PEER-REVIEWED ARTICLE

Food Protection Trends, Vol 42, No. 5, p. 362–376
https://doi.org/10.4315/FPT-21-041

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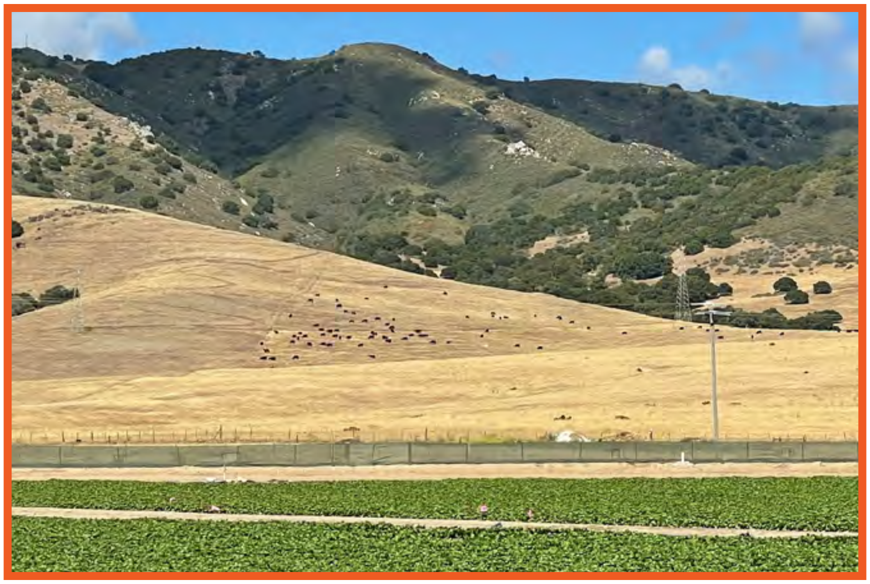

Environmental Risk Factors in the Human Pathogen Transmission Pathways between Animal Operations and Produce Crops

ABSTRACT

Once zoonotic pathogens leave their animal hosts, how they move through the environment and are deposited on unharvested produce remains a persistent industry challenge and research question related to produce safety. The proximity of animals to production areas, animal types and densities, an animal operation's management practices, and weather conditions are some of the areas explored by researchers to better understand how pathogens contaminate unharvested crops. Water, inputs, airborne particulates, wildlife, and insects may serve as vectors linking pathogens from their animal hosts to produce production areas. Studies have shown a positive correlation between rainfall and pathogen concentrations in agricultural water downstream from animal operations. Bacteria attached to airborne particulates can be deposited onto crops or open water sources. Wildlife and insects share habitat with domesticated animals in rangelands, pasture settings, pens, and feedlots. Plant conditions (injuries, disease) and characteristics (surface topography, genetic traits, age, native microbiota) and environmental conditions (relative humidity, moisture, temperature) play a major role in determining pathogen survival on unharvested produce. This article explores recent research findings elucidating human pathogen dispersion and deposition, subsequent transfer from animals to crops, and the various environmental risk factors along the way that play a role.

INTRODUCTION

In many regions, specialty crops are grown in proximity to or at an immediate interface with various types of domesticated animal operations *(105)*. Specialty crop production (fruits, vegetables, and nuts) in proximity to domesticated animal feeding and handling operations raises concerns about human pathogen transfer to crops. As well-recognized carriers of human pathogens, animals are one of the primary presumptive sources for direct or indirect risks to the safety of produce crops, and numerous studies have established an association between pathogen presence and proximity to domesticated animals in feedlots, dairies, pasturelands, etc. *(11, 13, 23, 29, 31, 51, 73, 96, 105, 116, 128)*.

