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Review

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 *Escherichia 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 whether 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 to 2018 and ranged from 8 to more than 8,500 cases. Detection of STEC in the food product was rare (4 of 35 outbreaks). For the remaining outbreaks, the determination of leafy greens as the source of the outbreak mainly relied on analytical epidemiology (20 of 35) or descriptive evidence (11 of 35). The traceback investigation in 21 of 32 outbreaks was not able to identify possible routes leading to 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 postharvest 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 traceback studies to inform control measures and future prevention.

HIGHLIGHTS

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

Key words: Epidemiology; Foodborne outbreaks; Food contamination; Public health; Shiga toxin–producing *Escherichia coli*; Sprouts

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Shiga toxin–producing *Escherichia coli* (STEC) causes gastrointestinal infections characterized by bloody diarrhea. Shiga toxins that enter the bloodstream can lead to hemolytic uremic syndrome (HUS), a serious complication of STEC infection that damages the kidneys and can lead to longer-term sequelae *(11)*. HUS most often affects children and is the leading cause of acute renal failure in children in the United Kingdom and elsewhere *(47)*. Given the severity of STEC infection and the potential for complications, STEC remains a pathogen undergoing public health research in an effort to reduce the number of infections.

The first documented outbreak of STEC O157:H7 occurred in 1982 and resulted from contaminated hamburgers from an American fast-food restaurant *(49),* leading to the nickname "burger bug" *(75)*. 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

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practices to reduce contamination both at slaughter and at retail sources *(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, 38, 46)*. In the United States, vegetable produce is the most frequent cause of STEC O157:H7 outbreaks after meat *(35, 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 *(44)*. Contamination during production could result from direct spread of animal feces via wildlife or rainwater runoff, particularly if animal reservoirs of STEC, such as cattle, sheep, or deer, are close to where produce is grown *(38, 45, 51)*. During cultivation, produce may also be contaminated by irrigation water sourced from rivers and/or ponds *(38)*. Because of nonhomogeneous distribution of STEC in the environment, cross-contamination during processing is possible if a batch of product from a contaminated field on a 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 *(76, 80),* increase the chance of an individual ingesting enough bacteria to cause illness. Because 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 farther up the food chain are important. Understanding the mechanisms of contamination involved and collating these data are required to inform better control measures. We performed a systematic review to determine whether there are identifiable recurrent features across STEC outbreaks caused by salad leaves, which 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 7 April 2019. The search strategy was limited to title, abstract, or keyword using the following medical subject heading terms or 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 gray literature, "stec salad outbreak" was searched in Google Scholar, and the first 100 hits were reviewed. The Web sites of the Centers 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 one of the authors (E.K.). To be considered for inclusion, articles had to describe an outbreak of STEC linked to salad, sprouts, or leaves. Full text of potential studies was 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 most 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 the outbreak, identified food item causing the outbreak, strain of STEC isolated, number of cases and associated hospitalizations, HUS cases and deaths, age range of cases, and gender ratio of cases. If analytical epidemiology studies were performed, 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 a traceback investigation was performed and the extent and results of food and environmental sampling in facilities associated with producing the food item linked to the outbreak. The full details extracted for each outbreak is available in the Supplemental Material.

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 used: suggestive evidence, descriptive results that implicated leafy greens; medium evidence, analytical epidemiological study with significant results that implicated leafy greens; strong evidence, microbiological evidence of STEC in the food item; and 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; and strong evidence, outbreak strain was identified in water or the environment near the 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 create graphs. A narrative synthesis approach was taken to summarize the results.

