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COMPREHENSIVE REVIEW

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Understanding potential cattle contribution to leafy green outbreaks: A scoping review of the literature and public health reports

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Abstract

Recently, multiple reports from regulatory agencies have linked leafy green outbreaks to nearby or adjacent cattle operations. While they have made logical explanations for this phenomenon, the reports and data should be summarized to determine if the association was based on empirical data, epidemiological association, or speculation. Therefore, this scoping review aims to gather data on the mechanisms of transmission for pathogens from livestock to produce, identify if direct evidence linking the two entities exists, and identify any knowledge gaps in the scientific literature and public health reports. Eight databases were searched systematically and 27 eligible primary research products, which focus on produce safety concerning proximity to livestock, provided empirical or epidemiological association and described mechanisms of transmission, qualitatively or quantitatively were retained. Fifteen public health reports were also covered. Results from the scientific articles provided evidence that proximity to livestock might be a risk factor; however, most lack quantitative data on the relative contribution of different pathways for contamination. Public health reports mainly indicate livestock presence as a possible source and encourage further research. Although the collected information regarding the proximity of cattle is a concern, data gaps indicate that more studies should be conducted to determine the relative contribution of different mechanisms of contamination and generate quantitative data to inform food safety risk analyses, regarding leafy greens produced nearby livestock areas.

KEYWORDS

cattle, *Escherichia coli* O157:H7, nearby/adjacent land use, *Salmonella*, vegetable row crops

1 | INTRODUCTION

Food safety risks due to the consumption of leafy greens and other fresh produce have become more pronounced as consumer demand increases. The increase in demand led to denser production in larger areas and greenhouses that caused supply chain disruptions and susceptibility to pathogenic contamination events (Gil et al., 2015). Foodborne outbreaks related to leafy greens, which are mainly consumed raw, are a major concern since the number of associated outbreaks and disease burden have seen an increase over the past few decades (Herman et al., 2015). Although several pathogens such as *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., and Norovirus are suspected or confirmed as the etiology of these outbreaks, *E. coli* O157:H7 and Shiga toxin-producing *E. coli* (STEC), in general, received particular attention as an emerging hazard in leafy green products. Between 2009 and 2018, 18 out of 40 outbreaks linked to STEC infections in the United States and Canada were confirmed to be associated with leafy greens, and 22 outbreaks were suspected to be related (Marshall et al., 2020). Foodborne disease source attribution estimates, by the Interagency Food Safety Analytics Collaboration (IFSAC, 2021), also indicate that the impact of vegetable row crops, including leafy greens, on the attribution of common pathogens, especially *E. coli* O157 and *Listeria monocytogenes*, has considerably increased within the past few years. Therefore, efforts to mitigate microbial risks related to the consumption of leafy greens have been an emerging focus of food safety studies and regulatory activities.

Although the records show that outbreaks of leafy greens were reported as early as 1973, when official surveillance of foodborne illnesses had been established in the United States, it was not until the modernization of the foodborne pathogen surveillance activities during the early 2000s when outbreaks were more frequently reported (Herman et al., 2015). In 2006, the deadliest leafy greens-associated *E. coli* O157:H7 outbreak in the United States occurred, causing five deaths and several hundred confirmed cases and hospitalizations (Gelting, n.d.). Furthermore, this was the first time an outbreak investigation report signaled a contribution of nearby cattle grazing to the contamination of fresh produce, in addition to other environmental and production factors. Since 2006, several leafy greens-associated outbreaks occurred in the United States, Canada, and the European Union, as well.

As a response to growing concerns about fresh produce contamination, the U.S. Food and Drug Administration (FDA) has implemented the Final Produce Safety Rule (FDA, 2016) in conjunction with the Food Safety Modernization Act (FSMA) and initiated the Leafy Greens STEC Action Plan (LGAP) (FDA, 2020b) for prevention, response, and addressing knowledge gaps regarding risks associated with leafy greens. Leafy green growers in California and Arizona also formed Leafy Green Products Handler Marketing Agreement (LGMA) programs in each state to voluntarily implement science-based food safety measures to control contamination of their produce (Arizona Leafy Greens Marketing Agreement, n.d.; California Leafy Greens Marketing Agreement, 2020). These programs require and/or recommend growers to adhere to certain standards regarding agriculture water, biological soil amendments in terms of raw manure and compost, domestic and wild animals, worker training and hygiene, equipment, tools, and buildings, indicating that the contamination of leafy greens is a multifactorial process that needs to be addressed by a holistic approach. The terms of distance or proximity to livestock operations, or adjacent and nearby land use are often mentioned in these documents as a risk factor for leafy green contamination. Currently, there are no enforced limits for the distance between produce fields and livestock operations; however, the LGMA recommends interim distances of 9.14 m (30 ft) from the edge of a crop field to grazing land and other domestic animals, 121.92 m (400 ft) to composting operations, and 365.76 m (1200 ft) to 1609.34 m (1 mile) to concentrated animal feeding operations (CAFOs) due to lack of scientific evidence at the time these recommendations were made (California Leafy Greens Marketing Agreement, 2020).

Major hazards of concern for leafy greens such as pathogenic *E. coli*, *Salmonella* spp., or *L. monocytogenes* are known to be harbored by cattle and other ruminants (Callaway et al., 2006). Therefore, it is mainly hypothesized that pathogens found on fresh produce are primarily sourced from livestock grown nearby or natural fertilizers that are transported to the fields. The proximity of cattle or livestock, in general, was reported as a risk factor for contamination in the surrounding environment by the major foodborne microbiological hazards; however, the exact mechanisms of transmission are still uncertain, limiting the efforts for risk-based decision-making to mitigate contamination. According to the updated LGAP document by the FDA (2020b), the possible pathways of contamination for leafy greens to human pathogens were identified as soil, fertilizers, direct animal contact, air/dust, and water. A lack of quantitative and qualitative information about the possible mechanisms of transmission from livestock to produce was also indicated in this report.

Increasing disease burden of STECs and other produce-related pathogens, which are associated with reoccurring outbreaks from the same type of products (mostly romaine lettuce and spinach), caught enormous attention from the public and news outlets in the United States. Since official traceback investigations and environmental assessments of leafy greens-associated outbreaks have unique challenges and take long periods of time to conduct, traceback investigations and risk communication are often delayed (Irvin et al., 2021), leaving room for interpretation by nonexperts.

This scoping review seeks to provide a comprehensive review of the scientific literature and public health reports from government agencies on the association of adjacent and/or nearby cattle with leafy green outbreaks from foodborne pathogens. This study specifically aims to (1) provide a scope of the current knowledge to support future research and systematic reviews and (2) address the gaps in knowledge on the possible mechanisms of transmission of pathogens from livestock to fresh produce and their significance. It is expected that this review will aid industry and regulatory authorities to implement risk assessments and mitigation measures to reduce the occurrence of leafy greens-associated diseases to better control environmental factors and direct future research in the areas of identified knowledge gaps.

2 | METHODOLOGY

2.1 | Review framework and question

A scoping review of the scientific literature, public health reports, and digital media was conducted based on the guidelines suggested by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines extension for scoping reviews (Arksey & O'Malley, 2005; Tricco et al., 2018). A review protocol was developed before conducting the review; however, it was not registered. The main research question was formulated based on the Population, Phenomena of Interest, Context (PICo) framework (Lockwood et al., 2015): "What is the current scope of the literature, public health reports, and social media on the potential cattle contribution (Phenomenon of Interest) to outbreaks associated to leafy greens (Population) grown on nearby or adjacent lands (Context)?" The review specifically sought to answer the questions:

1. Is there evidence that cattle production contributes to the contamination of leafy greens with foodborne pathogens?
2. What are the proposed or proven mechanisms of transmission between cattle operations and produce?
3. Does the consumption of leafy greens produced near cattle operations increase the risk of foodborne disease?
4. What are the knowledge gaps in mechanisms of transmission from cattle operations to produce fields and their contribution to foodborne diseases?

2.2 | Identification of relevant reports

Ten generic and agricultural, food science, or public health-specific databases (Agricola, Biological Abstracts, Biological & Agricultural Index Plus, BIOSIS Citation Index, CAB Abstracts, Food Science and Technology Abstracts, MEDLINE Complete, ProQuest, Scopus, and Web of Science Core Collection) and two secondary sources (Google Scholar and Microsoft Academic) were searched for the keywords given in Table 1. For public health reports, the location was limited to the United States and the Centers for Disease Control and Prevention (CDC), FDA, United States Department of Agriculture Food Safety and Inspection Service (USDA FSIS), and Animal and Plant Health Inspection Service (APHIS) databases were manually searched. All searches were finalized in December 2021, without any date, location, or language limitations. Records were exported to citation management software Endnote (Version 20.2.1, Clarivate, Philadelphia, PA, USA).

Records collected from the scientific literature were subject to a two-stage screening process by two independent reviewers (OD & MF) and confirmed by a third reviewer (MB) if two initial reviews conflicted. The first screening was based on the titles and abstracts and the second screening was done by retrieving and screening through full texts. Records were included if they (1) are primary research (peer-reviewed articles, dissertations, project reports, full-length conference papers), (2) focus on produce safety in relation to proximity to livestock operations, (3) provide an epidemiological or empirical association between cattle and produce, (4) describe mechanisms of transmissions of pathogens, and (5) were qualitative or quantitative. Exclusion criteria for scientific literature were (1) review articles, (2) no association between cattle and produce is described, (3) not available in English, and (4) full text cannot be retrieved. Selected articles were read and summarized by the two independent reviewers using predetermined data charting schemes given in the Supporting Information and compared for accuracy. Results are presented in the summary of findings tables and explained in the narrative.