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Because animals are well-known primary reservoirs or transient hosts for certain human pathogens, animal production, harvesting, and processing operations have for decades dealt with zoonotic pathogen infection and contamination in their food products. Much research has been conducted to investigate pathogen prevalence, loads, transmission, and dispersion in animal feeding and harvesting operations and methods to control and minimize subsequent contamination in by-products and end products. One meta-analysis of 46 studies published between 1980 and 2012 in North America revealed an *Escherichia coli* O157 prevalence of 7.35% among 110,641 cattle *(64)*. Results of multiple studies have indicated that environmental contamination within cattle operations greatly increases the spread of *E. coli* O157:H7 to uncolonized animals *(4, 48)*. Although several mechanisms and modes of transference are highly plausible, what is less well known is how specifically these zoonotic pathogens get from animal operations to specific produce fields, on a case-by-case basis.

However, as more research is published and contamination events and outbreaks are studied more thoroughly, the complexity of these events is becoming more evident. Rarely has the scientific evidence pointed to a simple transmission from pathogen source to preharvest crops. Despite these challenges, the empirical method has not failed but has provided volumes of evidence that when pieced together is methodically forming a clearer picture of how zoonotic pathogens pose a threat to the safety of preharvest crops. Research exploring how these individual risk factors contribute to and affect pathogen transmission from animals to in-field crops is revealing how multiple risk factors work in concert as a conduit for pathogen transmission. Therein lies the path to a better understanding of the contamination problem—capturing data from scientific research and real-world contamination events to use with analytical methods such as mathematical models.

This review includes an exploration of the published research for evidence of how human pathogens move from zoonotic origins in animal agriculture to in-field fruit and vegetable crops. Although pathogens from an animal operation may be confined to their immediate environment and never disperse at consequential levels beyond the pen or feedlot, numerous studies have documented pathogen dispersion beyond the confines of the animal operation *(13, 31, 45, 66, 67, 79, 112)*. The objective of this review was to explore and summarize recent research findings that shed light on human pathogen transmission pathways from animals to crops and the various factors that play a role in pathogen transfer, dispersion, deposition, survival, and persistence.

BACKGROUND: WHAT IS KNOWN ABOUT HOW HUMAN PATHOGENS MOVE FROM THEIR DOMESTIC ANIMAL HOSTS TO CROPS

After leaving the gastrointestinal tract of its animal host, a zoonotic pathogen begins a journey in the surrounding environment traveling primarily with the aid of various types of biological and nonbiological vectors. To study movement through the environment, researchers have used various genetic, chemical, and phage-based methods to help them identify pathogens in the environment and track their movements.

- Genetic tracking methods use host or pathogen subtype-specific gene sequences as markers or tags to follow pathogens from sources (i.e., concentrated animal operations and cow-calf operations) to surrounding areas within a region and sometimes even over long distances via dissemination pathways (e.g., watersheds).
- Chemical source tracking methods search for chemicals that are unique to a specific source and remain stable in the environment (e.g., metabolites of drugs given only to animals).
- Bacteriophages (phages) are viruses that infect specific bacterial hosts. Because phages are typically present in an environment where their bacterial host is also present, they could be used as a surrogate of sorts to investigate pathogen presence. Because a phage destroys its host after replicating within it, a negative correlation often exists between phage presence and bacterial host presence and, in some cases, phage presence without the presence of culturable bacterial hosts, and vice versa *(79, 81, 100, 111)*.

Much of this work has been pioneered and developed to identify and track human pathogens in drinking water or other water sources (e.g., irrigation water, watersheds, lakes, and canals) but is also applicable to tracking pathogens in media other than water *(52, 60)*. To assist pathogen transmission investigations, tracking methods are coupled with pathogen databases, such as that of the National Center for Biotechnology Information (https://www.ncbi.nlm.nih.gov/), that contain genetic and phenotypic data and descriptive information such as the location where and vehicle in which pathogens have been found *(109)*. Additional databases that have been useful for source tracking of pathogens are PulseNet, PulseNet-MLVA, and more recently GenomeTrakr *(49, 62, 132)*.

