard ratio) for the identified leafy greens were also extracted. To determine the possible source of contamination, details were recorded on whether or not a trace back investigation was performed and also the extent and results of food and environmental sampling in facilities associated with producing the food item linked to the outbreak.

**Levels of evidence.** The evidence for the food item associated with the outbreak and the source of the contamination of the food were determined separately. For identification of the food item causing the outbreak, the following criteria were followed- suggestive evidence: descriptive results implicated leafy greens; medium evidence: analytical epidemiological study with significant results implicated leafy greens; strong evidence: microbiological evidence of STEC in the food item; very strong evidence: analytical epidemiological evidence in addition to microbiological evidence.

For the identification of where the contamination of the food with STEC may have occurred, the following levels of evidence were used- no evidence: no microbiological evidence or faults in equipment or practice were identified; suggestive evidence: faults in equipment or practice were
identified during the investigation; strong evidence: outbreak strain identified in water or the environment near production of leafy greens.

Data synthesis. Data extracted from the studies were collected in a Microsoft Excel spreadsheet. Excel was also used to calculate summary statistics and for creating graphs. A narrative synthesis approach was taken to summarize the results.

Results

The searches of the databases returned a total of 1,612 articles, which reduced to 1,034 after de-duplication (Fig 1). The Google Scholar search did not return any studies not already found in the original search. Thirty-two references were identified from reviewing the bibliography of relevant literature or searching public health websites. One outbreak report meeting the inclusion criteria was provided directly by Public Health England. The full texts of 105 articles were obtained and screened for eligibility; articles were excluded if they were laboratory-based research, review articles, or the outbreak was caused by a food item other than sprouts, salad, or leafy greens. Based on the full-text screening, 62 articles were selected for inclusion in the final review. In total, 35 different outbreaks were described by the articles selected for inclusion from the literature search (Table 1).

Outbreaks attributed to STEC in leafy green products described in this review occurred between July 1995 and October 2018 (Fig 2). All the described outbreaks occurred in Europe (12 outbreaks), North America (22 outbreaks) or Japan (1 outbreak). For the 33 outbreaks that reported onset dates of illness, the outbreak duration ranged from 4 to 97 days with a median of 30 days. Seventeen of the outbreaks began during the months from June to September; six outbreaks began during colder winter months (Fig 3). Twenty-nine outbreaks were caused by STEC serogroup O157.

The size of the outbreaks ranged from 8 cases to over 8,500 (Fig 4). The highest number of HUS cases and deaths were recorded for the hybrid STEC enteroaggregative *E. coli* O104:H4 outbreak in Europe in 2011; this outbreak led to 854 cases of HUS and 54 deaths. Outside of this
2011 outbreak, hospitalizations ranged from 0-398 (up to 63.75% of overall cases), HUS cases ranged from 0-39 (up to 22.28% of cases), and deaths ranged from 0-5 (up to 2.22% of cases) for the outbreaks. The reported median age of cases within the outbreaks ranged from 16 years to 65, with a mean of 31.6 years. The percentage of cases that were female ranged from 45% to 97%, with an unweighted mean of 66.4%.

The levels of evidence for identifying the vehicle that caused the outbreak and the mechanism for contamination of product was determined for each outbreak (Table 1). Out of the 35 outbreaks, only four had a strong or very strong level of evidence associated with determining the food source; 20 had medium levels of evidence and eleven studies had only descriptive evidence. The 23 analytical epidemiological studies included a range of 16 to 521 participants (cases + controls or total cohort). Odds or risk ratios ranged from 1.93 (CI 1.38 - 2.7) to 100 (CI 6.47 - undefined) for identifying salad, sprouts, or leafy greens as the source of the outbreak.