3 | RESULTS AND DISCUSSION

3.1 | Scientific literature

A flow diagram of the scoping review process suggested by PRISMA (Tricco et al., 2018) is provided in Figure 1. After the screening process, 27 scientific reports, in the form of published peer-reviewed articles and project findings reports, were selected for extraction and inclusion in the review. Characteristics of included studies are listed in Table 2 and a detailed overview of the collected studies is provided in a summary of findings table in Table 3. A majority of the reported outcomes (28/34) were based on data collected in the United States; the microbiological hazard of interest was pathogenic or generic *E. coli* (33/50); and the target produce was several types of leafy greens (32/52) and animal source of various cattle (21/35).

Based on the findings of this review, the possible preharvest mechanisms of transmission from adjacent/nearby livestock to leafy greens were identified as biological soil amendments (fertilizer), water, direct animal contact, air/dust, wild animals/insects, and soil (Figure 2), as also indicated by the LGAP (FDA, 2020b). It is hypothesized that these pathways contribute to the contamination of either soil or the produce itself directly. Biological soil amendments or fertilization in general is a key factor

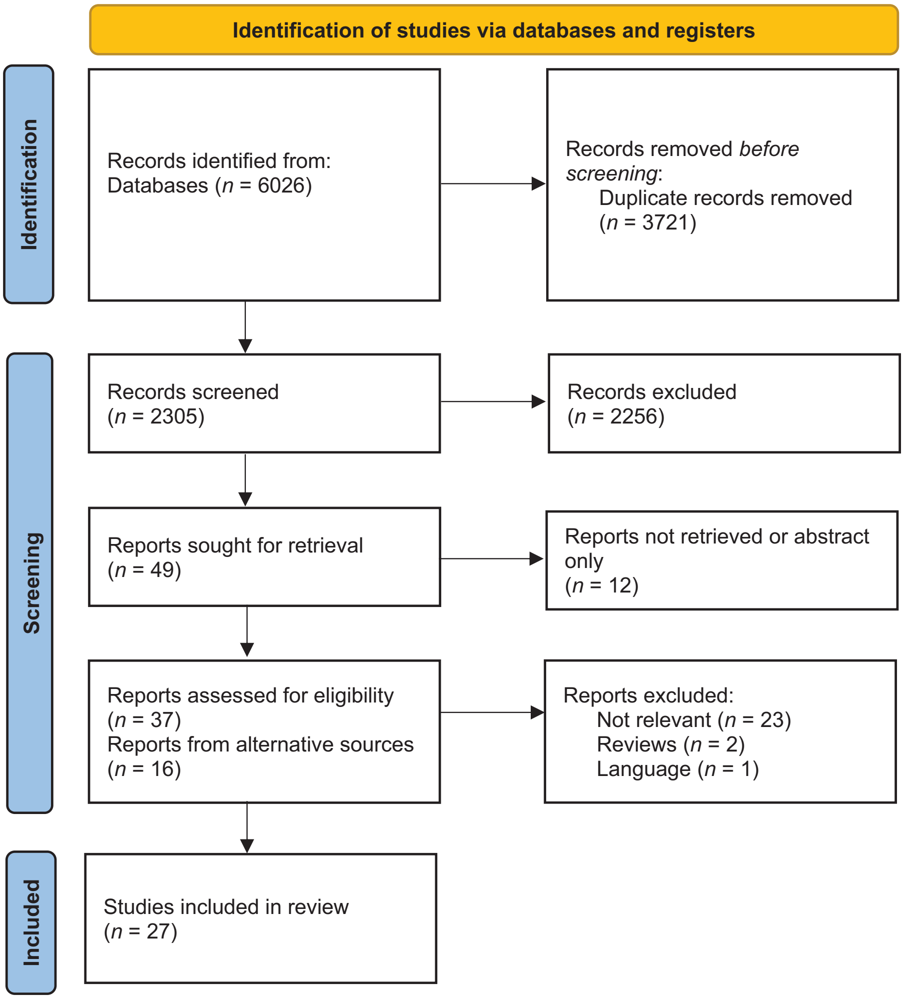

FIGURE 1 PRISMA 2020 flow diagram for scoping review flowchart.

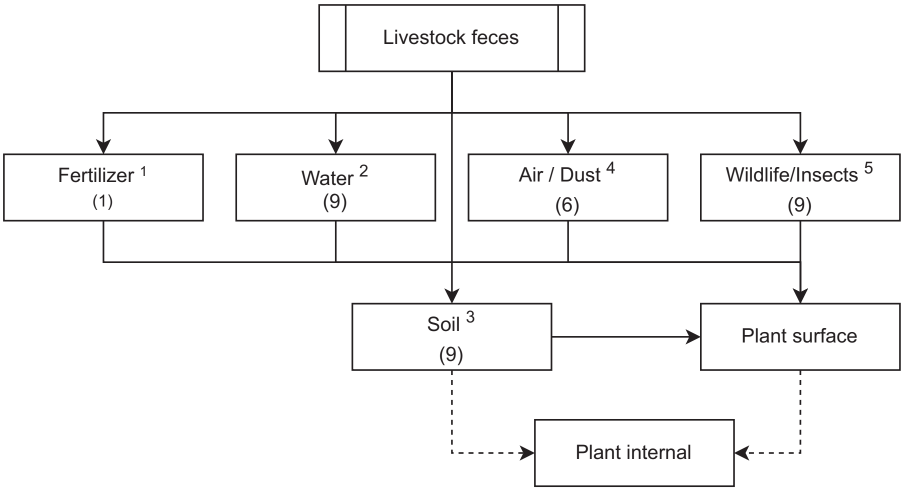

FIGURE 2 Proposed pathways of contamination of leafy greens originating from livestock feces. Numbers in parentheses indicate the number of scientific articles identified for the specified pathway. [1]Glaize et al. (2020). [2]Jay et al. (2007), Soderstrom et al. (2008), Gelting et al. (2011), Baloch (2014), Gelting et al. (2015), Kabiru et al. (2015), Mishra et al. (2017), Pang et al. (2017), and Weller et al. (2020). [3]Jay et al. (2007), Hoar et al. (2013), Strawn et al. (2013), Baloch (2014), Thakur et al. (2016), Weller et al. (2016), Mishra et al. (2017), Glaize et al. (2020), Weller et al. (2020), and Glaize et al. (2021). [4]Yanamala et al. (2011), Hoar et al. (2013), Berry et al. (2015), Thakur et al. (2016), Pang et al. (2017), and Glaize et al. (2021). [5]Jay et al. (2007), Talley et al. (2009), Jay-Russell et al. (2012), Baloch (2014), Thakur et al. (2016), Mishra et al. (2017), Berry et al. (2019), Glaize et al. (2020), and Hamilton et al. (2021).

TABLE 1 Breakdown of the research question to Population, Phenomena of Interest, Context (PICo) framework elements, main keywords, and synonyms used in the search algorithm.

PICo element	Explanation	Main keyword	Synonyms
(P)opulation	Leafy greens.	Leafy greens	("leafy greens" OR salad OR sprout* OR "pot herb" OR vegetable OR spinach OR cabbage OR arugula OR lettuce OR kale OR chard OR cress OR iceberg OR broccoli OR collard OR parsley OR coriander OR fruit)
Phenomenon of (I)nterest	Contribution of cattle to leafy green outbreaks.	Cattle	(cattle OR beef cattle OR cow OR livestock) AND (feedlot OR feedyard OR pen OR graz* OR ranch OR farm OR pasture)
(Co)ntext	Produce farming nearby or adjacent to cattle operations.	Proximity	(close OR "near*" OR proxim* OR adjacen* OR adjoin* OR contigu*)

that contributes to the contamination of produce. However, since manure can be hauled from any distance to the produce fields, studies focusing on the transfer from raw manure or compost applied to the soil were excluded from the scope of this review, and only included if the produce fields are located nearby or adjacent to manure stockpiling operations. Although the internalization of pathogens is also included in Figure 2, it is still unclear how the pathogens can attach and survive in or on the fresh produce, as the reports were conflicted about possible internalization or attachment of the pathogen to the surface of leafy greens. Although several reports indicate the possibility of internalization of pathogenic bacteria and viruses through the plant leaves and stems at high concentrations (6–8 $\log_{10}$ CFU/mL), some conclude that internalization would not occur at lower concentrations, since it is dependent on environmental and production variables such as temperature, moisture, and/or damage to the produce (Erickson, 2012; Hirneisen et al., 2012; Riggio et al., 2019).

Majority of the studies included in this section were conducted on the West Coast of the United States, including California and Arizona where majority of the fresh produce supply of the country is produced in large-scale, high-density farms under LGMA, whereas more small-scale farms exist in the other regions, especially the East Coast and some of the results summarized here come from experimental research stations and not commercial farms. Therefore, regional differences in terms of management and production practices could result in differences in food safety risks presented here. However, an analysis of these regional differences was out of the scope of this review.

3.1.1 | Water

Water is a major resource in leafy greens production and processing, where it is often sourced from the ground or surface water sources nearby produce operations. Although water is mainly needed for irrigation, it is also essential for the cooling, decontamination, and washing of the produce and equipment, as well as for pesticide and herbicide applications (Gerba, 2009). Therefore, if the water used in leafy green production and processing is contaminated with human pathogens, fresh produce can also become contaminated (Rock et al., 2019). Based on the results from our scoping review, water can contribute to preharvest contamination by using contaminated irrigation water or runoff from nearby/adjacent livestock areas.