RESULTS

The searches of the databases returned 1,612 articles, a number reduced to 1,034 after deduplication (Fig. 1). The Google Scholar search only returned studies already found in the original search. Thirty-two references were identified by reviewing the bibliography of relevant literature or searching public health Web sites. One outbreak report meeting the inclusion criteria was provided directly by Public Health England. The full text of 105 articles was obtained and screened for eligibility; articles were excluded if they were laboratory-based research or review articles or if 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 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 described outbreaks occurred in

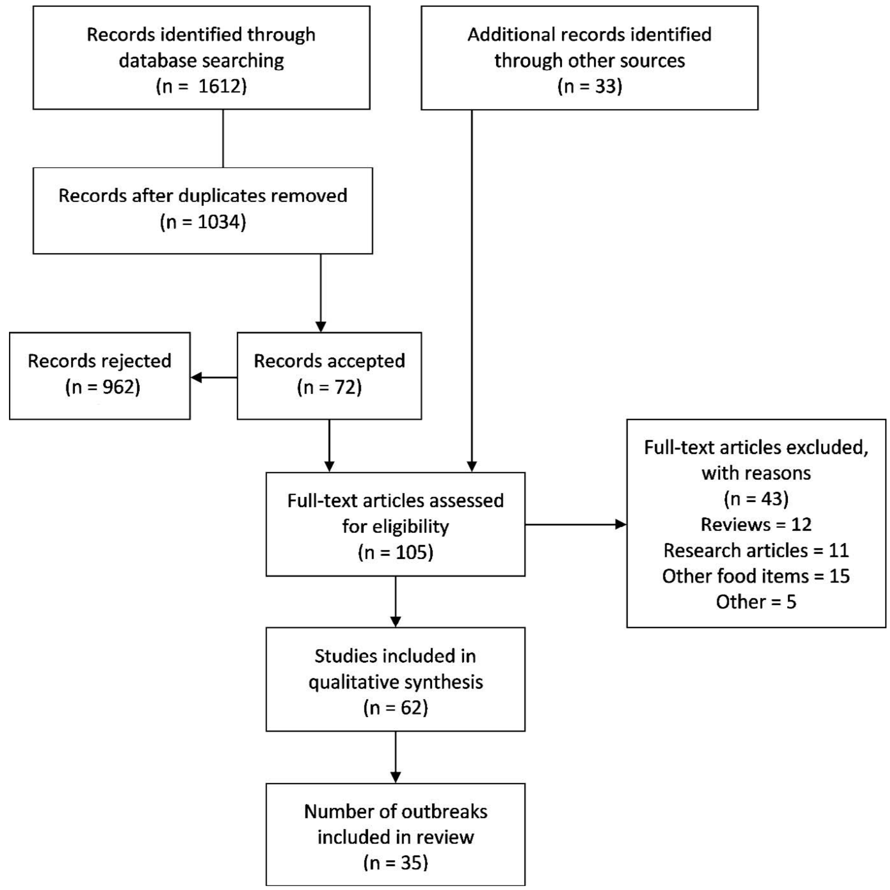

FIGURE 1. *Flow diagram of included studies.*

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 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 more than 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 to 398 (up to 63.75% of overall cases), HUS cases ranged from 0 to 39 (up to 22.28% of cases), and deaths ranged from 0 to 5 (up to 2.22% of cases). The reported median age of cases within the outbreaks ranged from 16 to 65 years, 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). Of the 35 outbreaks, only 4 had a strong or very strong level of evidence associated with determining the food source; 20 had medium levels of evidence, and 11 studies had only