Water pathways

Contaminated water can spread pathogens from animal sources directly to crops (i.e., through irrigation and other applications such as dilution of pesticides or nutrients) and has been studied possibly more than any other transmission pathway. Numerous surveys of watersheds supplying agricultural water to or in the vicinity of major specialty crop production areas have been published *(2, 10, 11, 29, 31, 32, 51, 70, 81, 128, 133)*. In one such survey, scientists from the U.S. Department of Agriculture (USDA) and the U.S. Food and Drug Administration (FDA) collected surface water samples at public access points in watersheds within California's Central Coast agricultural region over a 2-year period and tested these samples for Shiga toxin-producing *E. coli* (STEC), *Salmonella enterica*, and *Listeria monocytogenes* *(32)*. Of 1,386 samples tested for pathogenic *E. coli*, 11% were positive for STEC and 8% were positive for *E. coli* O157:H7 specifically. The highest *E. coli* O157:H7 prevalence was in water samples collected close to or downstream from cow-calf cattle operations. Of 1,405 water samples tested for *S. enterica* and *L. monocytogenes*, 65 and 43%, respectively, were positive. Twenty-four of the survey's 30 sampling sites were *Salmonella* positive 70 to 90% of the time, and some serotypes persisted over several years. (The watershed sources sampled in that study *(32)* are not used for produce production in the study region.) Benjamin et al. *(11)* collected samples from eight cow-calf ranches in Monterey, San Benito, and San Luis Obispo counties in California from June 2008 through late October 2010. Three of 204 surface water samples and 1 of 93 sediment samples were positive for *E. coli* O157. In approximately the same time frame, Benjamin et al. *(10)* collected agricultural water samples from streams and ponds near or on leafy greens farms in the California Central Coast area and reported that >15% ($n = 437$) were positive for *E. coli* O157 and 6% ($n = 96$) were positive for *Salmonella*.

Earlier surveys of California water sources also produced similar findings. In Monterey and San Benito counties, 7.1% of water samples ($n = 252$) collected from May 2008 through June 2009 were positive for *S. enterica (51)*. Starting in January 2005 and continuing through August 2006, Cooley et al. *(29)* sampled 22 locations in watersheds in the same area on 23 occasions for *E. coli* O157 prevalence. Over the 19-month study period, the group reported a 12.8% prevalence for an accumulated 584 samples taken at 1-month intervals. In a similar study, the same group *(31)* collected >13,000 samples from water, cattle, multiple species of wildlife, produce, and soil at multiple California Central Coast farms and identified *E. coli* O157 and non-O157 STEC at various prevalences, illustrating some of the potential transport systems in this region.

Another recent study was conducted to investigate the prevalence of STEC-specific bacteriophages in water samples collected in 20 watersheds throughout the produce-growing areas of the Salinas Valley (California) and found the 13 (9.9%) of 131 samples contained bacteriophages that were lytic against STEC strains, including serogroups O45, O145, O157, and O179 *(81)*. Researchers at Cornell University (Ithaca, NY) sampled water ($n = 74$) from produce-growing region watersheds in New York and reported that 11% were positive for *Salmonella* and 30% were positive for *L. monocytogenes (128)*. In a more recent study, the same research group reported a strong association between ruminant and human fecal source-tracking markers and *Salmonella* isolation and detection of STEC genes (*eaeA* and *stx*) associated with pathogenicity in agricultural water samples collected from 68 upstate New York streams between April and October 2018 *(138)*. Antaki et al. *(2)* sampled irrigation water for three growing seasons on three farms in a mixed produce-growing region of southern Georgia. Ten percent of surface water samples ($n = 39$) and 13.6% of subsurface samples ($n = 44$) from two farm ponds were positive for *Salmonella*. *Salmonella* was also found sporadically in water samples from farm ponds on Virginia's eastern shore, with 19% prevalence in both 2015 and 2016 ($n = 200$) *(133)*. In Oldman River Basin, southern Alberta (Canada), *Campylobacter*, *Salmonella*, and the swine-specific *Bacteroides* marker detection rates were significantly higher in water that was downstream from areas where livestock density was greatest than in water upstream from those areas *(70)*. These study results reveal the variation in microbial quality of environmental and agricultural water sources in the North American continent.