Jay et al. (2007) reported the findings from the 2006 spinach outbreak investigation with environmental assessment focusing on transmission via wild animals, water, and soil using pulsed-field gel electrophoresis (PFGE) and multilocus variable number tandem repeat analysis (MLVA). Investigators isolated the *E. coli* O157:H7 strain implicated in the outbreak from cattle feces approximately 1.6 km (reported as 1 U.S. mile) away from the produce fields and in 28 environmental samples, including two surface water samples. The exact mechanism of transmission from cattle to spinach could not be identified; however, it was hypothesized that fecal contamination of surface water by livestock could be one of the possible routes for contamination while ruling out the possibility of a runoff. Similarly, Gelting et al. (2011) reported solely on the issues related to irrigation water during the same outbreak and indicated that the hydrogeological factors, along with cattle and wild pigs having direct access to the San Benito River, contributed to the contamination of spinach. Gelting et al. (2015) also conducted a qualitative systems analysis of the same outbreak to describe the irrigation system and identify sources of contamination and susceptibility of the overall system to a failure that caused the outbreak. A possible pathway of contamination was identified as the wastewater from a nearby dairy farm, which was mixed with the wastewater lagoons and groundwater sources that

TABLE 2 Characteristics of 24 studies included in the review.

Characteristic	Count of each outcome[a]
Location	
United States	28
California	10
North Carolina	3
Tennessee	3
Arizona	2
Nebraska	2
Colorado	1
Maryland	1
Nevada	1
New York	4
Oregon	1
NR	2
Chile	1
Mexico	1
Nigeria	1
Republic of Korea	1
Microbiological hazard	
E. coli	33
E. coli O157:H7	13
Generic *E. coli*	9
STEC	5
E. coli O145	1
AMR non-O157 *E. coli*	1
AMR *E. coli*	1
EHEC	1
Other diarrheagenic *E. coli*	2
Salmonella	8
Salmonella spp.	7
S. enterica	1
Listeria	7
Listeria spp.	3
L. monocytogenes	4
Campylobacter spp.	1
Coliforms	1
Produce	
Various produce (non-LG)	20
Leafy greens total	32
Various lettuce	10
Spinach	8
Unspecified leafy greens	6
Turnip greens	3
Cabbage	2
Mustard greens	2
Brassica	1
Broccoli	1

(Continues)

TABLE 2 (Continued)

Characteristic	Count of each outcome[a]
Animal source	
Cattle (total)	21
Beef cattle	8
Unspecified cattle	7
Dairy cattle/cow	6
Unspecified/other livestock	6
Poultry	4
Small ruminants/sheep	2
Swine	2
Mechanism of transmission	
Water	9
Soil	9
Air	6
Not specified	6
Insects	5
Wild animals	4

[a]Each study can report multiple outcomes from multiple locations, populations, hazards, and mechanisms of transmission, therefore; the total counts will exceed the total number of included studies.

were also used for the irrigation of the produce. It was also noted that the wastewater system lacked controls against backflow and was not inspected properly. Baloch (2014) reported a case study of environmental assessment from the 2010 *E. coli* O145 outbreak from the consumption of lettuce, focusing on transmission of the pathogen via water, soil, and wild animals. During this study, a nearby recreational vehicle (RV) park was identified as the potential source, but no STECs were isolated from the water samples. However, a dairy feedlot sharing stormwater systems with the nearby lettuce ranch was considered as potential and permanent source of STEC. A "four-dimensional, spatial–temporal approach" was suggested to follow for future environmental assessment, taking all the environmental variables that can contribute to contamination into account. Kabiru et al. (2015) reported the prevalence of pathogenic *E. coli* in cattle feces, effluent from a cattle abattoir nearby produce fields, and water and vegetable samples in Nigeria. They provided evidence by polymerase chain reaction (PCR) that the untreated effluent from the abattoir could have been released into the river and used for irrigation, contaminating the produce irrigated by the low-quality water. Soderstrom et al. (2008) reported the findings from the 2005 verotoxin-producing *E. coli* (VTEC) outbreak from lettuce consumption in Sweden. The investigators were able to match the VTEC O157 Stx2 strain isolated from the patients to a cattle farm upslope at an unreported distance from the irrigation sources used for lettuce production. Although no water samples were

TABLE 3 Summary of findings for the scientific articles in chronological order.

Reference	Location	Produce type	Animal	Study type	Outbreak	Study settings	Duration	Distance to animal operation	Hazard	Mechanism of transmission	Outcome	Main conclusions	Recommended action	Association
Hilborn et al., 1999	Outbreaks in CT and IL, producer location not reported.	Mesclun lettuce	Not specified	Outbreak investigation (epidemiological, environmental, and field)	1997	Lettuce farm and adjacent small-scale cattle operation. Free-range chicken had access to both cattle and produce.	NR	Adjacent	*E. coli* O157:H7	Not specified	Matched case–control odds ratio, prevalence in produce and water samples.	Hazard was not detected during environmental assessment. Adjacent cattle and chicken were identified as potential source of contamination.	Improved surveillance.	Hypothesized
Jay et al., 2007	San Benito, CA	Spinach	Beef cattle	Outbreak investigation (environmental assessment)	2006	Spinach field on a ranch with cattle pasture separated by wire fence.	NR	1,609 m (1 mile)	*E. coli* O157:H7	Wild animals, water, soil	Prevalence in feral swine, cattle, water, sediment, soil. Genetic matching.	Mechanism of transmission is unclear and a multifactorial process, but wildlife is indicated.	Additional research to improve risk assessment and management practices.	Genetic similarity
Soderstrom et al., 2008	Sweden	Lettuce	Cattle	Outbreak investigation (epidemiological, environmental, and field)	2005 (Sweden)	Commercial farms. Eight cattle farms upstream from irrigation water source.	NR	NR	VTEC O157	Water (irrigation)	Prevalence in irrigation water, cattle fecal samples, lettuce, and other produce.	Hypothesized that the outbreak was caused by irrigation water with indication of contamination from upstream cattle operations.	Control of water quality and improvements in handling fresh produce.	Genetic similarity to outbreak strain
Talley et al., 2009	Salinas, CA	Spinach, lettuce, carrots, brassicas	Cattle or other livestock	Experimental, challenge test	–	Four leafy green fields adjacent to cattle rangeland	NR	Farm 1: adjacent, farm 2: 15 m, farm 3: adjacent, farm 4: adjacent	*E. coli* O157:H7	Insects (filth flies)	Prevalence of *E. coli* in collected flies, pathogen transmission events.	Flies can transmit pathogens from rangelands to leafy greens, justifying further investigation.	Additional research on association of flies with contamination of produce.	Experimental (tagged bacteria)
Yanamala et al., 2011	NR	Spinach	Cattle	Experimental	–	Raw spinach placed near feedyard within the path of wind.	6, 12, 24 h	0, 18.3, 45.7 m	Generic *E. coli*, *E. coli* O157:H7, *Salmonella*	Airborne (dust, wind)	Concentration of generic *E. coli*; prevalence of *E. coli* O157:H7 and *Salmonella*	Distance between feed yards and leafy greens should be carefully considered to mitigate risk of contamination.	Dust control and further research to determine safe distances.	Experimental (decreased contamination with increased distance)

(Continues)

TABLE 3 (Continued)

Reference	Location	Produce type	Animal	Study type	Outbreak	Study settings	Duration	Distance to animal operation	Hazard	Mechanism of transmission	Outcome	Main conclusions	Recommended action	Association
Gelting et al., 2011	San Benito, CA	Spinach	Cattle	Outbreak investigation (environmental assessment)	2006	Commercial farm located nearby livestock and other intense agriculture.	NR	NR	*E. coli* O157:H7	Water (irrigation, runoff)	PFGE patterns collected from produce, environment, and feces.	Surface water was potentially contaminated with cattle/wild pig feces, reaching irrigation walls and contaminating the produce.	Outbreak investigations should include spatial-temporal analysis and consider ground-surface water interactions.	Hypothesized
Jay-Russell et al., 2012	San Benito, CA	Spinach	Beef cattle	Outbreak investigation (environmental assessment)	2006	Spinach field on a ranch with cattle pasture separated by wire fence.	NR	1,609 m (1 mile)	*Campylobacter* spp.	Wild animals	Prevalence in feral swine.	Feral swine may acquire *Campylobacter* from cattle to theoretically contaminate produce fields.	Lethal and non-lethal methods to control feral swine (hunting, depredation, trapping, fencing)	Genetic similarity between cattle and feral swine isolates
Cooley et al., 2013	California central coast counties (Monterey, San Benito, San Luis Obispo)	Lettuce, spinach	Cows	Experimental, longitudinal	–	Private produce farms and ranches	3 years	NR	STEC, *E. coli* O157:H7, generic *E. coli*	Not specified	Prevalence in domestic and wild animals, soil produce, water, and sediment.	Cattle and feral pig isolates showed commonalities, leading to an indication of the role of wildlife in spread of STEC.	None	Genetic similarity to outbreak strain
Hoar et al., 2013	Imperial, CA	Unspecified leafy greens	Sheep	Longitudinal	–	Leafy green fields with adjacent sheep grazing areas.	11 months	2, 5, 10, 20, 50, 100 m (air samples)	*E. coli* O157:H7, *Salmonella*, generic *E. coli*, coliforms	Air, soil	Prevalence of *E. coli* O157:H7, *Salmonella* in sheep feces and soil. PFGE profiles of *E. coli* O157:H7. Concentration of generic *E. coli* and coliforms in feces and soil. Concentration of unspecified airborne bacteria.	Grazing sheep are considered "low risk" for leafy green contamination.	LGMA recommended buffer distance (30 ft) is justified and deemed more than enough.	Linear regression (fecal or soil concentration to aerosol bacterial concentration), prevalence

(Continues)

TABLE 3 (Continued)