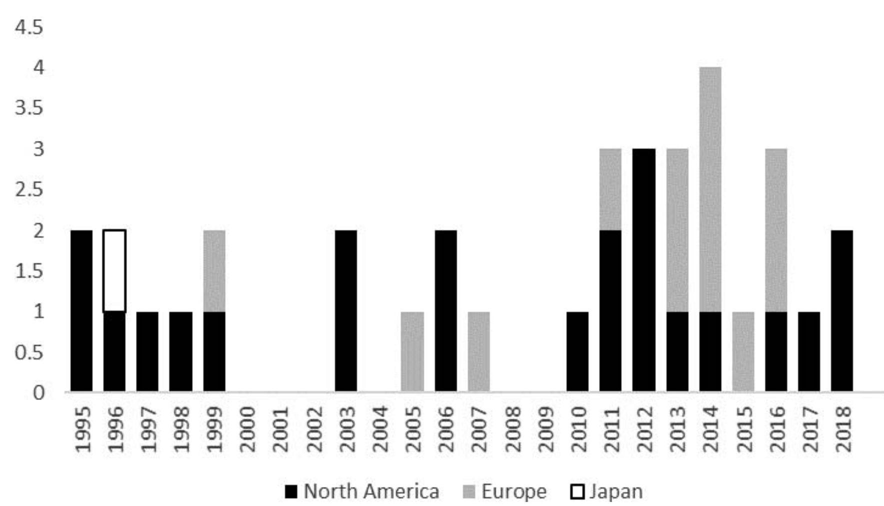

FIGURE 2. *Number of STEC outbreaks per year by location.*

TABLE 1. *Summary of STEC outbreaks attributed to sprouts, salad, and leafy green products*

					SofE[a]		
Year	Location	Food item	STEC	Cases	Food[b]	Contamination[c]	Reference(s)
1995	USA	Lettuce	O157	40	Medium	Suggestive	*1*
1995	Canada	Iceberg lettuce	O157	21	Medium	Suggestive	*55*
1996	USA	Mesclun lettuce	O157	61	Medium	Suggestive	*36*
1996	Japan	Radish sprouts	O157	8,500	Medium	No evidence	*39, 50*
1997	USA	Alfalfa sprouts	O157	51	Medium	No evidence	*6, 11*
1998	USA	Sprouts	O157	8	Medium	Suggestive	*52*
1999	USA	Lettuce	O111:H8	55	Medium	No evidence	*7*
1999	Sweden	Lettuce	O157	37	Suggestive	No evidence	*85*
2003	USA	Alfalfa sprouts	O157	20	Medium	Suggestive	*27*
2003	USA	Bagged mixed salad	O157	57	Medium	Strong	*79*
2005	Sweden	Lettuce	O157	135	Medium	Strong	*70, 71*
2006	USA	Spinach	O157	225	Very strong	Strong	*14, 15, 32, 34, 37, 54, 64, 86*
2006	USA	Lettuce	O157	80	Medium	No evidence	*13, 72*
2007	Netherlands and Iceland	Lettuce	O157	50	Medium	No evidence	*29, 30, 66*
2010	USA	Romaine lettuce	O145	31	Very strong	Suggestive	*4, 16, 78*
2011	Germany	Sprouts	O104:H4	3,816	Medium	No evidence	*3, 8, 26, 28, 31, 40, 62, 63, 81, 84*
2011	USA	Romaine lettuce	O157	58	Medium	No evidence	*69*
2011	USA	Clover sprouts	O26	29	Suggestive	No evidence	*17*
2012	USA	Bagged salad	O157	16	Medium	No evidence	*48*
2012	USA	Bagged mixed salad	O157	33	Strong	No evidence	*18*
2012	Canada	Shredded lettuce	O157	31	Suggestive	No evidence	*77*
2013	Sweden	Salad	O157	28	Medium	No evidence	*25*
2013	UK	Watercress	O157	22	Medium	Strong	*38, 45*
2013	USA	Salad	O157	33	Suggestive	No evidence	*19*
2014	USA	Clover sprouts	O121	19	Suggestive	Suggestive	*20*
2014	UK	Rocket	O157	10	Suggestive	No evidence	C.J. and L.B., personal communication
2014	UK	Slaw	O157	20	Suggestive	No evidence	*10*
2014	UK	Bagged mixed salad	O157	102	Medium	No evidence	*67*
2015	UK	Bagged mixed salad	O157	49	Medium	Strong	*51*
2016	USA	Alfalfa sprouts	O157	11	Suggestive	No evidence	*21*
2016	UK	Bagged mixed salad	O157	166	Medium	No evidence	*33*
2016	Finland	Rocket	ONT:H11	237	Very strong	No evidence	*41*
2017	USA and Canada	Leafy greens	O157	67	Suggestive	No evidence	*22, 56*
2018	USA and Canada	Romaine lettuce	O157	218	Suggestive	Strong	*23, 57*
2018	USA and Canada	Romaine lettuce	O157	91	Suggestive	Strong	*24, 58*