Six studies had strong and eight studies suggestive evidence for how the STEC bacteria contaminated the product (Table 1). In the six outbreaks with strong evidence, four were able to identify the outbreak strain in irrigation water, providing evidence for the likely contamination route. In the other two studies, the outbreak strain was found in animal faeces in the vicinity of the growing fields alongside detection of the stx2 gene in the irrigation water or use of untreated irrigation water. Detection of stx is an indicator of the presence of STEC although not conclusive evidence it belonged to the outbreak strain. For the eight outbreaks with suggestive evidence, five recorded either floods on the growing fields or noted a failure to adhere to disinfection protocols. A majority of the studies (21) did not identify a possible route of contamination; in 17 of these outbreaks, investigators did not find faults in either the growing fields or the processing facilities that might indicate where contamination had occurred.

In an effort to determine why so few outbreaks were able to identify the method of contamination, the investigations performed for each outbreak were recorded (Table 2). Trace back investigations were initiated in 32 outbreaks. Twenty-five of the 35 outbreaks included sampling of
the suspected food products. Only 17 outbreak investigations included food and environmental sampling, including water, at either the growing fields or production facility. Eight outbreak investigations included the sampling of animal faeces in the vicinity of the growing fields while five investigations collected stool samples from employees working at either production facilities or as kitchen staff.

Discussion

This paper describes 35 outbreaks caused by STEC contamination of sprouted or leafy green products. There was a wide variation between the outbreaks in terms of duration and the number of cases. The outbreak strain was detected within the salad product in only four outbreaks despite testing being carried out in 25 of the outbreaks reviewed. This may be due to the short-shelf life of the product, intermittent contamination, or levels of contamination below the detectable limits of the test (37). However, for the majority of the outbreaks (23/35), investigators were able to perform analytical epidemiology studies that implicated leafy greens as the vehicle in the outbreak. This suggests that requiring microbiological evidence for initiating recall of leafy greens identified as a likely vehicle by epidemiological studies could lead to an increase in both the length of time of the outbreak and thus the number of persons potentially falling ill. As for determining how the contamination of the product occurred, only 14 of the 35 outbreaks were able to identify possible routes or identify faults in the processing or handling of the produce that likely contributed to the contamination. It is noteworthy that in 11 of these 14 studies, water was somehow involved in the contamination process, whether it was contamination in the fields during growth or during the washing and disinfection of the seeds or produce. In only two of the outbreaks was evidence of STEC found in both animals near the growing fields, serving as the source, and in irrigation water, serving as the likely route of contamination. For a majority of the outbreaks (21/35), no production faults or source of the STEC contamination was identified, even though 32 studies initiated a trace back investigation. It is likely that contamination of fresh produce occurs following a sequence of both
natural events, such as flooding, and potentially preventable events, such as lack of biosecurity on the farm and animal ingress, irrigation using pond or river water, and failures in the manufacturing process.

A recent review investigated the sources of sporadic STEC infections (41). Only 2 of the 24 studies (8.3%) that investigated produce identified it as a significant risk factor. In the UK, O’Brien et al. identified watercress (OR 2.61 [1.24-5.47]) as a source of sporadic STEC infections (52); the only watercress-specific outbreak identified in this review also occurred in the UK over 16 years after this case-control study of sporadic STEC infections was performed (37, 44). In Canada, Wang et al. compared exposures for O157 vs non-O157 STEC infections and found that bagged greens were associated with sporadic non-O157 infections (p=0.014) (82). Only six outbreaks were due to non-O157 STEC strains in this systematic review, but the results from Wang et al. suggest we may be missing smaller salad outbreaks due to non-O157 strains, largely because they are less likely to be screened for in samples from ill patients.

There are several limitations to the current systematic review. One is that the main source of outbreak information relied on published papers and reports that contained enough extractable data to be included. Several review papers and governmental reports provided summary statistics on other STEC salad outbreaks (42, 68, 83), but without full details on the investigation or the outbreak characteristics, these were not included. Additionally, many of the reports obtained from public health websites on more recent outbreaks specifically mention ongoing investigations into the source of contamination, but further information was never made publicly available. Therefore, our summaries on the types of investigations performed for each outbreak and their results may be incomplete compared to the information held by public health agencies.