Reference	Location	Produce type	Animal	Study type	Outbreak	Study settings	Duration	Distance to animal operation	Hazard	Mechanism of transmission	Outcome	Main conclusions	Recommended action	Association
Strawn et al., 2013	New York State	Unspecified fruits and vegetables	Not specified	Longitudinal field study, geospatial models	–	Five produce farms	2 years	>62.6 m and <62.5 m	*L. monocytogenes*, *Salmonella*, STEC	Soil	Prevalence of *L. monocytogenes* at different locations on produce field surfaces.	Pasture proximity was a significant factor for increasing prevalence of *L. monocytogenes* and *Salmonella* in soil, but not for STEC.	GIS-enabled models can be used to determine land management and crop selection to reduce produce contamination.	Statistical association (classification tree)
Baloch, 2014	Yuma, AZ	Romaine lettuce	Beef cattle	Case-study based on outbreak investigation	2010	Commercial farm implicated in outbreak with a CAFO and a small dairy/beef cattle establishment nearby.	NR	NR	*E. coli* O145	Water (irrigation, runoff), soil, wild animals	Detection of STEC in water, soil, and wild animal feces.	Outbreak strain was not detected in any of the samples; however, soil samples yielded several STEC strains.	Develop standard methods for environmental assessments and consider spatial–temporal sources of variability.	Descriptive analysis
Chapin et al., 2014	New York State	Unspecified produce	Unspecified livestock	Longitudinal	–	Five produce farms and five natural sites across the state	2 years	NR	*Listeria* spp.	Not specified	Prevalence in production and natural environments. Correlation and random forest variable importance.	Proximity of pastures is identified as an important variable highly associated with *Listeria* detection in the environment. Livestock may be potential source of contamination of agriculture and other environments.	None.	Statistical association (random forest), prevalence
Berry et al., 2015	Clay Center, NE	Spinach, mustard greens, turnip greens	Beef cattle	Experimental, longitudinal	–	Research field: nine leafy green plots located 60, 120, and 180 m from a cattle feedlot with 6000 heads	2 years	60, 120, 180 m	*E. coli* O157:H7, generic *E. coli*	Airborne	Prevalence in feedlot surface manure and leafy greens. Concentration in air.	Current buffer zone (180 m) may not be enough.	Recommended buffer distance (120 m) may not be enough to reduce the risks and should be reevaluated.	Statistical association (GLM)
Benjamin et al., 2015	California central coast counties (Monterey, San Benito, San Luis Obispo)	Unspecified leafy greens	Beef cattle	Experimental, longitudinal	–	Cow-calf ranches nearby leafy greens	3 years	NR	*E. coli* O157:H7, generic *E. coli*	Not specified	Prevalence in cattle feces, water, and sediment.	Preharvest cattle interventions are suggested to reduce environmental transmission between cattle and produce.	Preharvest interventions for cattle (vaccination).	Genetic similarity, statistical association (logistic regression)

(Continues)

TABLE 3 (Continued)

Reference	Location	Produce type	Animal	Study type	Outbreak	Study settings	Duration	Distance to animal operation	Hazard	Mechanism of transmission	Outcome	Main conclusions	Recommended action	Association
Gelting et al., 2015	California	Iceberg lettuce	Dairy cattle	Systems analysis	2006	Lettuce farm with two nearby dairy farms sharing wastewater systems.	NR	NR	*E. coli* O157:H7	Irrigation water	Prevalence in environmental samples.	The exact source of contamination was not definitely identified. Risk-based irrigation water quality management approach is suggested.	Implement risk-based management approach for drinking water to manage irrigation water safety.	Hypothesized (systems analysis)
Thakur et al., 2016	North Carolina and Tennessee	Beans, cucumbers, red and green lettuce, turnip greens, squash, peppers, cabbage, broccoli	Dairy cattle and poultry	Experimental, longitudinal	–	Experimental research station, commercial diversified farming operation	1 year	Research station: 9.1, 61.0, 121.9, 243.8 m, Commercial: NR	Generic *E. coli*, STEC, *Salmonella* spp.	Insects, soil, air	Prevalence of *Salmonella* and STEC vegetable, soil, fecal water, and insect samples. Concentration of generic *E. coli*, coliforms, *Enterococci* in water, prevalence of *Salmonella*, STEC, generic *E. coli*, total coliforms in air.	Size of animal operation, proximity, and cultural practices impact pathogen transfer. Current buffer zone recommendations should be reevaluated.	Recommended buffer distance (120 m) may not be enough to reduce the risks and should be reevaluated.	Genetic similarity, descriptive analysis
Kabiru et al., 2015	Zaira, Nigeria	Lettuce, cabbage, carrots	Beef cattle	Longitudinal	–	Cattle abattoir	20 weeks	500 m	STEC, other diarrheagenic *E. coli*	Water (irrigation)	Prevalence in cattle fecal samples, produce, water.	Pathogen can contaminate the environment and thus the crops via irrigation.	None	Prevalence of virulence genes, descriptive

(Continues)

TABLE 3 (Continued)

Reference	Location	Produce type	Animal	Study type	Outbreak	Study settings	Duration	Distance to animal operation	Hazard	Mechanism of transmission	Outcome	Main conclusions	Recommended action	Association
Karp et al., 2015	United States (AZ, CA, CO, NV, OR), Mexico, Chile	Various leafy greens	Cattle	Longitudinal	–	An organic farm in California Central Coast region, several unspecified farms in other regions	6 years	NR	Generic *E. coli*, EHEC, *Salmonella* spp.	Not specified	Prevalence in leafy greens.	Removing vegetation does not reduce the risk of contamination of produce; prevalence was higher in farms closer to livestock grazing.	Vegetation zones around leafy green fields should not be removed. Growers, ranchers, and feedlot operators should collaboratively reduce food safety risks. Cattle vaccination, secondary treatment wetlands, fencing waterways.	Statistical association (likelihood ratio)
Weller et al., 2016	New York State	Unspecified produce	Not specified	Cross-sectional, geospatial model	–	Four produce farms	6 weeks	>62.6 m and <62.5 m	*L. monocyto-genes*	Soil	Prevalence of *L. monocytogenes* at different locations on produce field surfaces, predictive models.	Proximity to pasture was significantly associated with detection of *L. monocytogenes* isolation in fields.	Contamination is dependent on unique ecological conditions and geospatial models can help preventive measures tailored for individual produce farms.	Statistical association (logistic regression)
Mishra et al., 2017	Salinas, CA (hypotheti-cal)	Various leafy greens	Cattle	System model simulation	–	One-acre field containing lettuce, nearby cattle and free roaming feral pigs (hypothetical)	NR	NR	*E. coli* O157:H7	Soil, cattle, wild pigs, water (runoff, irrigation)	Simulated prevalence, mean and maximum concentration in produce.	Prevention of soil contamination by wild pig and cattle feces estimated to reduce concentration significantly	Contamination of soil with animal feces should be prevented.	Mathematical simulation
Pang et al., 2017	Clarksville, MD	Not specified	Dairy cattle	Experimental, longitudinal	–	Research farm: dairy with pasture, composting facility, vegetable production	14 months	NR	*Listeria* spp., *Listeria monocyto-genes*	Water (runoff), airborne (wind-driven dust)	Prevalence in preharvest environments at different locations and seasons.	Season, precipitation, and wind speed were statistically associated with contamination.	Meteorological variables can be used to predict preharvest contamination events.	Statistical association (logistic regression, random forest)

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TABLE 3 (Continued)

Reference	Location	Produce type	Animal	Study type	Outbreak	Study settings	Duration	Distance to animal operation	Hazard	Mechanism of transmission	Outcome	Main conclusions	Recommended action	Association
Berry et al., 2019	Clay Center, NE	Spinach, mustard greens, turnip greens	Beef cattle	Experimental, longitudinal	–	Research field	2 years	60, 120, 180 m	*E. coli* O157:H7	Insects (pest flies)	Prevalence in flies, carriage rates, PFGE patterns compared.	Although definitive role is not demonstrated, positive pest flies were common in fields nearby cattle feedlots.	Further research to define the role of files in transmission and implement pest control and determine distance from cattle production.	Statistical association (Fisher's test)
Cha et al., 2020	Republic of Korea	Various produce and leafy greens	Unspecified livestock	Field study, longitudinal	–	Commercial farms	2 years	NR	Generic *E. coli*, *E. coli* O157:H7, AMR *E. coli*	Not specified	Prevalence in livestock, environmental, human, and produce samples. AMR patterns and distribution of genes.	More than 60% of produce and livestock isolates were genetically similar indicating a potential contribution of cattle.	Good agricultural practices should be followed.	Descriptive analysis
Glaize et al., 2020	North Carolina and Tennessee	Leafy greens, tomatoes, melons, beans, and cucumbers	Poultry, cattle, swine, small ruminants	Experimental, longitudinal	–	Five commercial sustainable farm systems (CAFO)	1 year	NR	Antimicrobial resistant non-O157 *E. coli*	Soil, raw manure, insects	Prevalence of *E. coli* in farms, phylogenetic analysis, AMR profiles.	Transmission can occur in sustainable farming systems; however, there is no evidence of the effect of distance between CAFOs and produce fields.	Further research on transfer rates	Genetic similarity
Weller et al., 2020	New York State	Unspecified produce	Dairy cattle, pig, poultry	Longitudinal field study, hypothesis generation	–	Sixty-eight agricultural water streams	7 months	Various distances	*Listeria* spp., *L. monocyto-genes*, *Salmonella*, pathogenic *E. coli*	Water	Prevalence, odds of detection of microbial or molecular target.	Distance and upstream presence of dairy operations , upstream presence of pig farms were significant and distance to pastures, upstream distance to pig farms were marginally significant for the odds of detection of *Listeria* spp. Upstream density of dairy operations was significant and pasture presence within 100 m and distance to poultry operations were marginally significant for the odds of isolation of *Salmonella*.	Preventing contamination of agriculture water from potential sources by reducing overland flow.	Statistical association (generalized linear mixed models, conditional inference trees)

(Continues)

TABLE 3 (Continued)

Reference	Location	Produce type	Animal	Study type	Outbreak	Study settings	Duration	Distance to animal operation	Hazard	Mechanism of transmission	Outcome	Main conclusions	Recommended action	Association
Glaize et al., 2021	North Carolina	Romaine lettuce, tomatoes	Poultry and dairy	Experimental intervention study (vegetative buffer), longitudinal	–	Research farm	1 year	10, 61, 122 m	Generic *E. coli*, STEC, *Salmonella* spp.	Soil, air	Prevalence in fecal, environmental, and produce samples.	Vegetative barriers are effective against airborne transmission; remediation of contamination soil is advised.	Effective bioremediation, preventive controls, vegetative barriers	Descriptive analysis
Hamilton et al., 2021	Crossville, TN	Various fruit and vegetables	Beef cattle	Experimental, longitudinal	–	Research farm: one produce field near two beef cattle areas	2 years	300, 830 m (measured from figure)	*Salmonella enterica*	Insects (pest flies)	Prevalence in different types of flies caught at various distances.	Flies might be potential vectors for transmission; however, more work is needed to determine transmission rates to produce.	Fly control and environmental monitoring programs.	Statistical association (linear regression)

Abbreviations: CAFO, concentrated animal feeding operation; NR, not reported.

positive for the identical strain, it was reported that the irrigation source was unsanitary due to high concentrations of generic *E. coli* and 25 out of 77 samples were positive for VTEC O157, leading to a conclusion that the irrigation water contaminated by cattle fecal material was the cause.