Another microbiological aspect of agricultural water investigated by researchers is the release of microorganisms into the atmosphere via mechanical mechanisms. Bacteria attached to water droplets can be released from water bodies and become airborne. Droplets produced by mechanisms such as bubble bursting and fragmentation are transported into the atmosphere by wind, with many of the small droplets traveling over long distances *(1, 107)*. This phenomenon may be pertinent to lagoons located at concentrated animal operations. Droplets may be released from lagoons that are mechanically aerated, when it rains, and when effluent is added. A recent study documented the transfer of microorganisms in soil by microdroplets created by rainfall impingement. The microdroplets are transported immediately by wind or over potentially long distances after movement to the atmosphere *(72)*. However, as noted by Ravva et al. *(112)*, large volumes of contaminated aerosolized droplets would be required for pathogenic bacteria to pose a food safety risk to crops grown downwind of a microdroplet source.

Airborne particulate pathways

The question of whether pathogens associated with airborne particulates from concentrated animal operations and biosolid or manure spreading can cause disease in humans when inhaled has been explored by researchers over the past decades *(66, 112, 131, 146)*. However, the role that aerosols, airborne dust, and particulates in general play in pathogen dispersion to produce-growing environments has been grossly understudied *(36, 116)*. Bacteria use various mechanisms such as surface charge, weak molecular forces, cell hydrophobicity, and substances and structures on their membranes to attach to aerosols and go wherever the wind may carry them *(36, 41, 72)*. Numerous studies have been conducted to measure bacteria, including human pathogens, in aerosols such as dust samples *(26, 29, 41, 72, 87, 103, 110, 116, 130, 136, 145)*. Researchers have also studied transmission of microorganisms associated with airborne particulates produced by livestock and poultry operations in particular *(13, 40, 116, 131, 146)*. *E. coli* strains captured downwind from a swine house and dairy farm were closely related to those strains detected in feces and air within the pig and dairy facilities *(40, 116)*. Thiel et al. *(131)* found that manure applied and incorporated into fields also can become airborne and that bacteria attached to dried manure or feedlot surface material particles are more buoyant than bacteria attached to soil particles. In laboratory studies, Oni et al. *(103)* found that *S. enterica* remained viable for longer when attached to smaller turkey manure dust particles (125 µm) compared with larger particles (500 µm) and to particles with lower moisture levels (5 > 10 > 15%). They also reported that attachment to manure dust particles enhanced *Salmonella* survival after exposure to UV light, indicating that bacteria may benefit from their association with dust by acquiring some protection from environmental stresses such as UV light.

Because tree crops are grown off the ground and generally have no direct contact with the ground, orchards are naturally well suited for studying airborne pathogen transmission. Many orchards have switched from sprinkler to microirrigation systems, further reducing confounding factors from irrigation water when studying pathogen transmission to crops via dust and aerosolized particulates. Theofel et al. *(130)* sampled leaves from an almond orchard next to and downwind from a poultry operation. Both dust and bacteria (*Staphylococcaceae*) on tree leaves decreased as sampling progressed from the rows closest to the poultry operation (0 m) to rows 60 and 120 m into the orchard and away from the poultry operation. *E. coli* was present in significantly more air samples from orchards near the poultry operation than in samples from orchards farther away.

Row crops are also vulnerable to bacterial contamination associated with aerosols, dust, and droplets, but because particulates in these crops contact or are in close proximity to the ground, particulate distribution may differ from that in tree fruit and nut production. In their study of bacterial communities of romaine lettuce leaves from commercial production operations in Arizona and California, researchers from the University of California (UC), Davis noted that a severe dust storm during sampling in the Yuma area significantly affected the bacterial communities identified on the lettuce leaf samples collected that day *(110)*. These researchers also found that bacteria on leaves collected immediately after the dust storm passed differed from those collected later that same day. Based on this observation, they speculated that bacterial communities on crop surfaces may be transient at times. Research conducted in Salinas Valley fields over two growing seasons by other UC Davis scientists revealed day-to-day variations in native bacterial communities on romaine lettuce *(142)*, corroborating the previous findings in Yuma.