Another limitation of this systematic review is that the identification of an outbreak relies on linking cases together; the wide distribution and low infectious dose associated with salad and leaf products as a source of STEC indicate that these infections previously may have been
misidentified as sporadic infections (37). However, the advent of whole genome sequencing of STEC isolates means that these smaller, widespread outbreaks are now less likely to be missed in countries where this technology is routinely implemented. The information provided from the more sensitive detection of outbreaks could further help identify characteristics of STEC salad outbreaks that will better inform prevention measures.

Given the frequency, and potential scale and severity of STEC outbreaks caused by leafy greens, reducing the risk of STEC infection via this particular pathway would assist in the overall goal of reducing the burden of STEC infection. Since salad products are not cooked by consumers or pasteurized prior to sale to eliminate pathogens, interventions to reduce contamination of salad products by STEC bacteria will need to focus on the farm or production facility or possible methods for decontamination (5, 61). As noted above, a majority of the studies were not able to identify how the contamination took place. Nearby contaminated farm animals and potentially contaminated water stood out as important sources where evidence was identified. If interventions are to be successful, more effort needs to be placed on identifying the source of the original contamination during the trace back investigations. This paper recommends accepting epidemiological evidence to initiate trace back investigations when leafy green products are suspected as the source of an outbreak in order the increase the frequency with which contamination events are identified. As more outbreaks have their contamination events identified, the greater amount of data will hopefully reveal viable prevention strategies for reducing the number of leafy green outbreaks caused by STEC bacteria.

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Supplemental Materials

Full extraction information from all outbreaks is provided in Supplemental Table- All Info.xlsx

References


15. Centers for Disease Control and Prevention. 2010. Multistate Outbreak of E. coli O157:H7 Infections Linked to Romaine Lettuce (Final Update)


Figure Legends

Figure 1: Flow diagram of included studies.

Figure 2: Number of STEC outbreaks per year by location.

Figure 3: Month when first case of outbreak became ill.

Figure 4: Number of cases for STEC outbreaks per year by location.
Table 1: Summary of STEC outbreaks attributed to sprouts, salad, and leafy green products.