Weller et al. (2020) conducted a longitudinal field study for hypothesis generation on the effect of meteorological variables and land use near produce farms on the contamination of water streams to be used for agricultural purposes. The researchers collected water grab samples from 68 streams in New York State for the co-presence of *stx* and eaeA genes and the presence of *Listeria* spp. (excluding *L. monocytogenes*), *L. monocytogenes*, and *Salmonella*. The presence of dairy, pig, and poultry operations as well as land used as pasture was included in their statistical and machine learning models as a possible risk factor for contamination of water resources. The results showed that distance of streams to dairy operations (odds ratio [OR] = 0.84; 95% confidence interval [CI]: 0.03, 0.73), whether these operations were present upstream (OR = 0.26; 95% CI: 0.75, 0.98), and presence of upstream pig farms (OR = 0.16; 95% CI: 0.04, 0.69) significantly increased the odds of detection of *Listeria* spp. excluding *L. monocytogenes* ($p < 0.05$). According to the analysis, proximity to pasture lands (OR = 1.03; 95% CI: 1.00, 1.07) was marginally significant ($.05 \leq p < .10$). Pasture presence was also identified as a significant factor ($p < .05$) for *L. monocytogenes* isolation by the conditional inference tree approach and upstream dairy operation intensity was also associated with the chance of isolation only when it rained recently. For *Salmonella* isolation, upstream density of dairy operations was a significant factor (OR = 0.47; 95% CI: 0.28, 0.81; $p < .05$); however, pasture distance within 100 m upstream or distance of poultry operations was marginally significant ($.05 \leq p < .10$). Regarding the marginally significant results, it was noted that due to challenges in obtaining accurate geographical information, some animal operations such as dairy farms could have been excluded from the study; therefore, lack of statistical significance may not mean that these associations should be seen as a proof of lack of association. Overall, this study provided evidence that nearby animal operations have the potential to contaminate agricultural water sources; however, transmission from surface water resources to the fresh produce was out of their scope.

A quantitative, dynamic systems simulation model by Mishra et al. (2017) provided a hypothetical leafy greens production scenario in the Salinas Valley region of California, a region associated with most of the leafy green outbreaks in the United States that were identified in our review. Although the changes due to irrigation water were not explicitly reported and direct contamination from irrigation water was excluded, it was estimated by a sensitivity analysis that the changing amounts of runoff due to seasonal rainfall was a crucial factor, as a 100% increase in runoff would result in 1.64- to 6.86-fold increase in the counts of *E. coli* on the produce. However, it was estimated that wild animal intrusion into the fields would have a greater effect than runoff, which may also depend on the distance from the field. Pang et al. (2017) studied meteorological factors to determine the preharvest risk factors related to rainfall and wind that cause runoff and wind-driven transmission of *Listeria* spp., via logistic regression and random forest analysis. It was estimated that a higher prevalence of *Listeria* was statistically associated with seasonality, and precipitation had a "cumulative, long-term effect" on the prevalence, suggesting that runoff would be a potential contributing mechanism to the leafy green outbreaks.

Studies summarized here point toward the importance of water sources as a potential vehicle that can transmit foodborne pathogens from nearby livestock operations. Recommendations in scientific reports to mitigate pathogen transmission, via irrigation or runoff, are mainly focused on implementing risk-based water quality management and improved microbiological and physicochemical testing of agricultural water resources. Novel approaches, such as predicting contamination events based on the meteorological variables, as an early warning system, were also suggested by Pang et al. (2017); however, the lack of qualitative and quantitative knowledge on the degree and significance of different pathways is also acknowledged. Overall, it is important to note that none of the studies, from the scientific literature, investigating water as a source of contamination, provided a quantitative description of the magnitude or likeliness of the transfer of pathogens from cattle to produce, which could be used to assess risks that are extremely important inputs for risk assessments to evaluate mitigation efforts.

3.1.2 | Soil

Agricultural soil can naturally harbor foodborne pathogens or become contaminated by environmental factors and agricultural operations, causing cross-contamination of fresh produce and groundwater sources used for irrigation and processing (Gurtler, 2017). Foodborne pathogens, especially *E. coli* O157:H7, *Salmonella* spp., and *L. monocytogenes*, can be detectable in the soil for extended periods of up to 60–120 days, with time required for 1 decimal reduction (T_{90}) ranging from 12 to 25 days, depending on the soil type, environmental conditions, and experimental settings (Russell et al., 2022). Animal feces, directly or in the form of biological amendments, can contaminate the soil, which can contribute to contamination of water sources and air/dust and facilitate interspecies transmission between wildlife and livestock, including cattle, causing a persistent occurrence of pathogens in the environment, and repeatedly causing outbreaks related to both vegetable and meat products (Aslam et al., 2003; Fairbrother & Nadeau, 2006). Therefore, it is imperative to consider soil as a critical source of foodborne pathogen contamination in food chains.

Jay et al. (2007) reported that soil/sediment samples contained the *E. coli* O157 strain matching with the 2006 spinach outbreak (Gelting, n.d.) by PFGE and MLVA analysis, which indicated that the soil could be a potential common source of *E. coli* O157 among other possible sources, but the exact routes of contamination were not identified. Hoar et al. (2013) collected air, fecal, and soil samples around leafy green fields with adjacent sheep grazing areas. The distance or location of soil samples was not reported; however, based on the low prevalence of *E. coli* O157:H7 and *Salmonella* in soil, it was concluded that the soil on which the sheep graze had a lower risk than the "minimal risk" posed by the fresh sheep feces. In a case study by Baloch (2014), the outbreak strain was not isolated from the soil, but three samples were positive for non-O157 STEC. Even though the investigation ruled that the most likely cause of the outbreak was an RV park sewage nearby, it is also noted that the soil ground of the RV park was not suitable for septic absorption, pointing out that not only the agricultural soils, but also the surrounding environment is important for produce safety.

Thakur et al. (2016) collected soil samples from produce fields, dairy and broiler areas at an experimental research station at predetermined distances, and commercial sustainable farms with poultry houses and cattle pastures nearby. *Salmonella* and STEC were isolated from the soil samples, indicating a potential pathway for contamination of the produce. Samples positive for STEC were collected within 121.9 m (400 ft) from the animal source and PFGE analysis revealed that one soil isolate had an identical pattern with a fecal sample in the same area. Overall, insect, fecal, and soil isolates had a close genotypic relationship, signaling a need to reevaluate the 121.9m buffer zone recommendations by the California LGMA, at the time the study was conducted. It should also be noted that this study was conducted at a small-scale experimental research station and the second phase of the study included 10 commercial sustainable farms. Therefore, a direct comparison to larger scale, higher density farms and animal operations often located in the West Coast regions might not be possible. The same research group also reported the effect of vegetative barriers between livestock and produce (Glaize et al., 2021) and the transmission of antimicrobial-resistant *E. coli* in five commercial sustainable farm systems (Glaize et al., 2020) with nearby cattle, small ruminants, swine, and/or chicken operations. In both reports, although definitive pathways were not identified, soil was among one of the potential sources of transmission of *Salmonella*, STECs, generic *E. coli*, and antimicrobial-resistant *E. coli*. It was noted that the distance between livestock operations and produce fields was not a contributing factor, and vegetative barriers were effective in reducing the transmission; however, if the land to be used for produce farming was exposed to nearby livestock operations for long periods, remediation of soil is deemed necessary to ensure produce safety in addition to implementing the vegetative barriers as the pathogens can be persistent in the soil for extended periods.

Strawn et al. (2013) and Weller et al. (2016) utilized geographical information system data to address the effect of meteorological and landscape factors including proximity to pasture-designated land on contamination of produce farms. In a longitudinal field study over 2 years, Strawn et al. (2013) collect soil drag swabs and water and fecal samples to test the prevalence of *L. monocytogenes*, *Salmonella*, and STECs and recorded coordinates to measure distances from pastures. The results showed that at one particular farm, where produce and livestock operations nearby were comanaged, *Salmonella* prevalence was significantly higher than the other four farms sampled, and pasture proximity was reported as an important predictive factor for the presence of *L. monocytogenes* on farms. On the other hand, no significant statistical association was found between any of the factors including proximity to pastures and STEC prevalence. It was concluded that the transfer of foodborne pathogens in the preharvest setting is a multifaceted procedure; therefore, food safety hazards at individual farms should be evaluated considering the unique environmental and geographical conditions. Weller et al. (2016) further validated the statistical models from the Strawn et al. (2013) study and collected extra samples to measure *L. monocytogenes* prevalence over 6 weeks on four produce farms in New York State and provided predictive models based on geographical factors including pasture proximity. The two major factors that significantly increased the odds of isolation of *L. monocytogenes* were the proximity of sampled locations to water and pasture. It was concluded that multiple environmental conditions, including the proximity of pastures, should be considered when making risk-based decisions for produce safety.