Bacteria attached to dust may also internalize if they attach at locations on the plant surface that allows entry into the fruit or vegetable interior. In a study exploring the role dust may play in specialty crop contamination, researchers exposed tomato plants in bloom to soil particulates contaminated with *Salmonella* Newport and used compressed air to simulate wind currents. One week after exposure, 29% of the blossoms were positive for *Salmonella* Newport; sterilized fruit also tested positive, indicating pathogen internalization within the tomatoes *(37)*.

Insects

Similar to dust and particulates, insects may play a role in pathogen transmission pathways between animal operations and crops, but this possibility has not been widely studied. We know human pathogens can survive in and on insects. The FDA has identified 21 species of "filth flies" that represent a potential human health threat as "scientifically proven causative agents" of foodborne illness or as carriers of *E. coli*, *Salmonella*, *Shigella*, and other foodborne pathogens *(101)*. Filth flies that breed in animal and human feces and decaying food and vegetation can serve as vectors for transmitting human pathogens. Efforts to control houseflies on military bases have resulted in significant reductions of diarrheal diseases caused by foodborne pathogens (e.g., *Shigella* and pathogenic *E. coli*), and an upsurge in fly density was positively associated with increased disease incidence in Bangladeshi children *(28, 46)*.

Studies in produce-growing regions have also provided evidence that insects can pose a food safety risk in areas where animals are present. Talley et al. *(129)* caught 34 filth flies in a field of leafy greens adjacent to cattle-occupied rangeland in the Salinas Valley. Of 18 flies tested with a PCR assay for the *E. coli* O157:H7 gene *eae*, 61% were positive for the pathogen. Other studies of flies in proximity to domesticated animals and other sources of foodborne pathogens (e.g., landfills, compost operations, and dumpsters) have revealed that flies can transfer pathogens from contamination sources to other surfaces *(19, 34, 53)*. Wasala et al. *(137)* found that filth flies that acquired *E. coli* O157:H7 from contaminated cattle manure deposited the pathogen by regurgitation onto spinach, where the *E. coli* survived and multiplied. Janisiewicz et al. *(68)* used a fluorescently labelled nonpathogenic *E. coli* strain to demonstrate that fruit flies can transmit bacteria from refuse piles of decaying fruit to uncontaminated apple wounds, indicating that the flies were contaminated both externally and internally. In laboratory experiments, Talley et al. *(129)* used a similar labeling method to investigate *E. coli* O157:H7 transfer from flies to spinach and found the fluorescently labelled bacteria on 50 to 100% of leaves examined with a fluorescence microscope.

But not all insects are equal in the threat they pose as vectors for transferring human pathogens. In their 2017 study of houseflies and blow flies, Pace et al. *(104)* found that blow flies were more efficient than houseflies at transmitting *E. coli* O157:H7 and *S. enterica* from manure to leafy greens. The risk to produce fields from insects may also be dependent on the presence of risk factors in the surrounding environment in addition to the insect species. Barreiro et al. *(7)* tested flies from various rural areas in Portugal and reported that flies in proximity to animals had higher human pathogen prevalence than those collected from kitchens. USDA researchers measured *E. coli* O157:H7 carriage rates in five different types of flies (house, face, flesh, blow, and stable flies) collected at their 6,000-head-capacity feedlot and adjacent leafy greens research fields in Clay Center, NE during summer 2011 and summer 2012 *(14)*. Excluding stable flies, which had an *E. coli* O157:H7 carriage rate of 1.1 flies per 1,000 flies sampled, all had similar carriage rates of 22.3 to 29.0 per 1,000 (*n* = 6,228). Flies collected at 0, 60, 120, and 180 m from the feedlot were not significantly different in their carriage rates.