[a] SofE, strength of evidence.
[b] Suggestive, only descriptive evidence is available; medium, odds or risk ratio from epidemiological studies supports salad or leaves; strong, microbiological evidence from salad or leaves; very strong, analytical epidemiological evidence and microbiological evidence.
[c] No evidence, no evidence of STEC bacteria or faults in growing and/or production were found; suggestive, faults in production that could have contributed to contamination were identified; strong, traceback identified the outbreak strain in animals near farms or within production facilities.

descriptive evidence. The 23 analytical epidemiological studies included a range of 16 to 521 participants (cases plus controls or total cohort). Odds or risk ratios ranged from 1.93 (confidence interval of 1.38 to 2.7) to 100 (confidence interval of 6.47 to undefined) for identifying salad, sprouts, or leafy greens as the source of the outbreak.

Six studies had strong evidence and eight studies suggestive evidence for how the STEC bacteria contaminated the product (Table 1). For the six outbreaks with strong evidence, four studies 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 feces near the growing fields, alongside detection of the stx_2 gene in the irrigation water or use of untreated irrigation water. Detection of *stx* is an indicator of the presence of STEC even when there is not conclusive evidence it belonged to the outbreak strain. For the eight outbreaks with suggestive evidence, five studies recorded either floods on the growing fields or noted a failure to adhere to disinfection protocols. Most studies *(22)* 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 investi-

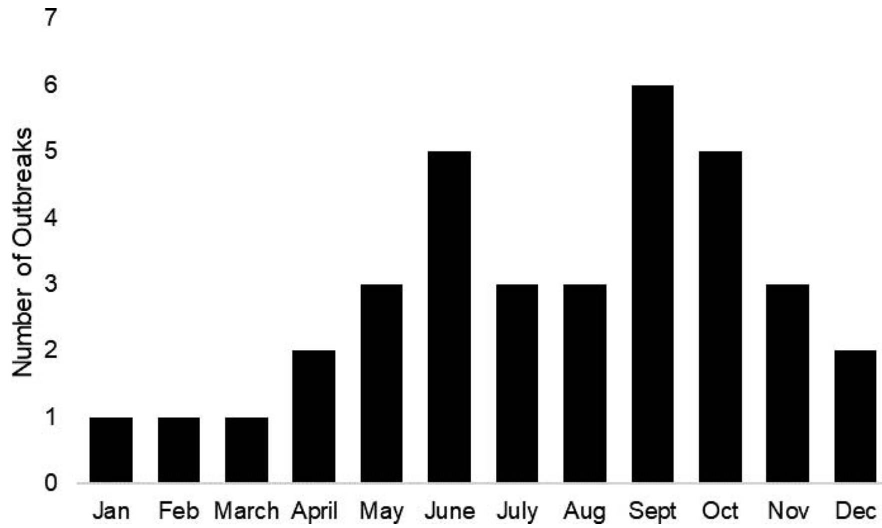

FIGURE 3. *Month when first case of outbreak became ill.*

gations performed for each outbreak were recorded (Table 2). Traceback 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 feces near the growing fields, while five investigations collected stool samples from employees working at either production facilities or as kitchen staff.