In the systems model simulation developed by Mishra et al. (2017), the soil had a vital role in the transmission of *E. coli* O157:H7 to the produce from a variety of animal feces, due to runoff from nearby animal operations. The model included precipitation, animal feces, irrigation, and plant wastes as sources of soil contamination. While direct contamination from the soil, via uptake from roots or direct contact, was not included in the model, soil splashing due to irrigation was included in the model as a possible mechanism of transmission. Different scenarios evaluated through the model estimated that *E. coli* O157:H7 contamination on leafy greens can be significantly reduced if the contamination of soil from wild animals and cattle feces could be prevented.

3.1.3 | Air

Cattle operations can create a considerable amount of dust that can be disseminated through the environment by wind and possibly carry foodborne pathogens to nearby produce fields. Six articles were retrieved focusing on the airborne transmission of pathogens from various livestock in this review. Yanamala et al. (2011) placed spinach plants near a cattle loadout area to determine the effects of distance from the feed yard and duration of exposure to the transmission of generic *E. coli*, *E. coli* O157:H7, and *Salmonella*, especially around high-traffic locations such as the loadout areas. It was observed that at the maximum studied distance of 45.72 m (reported as 50 yards), there was significant contamination of all microorganisms tested, indicating that the crops should be placed farther than 45.72 m. However, for both pathogens tested, no obvious trends were observed with respect to time and distance, leading to a conclusion by the authors that the transmission can randomly occur, and the buffer distance should be carefully considered. Berry et al. (2015) planted various leafy greens in plots located 60, 120, and 180 m away from a research cattle feedlot and sampled leafy greens and air at predetermined distances to evaluate the possibility of airborne transmission. The results indicated airborne dissemination of generic *E. coli* and *E. coli* O157:H7 as these were detected even at 180 m, although the other possible mechanisms were not dismissed. It was noted that the risk of airborne transmission is increased when the cattle pen surfaces are dry, compared to when the surfaces are muddy. The conclusion was that the buffer zone recommendations at the time of the study (120 m) would not be enough to mitigate airborne transmission of pathogens. In addition to soil samples, Thakur et al. (2016) also sampled air from only one of the farms in North Carolina at 0 and 121.9 m distances. However, no *Salmonella* or STECs were isolated from any air samples collected, and they reported a 40% prevalence of *E. coli* and coliforms. Therefore, airborne transmission was not considered a risk factor, and the importance of farm management was emphasized. Glaize et al. (2021) investigated vegetative barriers, as an intervention against wind-driven transmission, via a longitudinal study over 2 years and a challenge test with artificially contaminated vegetative zones. Although no samples tested positive for STECs, generic *E. coli* was detected in air samples located from 10 to 122 m in distance from the vegetative barrier between cattle and poultry operations located nearby lettuce fields. The prevalence in air samples was similar at three different distances, and overall contamination increased when the distance from the vegetation was also increased. Therefore, suggesting that the animals were not the only sources of contamination and the transmission can occur from the environment to the leafy greens.

Animal operations, other than cattle, and less-frequent microbiological hazards were also included in this review to capture evidence for the explanation of the transmission in similar settings. Hoar et al. (2013) studied the distance between sheep grazing and vegetable fields and measured total coliform counts in the air to be at minimal levels (16.50 CFU/m^3 maximum at 2 m distance). Although the results from the air samples were not conclusive, based on the analysis of soil and animal feces, the authors recommended that a 30 ft (9.14 m) distance was enough to mitigate the risk of cross-contamination. In an attempt to address the meteorological risk factors for *Listeria* spp. contamination of produce sourcing from a nearby dairy farm, Pang et al. (2017) estimated that wind speed and precipitation were important factors for contamination of the produce, as these could increase the chances of runoff and airborne transmission and the effect of wind speed was more "instant," compared to the effect of precipitation.

3.1.4 | Wild animals and insects

Four studies investigated wild animals as sources or carriers of foodborne pathogens from nearby livestock to produce at the preharvest stage. Jay et al. (2007) hypothesized transmission via feral swine or other wildlife from the results of an environmental assessment following the 2006 *E. coli* O157 spinach outbreak (Gelting, n.d.). The original outbreak strain was first isolated from cattle feces approximately 1.6 km (1 mile) away from the spinach fields. The same strain was isolated from feral swine as well as other samples, and the molecular typing indicated that the strain remained detectable in samples for up to 3 months, and swine-to-swine and cattle-to-swine transmission was possible. However, the exact mechanism was still unclear as water and soil were also mentioned as possible sources of contamination for implicated spinach and wild animals. During the same investigation, Jay-Russell et al. (2012) also reported a high prevalence of *Campylobacter* spp. in the feral swine population and noted that the majority of isolates were either *Campylobacter jejuni* or *Campylobacter lanienae*, which were the most abundant species that were previously reported to be common in cattle. Therefore, the authors recommended that fresh produce fields should be protected from wild animal intrusion, although no comparison was made between swine, cattle, or produce samples and the proposed pathway was still theoretical. A case study by Baloch (2014), based on the 2010 *E. coli* O145 outbreak due to the consumption of shredded lettuce, suspected wildlife as a potential source of contamination as the investigation team collected coyote, bobcat, rabbit, and rodent fecal samples around the implicated field in Yuma, AZ in proximity to a CAFO and a dairy cattle farm. All samples from the wildlife were negative for *E. coli* O145; therefore, this pathway was largely dismissed. Mishra et al. (2017) systems model simulation also included wild pig intrusion as a direct source of contamination for soil. Sensitivity analysis estimated that a 100% decrease in wild animal defecation in the produce field would decrease mean counts of *E. coli* on the crops in a range from 1.66- to 11.62-fold, corresponding to 0.22–1.07 $\log_{10}$ reduction.

Flies and other insects are also theorized to carry pathogens from livestock operations to nearby produce fields. Berry et al. (2019) reported *E. coli* O157:H7 carriage rates for five species of flies and compared PFGE profiles of *E. coli* O157:H7 isolated from flies, manure, and leafy green samples. Although a definitive and quantitative explanation was not possible at the time, it was reported that flies were capable of transmitting the acquired pathogens to the plant surface at distances greater than the LGMA recommendations at the time the study was conducted (366 m [1200 ft]).

Hamilton et al. (2021) reported the prevalence of *Salmonella* in different fly families, around a produce plot nearby two low-density beef cattle operations, as 2.2%–15.2%. Trap location was not a significant predictor for *Salmonella* prevalence and the prevalence estimations from this study were different from that of Berry et al. (2019). It was also noted that the origin of contamination in flies could result from different environmental sources, as well as direct contamination via fecal material. Thakur et al. (2016) isolated STEC, *E. coli* O157:H7, and *Salmonella* from insects collected from an experimental research station and observed closely related PFGE patterns from STEC isolated from insect, fecal, and soil samples. Glaize et al. (2020) collected *E. coli*-positive insects (12%, $n = 227$) 2655–3219 m away from a cucumber field on one of the farms sampled, and the isolated strains originating from insects were genetically closely related to produce, soil, and raw chicken manure isolates, concluding that insects could be a possible environmental transmission route for AMR *E. coli*. Talley et al. (2009) utilized fluorescent-tagged *E. coli* O157:H7 to experimentally show that house flies can carry pathogens from manure to the surface of produce. It was also shown by PCR and DNA sequencing that some random flies caught in the fields were positive for *E. coli*

O157:H7; however, a quantitative explanation in terms of the transfer rates and survival of pathogens in insects is still lacking.

3.1.5 | Other or nonspecified mechanisms of transmission

Eight studies identified during the review process only considered nearby/adjacent livestock as a source, without specifying any mechanisms of transmission and therefore not falling into any of the aforementioned categories.

Hilborn et al. (1999) provided a detailed report of the 1996 *E. coli* O157:H7 outbreak where the source of the pathogen was traced back to mesclun lettuce produced by a single producer through a case–control study. It was implied that the nearby cattle ranch or free-range chickens, having direct access to both the cattle and produce areas, would be the possible sources. No *E. coli* O157:H7 was isolated during the investigation; however, non-O157:H7 *E. coli* was detected in water sources, which led the investigators to suggest both cattle and chickens as potential sources.

Benjamin et al. (2015) examined climate and management risk factors for *E. coli* O157 contamination by molecular subtyping in multiple cattle locations. It was noted that "minimal movement" of strains occurred between different ranches, and the specific strain from the 2006 spinach outbreak was not detected in any of the samples. However, the outbreak strain isolated from the patients was closely related to some strains isolated from cattle and water shown by the MLVA, suggesting preharvest cattle interventions to mitigate the risk of contamination of fresh produce.

Cha et al. (2020) observed the phylogenetic distribution of *E. coli* from fresh produce and other agricultural samples to make a comparison between produce and livestock isolates in the Republic of Korea. More than 60% of isolates from the various produce observed were from the phylogenetic groups similar to isolates from meat products, suggesting a link between produce contamination and livestock. It was also observed that the produce isolates had different virulence factors and reduced antimicrobial resistance, when compared to cattle and human isolates. Antimicrobial resistance patterns in isolates from Chinese cabbage and water sources for ESBL-producing *E. coli* were concluded as genetic evidence that the water sources can contribute to the spread of antimicrobial resistance.

Chapin et al. (2014) studied the geographical and meteorological factors that contribute to *Listeria* spp. contamination in produce production environments, via a random forest analysis. Proximity of pastures to production environments was highly associated with the isolation of *Listeria* and identified as an important variable, via the analysis. The data suggested that livestock were a source of dissemination for pathogenic bacteria to agriculture and other environments.