Wildlife

Wildlife also can serve as human pathogen vectors between animal agriculture and specialty crop production areas. Aside from birds, wildlife come in contact with animal agriculture more often in pasture settings, but even in facilities and feedlots, other wildlife such as rodents have frequent contact with production animals. Numerous surveys have been used to track pathogen prevalence in wildlife with habitat and migratory pathways in proximity to produce-growing regions *(31, 54, 69, 74, 77, 84, 124)*. Indistinguishable pathogen strains are frequently present among wildlife and domestic animals in the same geographical area, suggesting transmission among species and/or contact with a common vector(s) or reservoir(s) of contamination in the environment. Deer that share habitat with cattle are often thought to be more susceptible to pathogenic *E. coli* colonization. Díaz-Sánchez et al. *(38)* found a positive association between STEC prevalence in red deer feces and the presence of livestock. In other studies, STEC-positive deer have been found in proximity to dairy and cattle operations *(47, 117, 124)*. Kilonzo et al. *(75)* screened fecal samples from wild rodents trapped on 13 agricultural farms (9 produce farms, 3 cow-calf operations, and 1 beef cattle feedlot) in Monterey and San Benito counties, California to investigate the prevalence and risk factors for shedding of several foodborne pathogens. *Cryptosporidium* spp. (26.0% prevalence), *Giardia* spp. (24.2%), *S. enterica* serovars (2.9%), and *E. coli* O157:H7 (0.2%) were detected in rodent fecal samples. These researchers also discovered that pathogen presence was higher in rodent communities with a higher number of deer mice and lower diversity than in more diverse rodent communities. During studies of the ranch associated with a major outbreak of *E. coli* O157:H7 infection linked to baby spinach in 2006, a high percentage of feral pigs captured on the ranch carried the outbreak strain, which was also present in a high percentage of cattle fecal samples *(29, 69)*. The same outbreak strain was isolated 5 years later from a bird (Dark-eyed Junco) trapped ca. 10 miles (16 km) from the outbreak ranch *(30)* (unpublished data).

With extensive sampling, Cooley et al. *(29, 31, 32)* used multiple locus variable number of tandem repeats analysis (MLVA) to identify additional environmental *E. coli* O157:H7 strains with identical 11-loci genotypes isolated many months apart from the same sites and from different sites (water, cattle, and wildlife), indicating the stability of at least some microbial populations. Multiple outbreak-associated *E. coli* O157:H7 strains had the same 11-loci genotype as strains isolated during these studies. For example, a strain associated with an outbreak caused by contaminated leafy greens grown on the island of Kauai was indistinguishable by pulsed-field gel electrophoresis and highly related by MLVA to a strain isolated many months before from cows in Monterey County, California. One hypothesis for this distant relationship between strains is the common transporting of cows from Kauai on container ships for fattening in California, which again suggests genetic stability of some strains of *E. coli* O157:H7 (unpublished data). These findings illustrate that pathogens are transported by comingled animals and emphasize how animal populations and interactions can affect pathogen transmission pathways. A better understanding of how the ratios of hosts (livestock) to wildlife vectors (e.g., rodents, feral pigs, birds, coyotes, and insects) and vector-host, host-host, and vector-vector interactions affect pathogen transfer will play a critical role in estimating the risk of transmission in any given environment.

A study of 21 New York produce farms was conducted to investigate the association between field-level management practices and field samples positive for *Salmonella* and *L. monocytogenes*. The researchers reported that wildlife observation within 3 days of sample collection increased the likelihood of a *L. monocytogenes*-positive field *(133)*. Researchers at the USDA Meat Animal Research Center in Nebraska sampled water from a stream flowing through a cattle pasture and determined that waterfowl density significantly affected the stream *E. coli* levels after a storm event in the fall *(56)*. In a year-long survey of birds in an agricultural region of California, Navarro-Gonzalez et al. *(97)* reported finding similar strains of STEC in wild geese and free-range cattle that comingled and/or were found in the same geographical area. Carlson et al. *(21)* researched how bird-livestock interactions affected the spread of ciprofloxacin-resistant *E. coli* in cattle feedlots across the United States and found European Starlings positive for ciprofloxacin-resistant *E. coli* strains in every feedlot tested. The total number of European Starlings was positively associated with increased cattle fecal shedding of the same antibiotic-resistant *E. coli* strains. These studies provide evidence of the role wildlife may play in human pathogen transmission pathways between animal agriculture and specialty crops.