DISCUSSION

This article describes 35 outbreaks caused by STEC contamination of sprouted or leafy green products. There was variation among the outbreaks in terms of duration and number of cases. The outbreak strain was detected within the salad product in only 4 outbreaks despite testing being carried out in 25 of the outbreaks reviewed. This may be because of the short shelf life of the product, intermittent contamination, or levels of contamination below the detectable limits of the test *(38)*. However, for most outbreaks (23 of 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 the length of time of the outbreak and thus the number of people 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. 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 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 irrigation water, serving as the likely route of contamination. For most outbreaks (21 of 35), no production faults or source of the STEC contamination was identified, even though 32 studies initiated a traceback 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 review investigated the sources of sporadic STEC infections *(42)*. Only 2 (8.3%) of the 24 studies that

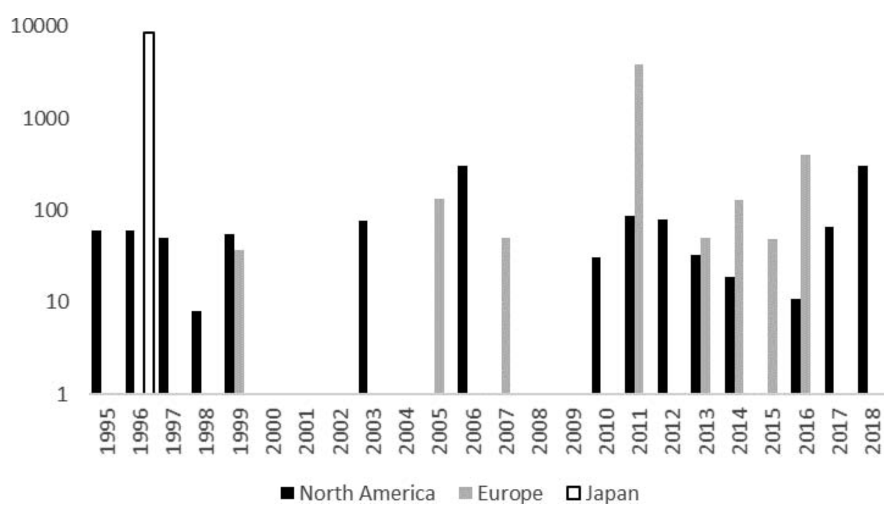

FIGURE 4. *Number of cases for STEC outbreaks per year by location.*

TABLE 2. *Investigations performed to help identify sources of contamination in STEC outbreaks attributed to sprouts, salad, and leafy green products*

Investigation performed	No. of outbreaks performing
Traceback through supply chain	32
Sampling of food products	25
Sampling of environment or water at either farm or production facility	17
Sampling of animal feces around farms	8
Stool samples from employees	5

investigated produce identified it as a significant risk factor. In the United Kingdom, O'Brien et al. *(53)* identified watercress (odds ratio of 2.61 [1.24 to 5.47]) as a source of sporadic STEC infections; the only watercress-specific outbreak identified in this review also occurred in the United Kingdom more than 16 years after this case-control study of sporadic STEC infections was performed *(38, 45)*. In Canada, Wang et al. *(82)* compared exposures for O157 versus non-O157 STEC infections and found that bagged greens were associated with sporadic non-O157 infections ($P = 0.014$). Only six outbreaks resulted from non-O157 STEC strains in this systematic review, but the results from Wang et al. suggest we may be missing smaller salad outbreaks because of 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 articles and reports that contained enough extractable data to be included. Several review articles and governmental reports provided summary statistics on other STEC salad outbreaks *(43, 68, 83),* but without full details on the investigation or the outbreak characteristics, these were not included. In addition, many reports obtained from public health Web sites on 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 with 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 *(38)*. However, the advent of whole genome sequencing of STEC isolates means that these smaller, widespread outbreaks are less likely to be missed in countries where this technology is routinely implemented. The information provided from the more sensitive detection of outbreaks could 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. Because salad products are not cooked by consumers or pasteurized before 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 earlier, most 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 traceback investigations. This article recommends accepting epidemiological evidence to initiate traceback investigations when leafy green products are suspected as the source of an outbreak to 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 MATERIAL

Supplemental material associated with this article can be found online at: https://doi.org/10.4315/0362-028X.JFP-19-014.s1

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