Cooley et al. (2013) mainly focused on the method development for STEC isolation in production environments. At the time of the study, a lack of standardized methodology to detect and isolate non-O157 STECs from different environmental agricultural samples including soil, fecal material, and leafy greens was noted. The authors conducted a longitudinal study by taking produce, soil, livestock, and water samples from 33 farms and ranches over a 2.5-year period. In addition to the method development, the results offered insights into the phylogenetic similarity of food, environmental, and clinical samples collected. Some strains collected from cattle and feral pigs during the study were identical, or closely related, to outbreak strains from three previous outbreaks with suspected leafy greens connection. No specific mechanism of transmission was suggested as the origin of exposure to animals could not be determined; however, co-mingling of cattle and wildlife was indicated as a possible reason for genetic similarities.

Karp et al. (2015) reported the effect of landscape use around produce farms on *E. coli*, Enterohemorrhagic *E. coli* (EHEC), and *Salmonella* prevalence. It was found that EHEC prevalence in leafy greens significantly increased, corresponding to the pressure on farmers to remove vegetation around the produce fields between 2005 and 2012 as a mitigation measure. The model predicted that EHEC detection was about 100 times more likely near grazable land within 1500 m and removing vegetation would not help to reduce EHEC prevalence over time.

3.1.6 | Main conclusions and suggested interventions

Findings from the scientific literature mostly confirm that nearby or adjacent livestock can be a risk factor for the contamination of fresh produce at preharvest; however, the relative contribution of each mechanism is still largely unknown. Therefore, a common conclusion among the included studies was to encourage further research on the mechanisms of transmission to provide quantitative evidence, which will help address risk management questions. Furthermore, interactions between livestock, fresh produce, and the environment are complex and unique to the given environmental conditions; therefore, instead of concentrating on individual pathways, further studies should consider the produce and meat supply chains, as a whole, to encompass all possible loopholes. Lastly, most research focuses mainly on pathogenic *E. coli* (especially STECs) as the main hazard; however, the public health impact of other foodborne pathogens such as

Salmonella and *Listeria* spp. or viruses may have been overlooked.

Several suggestions to mitigate the contamination of produce with foodborne pathogens were provided in the literature included in this review. These suggestions were mainly categorized as risk-based water and farm management approaches, preharvest cattle interventions, preharvest produce interventions, and surveillance and monitoring programs. In terms of risk-based water and farm management approaches, Gelting et al. (2015) suggested implementing risk-based criteria for irrigation water, based on World Health Organization guidelines for drinking water (WHO, 2017). Later, the FDA (2021d) published the Proposed Rule for Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption Relating to Agricultural Water, to establish science-based limits for agriculture water. Pang et al. (2017) suggested their model be improved and used for predicting preharvest contamination events based on meteorological predictions. Karp et al. (2015) pointed out the collaboration between produce growers, ranchers, and feedlot operators to reduce food safety risks by coordinated risk management practices. It was also urged that the safe distances between produce and nearby livestock should be carefully adjusted based on the findings from future studies (Berry et al., 2015, 2019; Thakur et al., 2016; Yanamala et al., 2011).

The only preharvest intervention targeting cattle was vaccination programs, as suggested by Benjamin et al. (2015) and Karp et al. (2015). Although a few vaccines were shown to be effective against *E. coli* O157:H7 colonization of cattle, vaccines have not been used extensively in cattle production and vaccine efficacy has not been demonstrated clearly up to date. Other preharvest strategies include controlling wildlife access to produce fields by lethal and nonlethal methods such as hunting, trapping, and fencing (Jay-Russell et al., 2012; Karp et al., 2015; Mishra et al., 2017), pest control (Berry et al., 2019; Hamilton et al., 2021), dust control (Yanamala et al., 2011), implementation of vegetative buffer zones (Glaize et al., 2021; Karp et al., 2015), and remediation and treatment of soil surrounding production areas (Glaize et al., 2021; Karp et al., 2015). Among these interventions, only the vegetative buffer zones were tested experimentally and reported as effective (Glaize et al., 2021).

3.2 | Public health reports

Although numerous foodborne outbreaks related to leafy greens and other produce have been reported, only the reports mentioning a contribution of cattle or any other livestock were included in this review. Fifteen official documents were retrieved in the form of outbreak investigations, environmental assessments following foodborne outbreaks, statements, and action plans as outlined in Table 4. Although the main scope of this review was to address cattle contribution to leafy green outbreaks, information on different products (cantaloupes, peaches, and red onions) and the contribution of other animals such as small ruminants and broilers was also collected to address interactions between animal and produce operations. For all the outbreak reports related to leafy greens, the most common etiology was pathogenic *E. coli* (O157:H7 and O145), while reports related to fruits identified the etiology as *Salmonella* spp. Although the definitive role and relative contribution of the transmission pathways were not explicitly reported in majority of the outbreak investigation reports, they mostly focused on possible contributing factors such as runoff from adjacent or nearby lands occupied by animal operations, direct transmission via dust and wind, intrusion of wild animals, and use of contaminated water for irrigation or other purposes or a combination of some of these factors.

The earliest outbreak investigation suspecting the contribution of cattle to leafy green outbreaks was reported by Hilborn et al. (1999). Clinical samples from 49 cases related to the consumption of mesclun lettuce, in Connecticut and Illinois, were analyzed using PFGE where the investigation team was able to trace the source of the contamination to a single processor and grower, but the location of the processing and growing sites was not reported. The environmental assessment identified potential sources of contamination as a neighboring cattle ranch, free-range chickens that had access to the cattle, water management issues, lack of sanitation and hygiene practices, and an open-environment processing facility. However, *E. coli* O157:H7 was not detected in any of the collected environmental samples including processing surfaces, wash water, wash tanks and pipes, and finished product. On the other hand, water used for washing the lettuces and finished lettuce samples yielded generic *E. coli*, indicating that the wash water might be a plausible source of contamination. It was also noted that the lettuce was washed manually in tanks filled with recirculated unchlorinated well water and filtering systems in the facility were faulty around the time the outbreak-related produce was processed. Therefore, the suspicion of livestock contribution to this outbreak was not confirmed. Similarly, in outbreak investigations related to leafy greens and other fresh produce, cattle or other animal operations were suspected or hypothesized to contribute to contamination; however, the contribution of nearby/adjacent animal operations was dismissed or reported unclear due to a lack of data in these reports (Crawford et al., 2010; FDA, 2013a, 2018, 2019, 2021c).

The multistate *E. coli* O157:H7 outbreak related to spinach in 2006 was one of the largest leafy greens

TABLE 4 Summary of findings for the public health reports in chronological order.

Document type	Outbreak date	Location	Hazard	Produce	Involvement of livestock	Reference
Outbreak investigation	May–June 1996	Not reported	*E. coli* O157:H7	Mesclun lettuce	Suspected, not identified	Hilborn et al., 1999
Outbreak investigation	September 2006	San Benito, CA	*E. coli* O157:H7	Spinach	Potential source	Gelting, n.d.
Environmental assessment	Spring 2010	Welton, AZ	*E. coli* O145	Romaine	Suspected, not likely	Crawford et al., 2010
Environmental assessment	August 2012	Indiana	Salmonella	Whole cantaloupe	Suspected, no link found	FDA, 2013a
Outbreak investigation	November 2013	California	*E. coli* O157:H7	Lettuce (ready-to-eat salad)	Possible source	FDA, 2013b
Environmental assessment	April 2018	Yuma, AZ	*E. coli* O157:H7	Romaine	Suspected, not identified	FDA, 2018
Outbreak investigation	Fall 2018	Santa Barbara, CA	*E. coli* O157:H7	Romaine	Hypothesized	FDA, 2019
Outbreak investigation	November 2019	Salinas, CA	*E. coli* O157:H7	Romaine	Potential source	FDA, 2020a, 2020c
Outbreak investigation	December 2019	Salinas, CA	*E. coli* O157:H7	Salad mix	Potential source	FDA, 2020a, 2020d
Outbreak investigation	Fall 2019	Salinas, CA	*E. coli* O157:H7	Romaine	Potential source	FDA, 2020a
Outbreak investigation	June–October 2020	Holtville, CA	*Salmonella* Newport	Red onions	Suspected, not identified	FDA, 2021c
Outbreak investigation	Summer 2020	California	*Salmonella* Enteritidis	Peaches	Potential source	FDA, 2021b
Outbreak investigation	Fall 2020	California	*E. coli* O157:H7	Leafy greens (various)	Potential source	FDA, 2021a
Statement	Fall 2020	–	*E. coli* O157:H7	Leafy greens	–	Yiannas, 2021
Action plan	Not an outbreak	–	STEC	Leafy greens (various)	–	FDA, 2020b

outbreak in history as 199 cases were identified with 102 hospitalizations, 31 developed hemolytic–uremic syndrome, and five people died (Gelting, n.d.). The outbreak investigation conducted by the CDC focused on the impact of runoff from nearby grazing areas and the possibility of nearby cattle contaminating ground and surface water sources used for irrigation of spinach and wild pigs as a transporter of the pathogen from cattle grazing areas to spinach fields. The investigation team found that fecal samples from cattle and pigs, one water sample, and one sediment sample from the San Benito River matched the outbreak strain isolated from clinical samples and implicated produce. The report mainly focused on irrigation water as a contamination source and did not report an investigation of airborne transmission or direct intrusion of cattle or wild animals. However, it was indicated that the animals had direct access to surface waters (mainly the river), which contaminated the surface water, and it could potentially reach the wells on the ranch that are used for irrigation. As a result, the agency recommended actions to be taken to monitor and improve irrigation quality, implement buffer zones, and limit animal access to surface water sources. This major outbreak, along with other leafy green outbreaks, led growers in California and Arizona to establish the LGMA to improve food safety practices.