RISK FACTORS: HOW ENVIRONMENTAL CONDITIONS INFLUENCE PATHOGEN DISPERSION, TRANSMISSION, AND DEPOSITION ON CROPS

Even when a human pathogen is attached to a dust particle or an aerosolized droplet or droplet nucleus, its dispersion to and deposition on crops is also greatly influenced by inherent determinants of buoyancy and various environmental conditions such as landscape topographical features, gravitational settling, regional weather, and the effect of crop traits on boundary layer surface dynamics. The roles of some of these conditions are obvious, for example, the susceptibility of a row crops located directly downslope from a cattle feedlot. Other conditions are less obvious, for example, a row crop ranch located 2 miles (3.2 km) downwind from a swine production facility. The distance that a crop production facility should be from an animal agriculture facility to prevent contamination is an ongoing and somewhat controversial discussion among fresh produce industry stakeholders. In one study conducted in Colorado and Texas, *E. coli* counts on in-field spinach plants were significantly increased by proximity (within 10 miles) to dairy, beef, and poultry farms *(105)*. In another study, a relationship was found between pathogenic *E. coli* presence in agriculture water and how much of the waterway was bordered by animal operations *(44)*. These studies have provided evidence of how environmental factors may influence bacterial dispersion, and they provide valuable information that can be used to assess the contamination risk from microbial hazards. However, considering the complexity and perhaps uniqueness of each agricultural region, the results do not provide equal value for risk assessment.

Animal operation features

Considering the numerous studies reporting various levels of human pathogens in animal agriculture, controlling pathogen concentrations at the animal operation level would undoubtedly have a beneficial effect on transfer of pathogens to food crops. An extensive review of research on human pathogen prevalence in animals and risk factors associated with high levels of pathogens such as STEC in grazing and feedlot cattle is beyond the scope of this review, and published reviews of those studies are readily available *(20, 42, 92)*. Nevertheless, certain risk factors are related to features and conditions of an animal operation and of the animals themselves.

Distance between specialty crop production and animal operations is an obvious risk factor for human pathogen transmission. Berry et al. *(13)*, Park et al. *(105)*, Sanz et al. *(116)*, and Theofel et al. *(130)* described the proximity to animal operations as an influence on the microbial profiles of nearby specialty crops and on human pathogen contamination of crops. Connections between crops and animals, such as waterways or landscape topographical features creating wind tunnels, also influence the risk of pathogen transfer from animal operations. In a study in British Columbia of connections between pathogens in surface water used for irrigation and the length of upstream borders with animal operations *(44)*, the researchers described the significant correlation between pathogenic *E. coli* levels in irrigation water and the length of a waterway (2 to 3 km) bordering properties containing cow or poultry operations.

Animal density is another well-established risk factor in pathogen transmission; areas with multiple animal operations or individual high-density animal operations pose a higher risk of pathogen transfer *(35, 50, 70, 80, 130, 143)*. However, independent of density, high levels of human pathogens have been measured at some animal facilities and not at other similar operations. Thus, some characteristics of the operation and/or the resident animals affect the dispersion and the concentration of human pathogens. Researchers have explored conditions other than density, such as management practices, related to animal operations to see how they might contribute to pathogen transmission to preharvest in-field specialty crops. Contamination can spread to the hides of additional animals in the feedlot via pathogen-containing dust particles *(93)*. Many animal operations spray water to control dust. At the USDA research facility in Nebraska, Berry et al. *(13)* noted that cattle pen dryness and animal activity affected the amount of dust that was dispersed to nearby test fields of leafy greens and the detection of *E. coli* O157:H7 on test plants. In experiments on which uninfected cattle were exposed to cattle contaminated with *E. coli* O157:H7, pathogen transmission rates were significantly increased by contamination in the surrounding environment *(48)*.

In numerous studies, pathogens have been found in water troughs, feedstuffs, feedbunks, incoming water, and silage *(59, 76, 98, 99, 113, 118, 134, 135)*. However, water troughs may affect human pathogen levels in cattle even when the water is not contaminated. Beauvais et al. *(8)* found a positive relationship between water levels in automatic refilling troughs in a Texas feedlot and the prevalence of *E. coli* O157:H7 fecal shedding in the feedlot cattle. When water levels were low, *E. coli* O157:H7 shedding by cattle increased, and when water levels were high, *E. coli* O157:H7 shedding decreased. The study authors proposed some explanations for this finding but were admittedly unsure of the cause of the association.