<table>
<thead>
<tr>
<th>Year</th>
<th>Location</th>
<th>Food Item</th>
<th>STEC</th>
<th>Cases</th>
<th>SofE: food</th>
<th>SofE: contamination</th>
<th>References</th>
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<td>1995</td>
<td>USA</td>
<td>Lettuce</td>
<td>O157</td>
<td>40</td>
<td>medium</td>
<td>suggestive</td>
<td>(1)</td>
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<td>1995</td>
<td>Canada</td>
<td>Iceberg lettuce</td>
<td>O157</td>
<td>21</td>
<td>medium</td>
<td>suggestive</td>
<td>(55)</td>
</tr>
<tr>
<td>1996</td>
<td>USA</td>
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<td>O157</td>
<td>61</td>
<td>medium</td>
<td>suggestive</td>
<td>(35)</td>
</tr>
<tr>
<td>1996</td>
<td>Japan</td>
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<td>O157</td>
<td>8500</td>
<td>medium</td>
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<td>(38, 49)</td>
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<td>51</td>
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<td>no evidence</td>
<td>(6, 53)</td>
</tr>
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<td>USA</td>
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<td>O157</td>
<td>8</td>
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<td>(51)</td>
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<td>55</td>
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<td>(7)</td>
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<td>1999</td>
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<td>O157</td>
<td>37</td>
<td>suggestive</td>
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<td>(85)</td>
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<td>2003</td>
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<td>O157</td>
<td>20</td>
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<td>suggestive</td>
<td>(26)</td>
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<td>2003</td>
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<td>O157</td>
<td>57</td>
<td>medium</td>
<td>strong</td>
<td>(79)</td>
</tr>
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<td>2005</td>
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<td>O157</td>
<td>135</td>
<td>medium</td>
<td>strong</td>
<td>(70, 71)</td>
</tr>
<tr>
<td>2006</td>
<td>USA</td>
<td>Spinach</td>
<td>O157</td>
<td>225</td>
<td>very strong</td>
<td>strong</td>
<td>(13, 14, 31, 33, 36, 54, 64, 86)</td>
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<tr>
<td>Year</td>
<td>Country</td>
<td>Food Description</td>
<td>O-Number</td>
<td>Clinically Related Incidence</td>
<td>Virulence Markers</td>
<td>Evidence</td>
<td>Reference(s)</td>
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<td>50</td>
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<tr>
<td></td>
<td>and Iceland</td>
<td></td>
<td></td>
<td></td>
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<td>2010</td>
<td>USA</td>
<td>Romaine lettuce</td>
<td>O145</td>
<td>31</td>
<td>very strong</td>
<td>suggestive</td>
<td>(4, 15, 78)</td>
</tr>
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<td>Germany</td>
<td>Sprouts</td>
<td>O104:H4</td>
<td>3816</td>
<td>medium</td>
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<td>(3, 8, 25, 27, 30, 39, 62, 63, 81, 84)</td>
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<td>O157</td>
<td>58</td>
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<td>(69)</td>
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<td>Clover sprouts</td>
<td>O26</td>
<td>29</td>
<td>suggestive</td>
<td>no evidence</td>
<td>(16)</td>
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<td>USA</td>
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<td>O157</td>
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<td>medium</td>
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<td>(47)</td>
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<td>strong</td>
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<td>(18)</td>
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<td>Product</td>
<td>Strain</td>
<td>E. coli O:Antigen</td>
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<td>Evidence Type</td>
<td>Notes</td>
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<td>19</td>
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<td>10</td>
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<td>UK</td>
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<td>O157</td>
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<td>UK</td>
<td>Bagged mixed salad</td>
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<td>102</td>
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<td>(67)</td>
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<td>medium</td>
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<td>(23)</td>
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<td>medium</td>
<td>no evidence</td>
<td>(32)</td>
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<td>2016</td>
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<td>Rocket</td>
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<td>237</td>
<td>very Strong</td>
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<td>(40)</td>
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<td>67</td>
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<td>no evidence</td>
<td>(21, 56)</td>
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<td>Romaine lettuce</td>
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<td>218</td>
<td>suggestive</td>
<td>strong</td>
<td>(22, 58)</td>
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<td>91</td>
<td>suggestive</td>
<td>strong</td>
<td>(20, 57)</td>
</tr>
</tbody>
</table>

*a: SoE = strength of evidence*  
b: suggestive: only descriptive evidence available; medium: odds or risk ratio from epidemiological studies support salad/leaves; strong: microbiological evidence from salad/leaves; very strong: analytical epidemiological evidence and microbiological evidence  
c: no evidence: no evidence of STEC bacteria or faults in growing and/or production found; suggestive: faults in production that could have contributed to contamination identified; strong: trace back identified outbreak strain in animals near farms or within production facilities
Table 2: Investigations performed to help identify sources of contamination in STEC outbreaks attributed to sprouts, salads, and leafy green products

<table>
<thead>
<tr>
<th>Investigation performed</th>
<th>Number of outbreaks undertaking</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trace back through supply chain</td>
<td>32</td>
</tr>
<tr>
<td>Sampling of food products</td>
<td>25</td>
</tr>
<tr>
<td>Sampling of environment/water at either farm or production facility</td>
<td>17</td>
</tr>
<tr>
<td>Sampling of animal feces around farms</td>
<td>8</td>
</tr>
<tr>
<td>Stool Samples from employees</td>
<td>5</td>
</tr>
</tbody>
</table>