In 2013, a multistate outbreak of *E. coli* O157:H7 was investigated by the FDA, CDC, and California Department of Health, related to the consumption of ready-to-eat (RTE) salad containing romaine lettuce where 33 laboratory-confirmed cases were identified (FDA, 2013b). The investigators identified two cattle operations near the field where implicated products originated as a "possible source of cross-contamination." During the environmental investigation, five out of 10 soil and water samples tested positive for *E. coli* O157:H7 that did not match with the outbreak strain. Nonetheless, the report concluded that based on epidemiologic and traceback investigations, the proximity of cattle operations was considered a risk factor for cross-contamination and the company involved was warned against the risks of growing RTE leafy greens nearby.

The proximity of cattle operations was explicitly identified by the FDA as a potential contributing factor to leafy green outbreaks for the first time, following three distinct outbreaks of *E. coli* O157:H7 attributed to romaine lettuce and RTE chopped salad kits, in Fall 2019 (FDA, 2020a, 2020c, 2020d). The implicated romaine lettuce products were traced back to a leafy green grower, with multiple fields, in California within close proximity to two cattle operations, which were identified as a possible source of cross-contamination. During the investigation, samples from clinical isolates, soil, animal feces, biological soil amendments, and agricultural water were compared using whole-genome sequencing (WGS); however, WGS information provided by the FDA was not publicly accessible at the date this review was conducted. One of the outbreak strains was found in a fecal–soil composite sample less than 3.2 km (2 miles) away from the production area near a cattle grate, which led the investigation team to suggest adjacent and nearby cattle operations as a potential source. Furthermore, one nearby water sample was positive for a non-outbreak-related strain of *E. coli* O157:H7 and several environmental samples yielded other strains of STEC. Although these findings were considered indicators of the persistence of *E. coli* O157:H7 in the Salinas River area animal reservoir, the reports were inconclusive about the potential sources of transmission from animals to the produce. However, after revisiting data from the previous leafy green outbreaks starting from 2013 (FDA, 2013b), an association between persistent STEC contamination in the environment and proximity of cattle, as a contributing factor, was suggested. As a result of these outbreak investigations, FDA suggested leafy green growers implement and improve current prevention efforts, improve traceability, and implement industry-led root cause analyses to address issues specific to individual growers. In response to the 2019 outbreaks, LGAP (FDA, 2020b) was published to improve collaboration in prevention, response, and addressing knowledge gaps to control contamination of leafy greens, including possible transmission from nearby or adjacent cattle operations.

Outbreaks in 2019 were followed by several *E. coli* O57:H7 and *Salmonella* outbreaks in 2020 where involvement of adjacent/nearby animal operations was suspected or declared as a potential source. In the Fall of 2020, another *E. coli* O157:H7 outbreak related to leafy greens occurred, causing 40 cases of illness (FDA, 2021a) in the same region as the 2019 reported outbreaks. Similar to the investigation that took place in 2019, cattle operations were identified as the "most likely" source of contamination of the produce, as the outbreak strain was detected from a fecal composite sample on a road approximately 2.1 km (1.3 miles) upslope from the produce farm. Although the outbreak strain was not isolated from any leafy green samples and the exact mechanism of transmission was not clear, it was concluded that this "reoccurring strain of concern," "reoccurring region," and "reoccurring issues with adjacent land use" were indicators that the cattle were a contributing factor for leafy green outbreaks. During the summer of 2020, peaches grown in California, with adjacent broiler and dairy cattle operations, were identified to cause an outbreak of *Salmonella* Enteritidis with 101 laboratory-confirmed cases and 28 hospitalizations (FDA, 2021b). The investigators sampled peaches, tree leaves, and soil in the orchard for the presence of the outbreak strain, but none of the *Salmonella* isolates were related to the outbreak strain. However, several leaf samples at the edge of the orchard facing the broiler and cattle operations were positive for *Salmonella* Alachua and *Salmonella* Montevideo, determined to be closely related to historical chicken and cattle isolates by WGS. In light of this information, airborne transmission of pathogens, mainly due to fugitive dust from adjacent animal operations, was considered a "plausible" factor for the contamination of produce. Although the air was not sampled, considering the height of the tree leaves, the most plausible mechanism of transmission was identified as fugitive dust from nearby animal facilities. Even though the implicated produce was not leafy greens, this investigation pointed toward the importance of controlling dust in the environment for food safety protection.

Following the findings from the repetitive *E. coli* O157:H7 and STEC outbreaks and the extensive media coverage regarding the safety of leafy greens, the FDA and leafy greens sector stakeholders published the 2020 LGAP (FDA, 2020b) to address produce safety issues and improve collaboration. The action plan prioritized prevention methods and response to future outbreaks and addressed the knowledge gaps regarding the contamination of leafy greens. The plan identified contaminated soil, biological soil amendments (fertilizers), animals, air/dust, and water as possible sources for STEC contamination in leafy greens. The plan also provided action items covering advanced agricultural water safety, inspection, auditing, and certification, buyer specifications, leafy greens data trust, microbiological methods for detection and sampling, adjacent and nearby land use, and outreach and communication programs with stakeholders. Among these action items, adjacent and nearby land use considered nearby livestock production as a risk factor for contamination, and the document cited a lack of understanding of the mechanisms of transmission and how to mitigate the potential hazards. In 2020, the agency collaborated with stakeholders to collect existing data and focused on generating new data about nearby and adjacent land use. Furthermore, studies on the practicality of implementing preharvest cattle interventions were encouraged.

Traceback investigations conducted by regulatory agencies are crucial for understanding the sources of produce contamination. However, these investigations inherently face challenges that can cause delays (Irvin et al., 2021). Due to the time required for processing, distribution, and storage of raw leafy greens, as well as the delay between exposure and disease onset, outbreak investigations often require a significant time after the contamination occurs. Once the first cases of an outbreak are reported, epidemiological investigations identify the food sources of exposure, and the product's history is traced back throughout the entire supply chain. However, conducting environmental investigations in preharvest settings is only possible once the product has been successfully traced back to its producer. Data gaps in traceability can further delay the investigation process. By the time an environmental investigation begins, the conditions that facilitate contamination events may have changed, thereby reducing the likelihood of detecting the outbreak strain in the environment. Therefore, it is advisable to harmonize traceability systems within the produce industry, improve outbreak tracking systems, and promote data sharing between the livestock, produce industries, and government agencies.

4 | CONCLUSIONS

The results from this scoping review conclude that both the scientific literature and public health reports suggested livestock operations nearby, especially cattle, as a potential source of contamination of leafy greens; however, knowledge gaps in the relative contribution of specific mechanisms of contamination were also identified. Several studies report that contamination events seem to occur through multiple pathways (water, soil, air, wildlife, and others), which require the consideration of the produce supply chain as a whole. Foodborne diseases are rare events that often occur when multiple failures or extreme events happen, which justifies the need for complex systems analyses, quantitative risk assessments, and sensitivity analyses to better control the interactions between the environment, livestock, and produce farms.

Recommendations specific to the involved industries can also be made based on the data collected here. Studies investigating the buffer distances between produce farms and livestock operations report that the interim distances proposed by both LGMAs might not be enough, except for a single study. The produce industry should continue to monitor new information and update the recommendations accordingly. The distance recommendations should also consider geographical risk factors and establishment of vegetative buffer zones as well as the distance of agricultural water resources from animal operations. Regular monitoring of the transmission pathways, especially water, air, and wildlife, should be encouraged and funded by industry and government organizations to expand quantitative knowledge that can aid facility-specific risk assessments that address unique environmental and management conditions of farms in different regions. A very common conclusion among the studies was that the transmission of pathogens is most likely a multifactorial process that includes fertilizers, water, soil, air, and wildlife that can also be facilitated by meteorological and geographical conditions; therefore, future research should focus on complex interactions between these. On the other hand, livestock operations should also take proactive steps to control preharvest contamination based on the availability and feasibility of methods such as dust control, preventing insect and wild animal intrusion to pastures and feedlots, use of direct-fed microbials, vaccinations, or any other suitable methods. Controlling preharvest livestock contamination not only prevents the environmental dissemination of pathogens but also prevents cross-contamination between animals, thereby improving the microbiological safety and quality of meat and poultry products. Last but not least, in accordance with the data-sharing initiatives by the regulatory agencies, these two major industries should share information and collaborate in terms of reducing food safety risks at the livestock–fresh produce interface.

Few studies identified proximity to livestock as a risk factor for contamination of the produce, but its contribution to the risk of foodborne illness was not assessed. Therefore, the current evidence regarding mechanisms of transmission from cattle to produce is not fully understood, and more research should be encouraged to determine the relative contribution of the mechanisms and the effect of agricultural and environmental factors on the contamination events. Although suggestions for further research are common conclusions in majority of scientific studies, it is particularly important to emphasize the need for more quantitative evidence for the transmission of foodborne pathogens to leafy greens in order to develop robust risk management systems for unique environmental conditions. Because of this, the FDA initiated large-scale longitudinal research programs in California and Arizona with the goal of understanding the persistence and movements of foodborne pathogens in produce farms in conjunction with the goals of the LGAP. It is crucial that both the produce and livestock industries collaborate and provide support for the ongoing and future efforts for longitudinal environmental studies.

AUTHOR CONTRIBUTIONS

Onay B. Dogan: Investigation; writing – original draft; writing – review & editing; formal analysis; data curation; methodology; visualization; conceptualization; validation. **Makenzie G. Flach**: Conceptualization; investigation; writing – original draft; writing –review & editing; formal analysis; data curation; validation. **Markus F. Miller**: Conceptualization; funding acquisition; writing – review & editing; project administration; supervision. **Mindy M. Brashears**: Conceptualization; methodology; writing – review & editing; funding acquisition; investigation; project administration; supervision; resources.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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