The presence of supershedders in a cattle herd has also been associated with increased pathogen prevalence within cattle operations. Cattle shedding *E. coli* O157:H7 in their feces at $\geq 10^4$ CFU/g are labeled supershedders and transmit the pathogen to other cattle (horizontal transmission) in feedlots *(3, 4, 25, 126)*. Matthews et al. (*88*) presented data that suggest that the spread of *E. coli* O157:H7 in cattle herds could be effectively controlled by prevention strategies targeted at the top 5% of cattle that shed high levels (10^4 to 10^5 CFU/g). Findings from other studies support this strategy of controlling the supershedders in a cattle herd to prevent widespread infection throughout the herd *(27, 89, 102)*. Supershedder strains become the predominant strains in the environment, and when they also are highly virulent they can cause illnesses.

In studies of herds in pasture, cattle access and density was significantly associated with increased *E. coli* levels in water sources and feces *(11, 56, 138, 140, 141, 143)*. At the USDA research facility in Nebraska, Hansen et al. *(56)* found higher *E. coli* levels in a stream flowing through the pasture when more cattle were grazing close to the water during the summer months. Benjamin et al. *(11)* also reported increased odds of detecting *E. coli* O157 in feces in larger herds. Wilkes et al. *(140, 141)* found higher levels of ruminant Bacteriodales markers and *E. coli* O157:H7 prevalence in water samples from streams where cattle had unrestricted access compared with streams where cattle access was restricted. Contamination flows both ways; cattle on California ranches that used surface water sources for drinking had a 4.2 times higher risk of testing positive for fecal *E. coli* O157:H7 than did cattle on ranches without surface water access *(143)*. Cooley et. al. *(29, 31, 32)* described transport of foodborne pathogens as likely fluid and bidirectional from water runoff, wildlife, manure, and water transport or flooding to wildlife, farms, and ranches.

Weather

Researchers have investigated various climate- and weather-related conditions that influence pathogen dispersion and deposition. Surface water flows due to rainfall serve as a natural conduit for pathogen dispersion. Rainfall is associated with increased bacterial levels in agricultural water *(2, 29, 31, 56, 114, 133, 138)*. USDA researchers at the Meat Animal Research Center in Nebraska studied the effects of animal and waterfowl presence and rainstorm events on *E. coli* levels in a stream traversing a pasture. The presence of cattle in pastures adjacent to the stream the day of a rainstorm and the increasing accumulation of cattle (density) throughout the growing season had a significant impact on *E. coli* levels in the stream following summer storm events *(56)*. In the fall, a significant positive correlation was found between waterfowl presence and *E. coli* levels in the stream following rainstorms. Weller et al. *(138)* also found a positive correlation between rainfall and *L. monocytogenes* level in agricultural water downstream from dairy operations in upstate New York. Cooley et al. *(29)* reported increased incidence of *E. coli* O157 isolates in rivers when heavy rain caused increased flow rates, and heavy rainfall in elevated watersheds resulted in some indistinguishable strains being isolated from sites in the contiguous watershed up to 32 m from a point source. Researchers studying land use effects on *E. coli* levels in water sources also found increased prevalence and higher levels when storms and overland water flows occurred more frequently *(114)*. In their study of farm ponds on Virginia's eastern shore, Truitt et al. *(133)* found a significant effect on the probability of detecting *Salmonella* when precipitation occurred the day before or the day of sampling.

Wind can have a variable effect on pathogen transmission and deposition depending on the medium in which the pathogen resides and the environment in which it is located. The importance of wind direction is self-evident. When specialty crops are grown downwind from animal operations, the contamination risk is higher than it would be if the crops were grown upwind. Wind can carry pathogen-contaminated dust particles from an animal operation and deposit them in the surrounding environment. Sanz et al. *(116)* analyzed air samples taken in November and July at various distances from a dairy farm in all directions and at three elevations for the presence of *E. coli*. Both higher temperatures and wind direction positively affected the number of *E. coli* isolates captured in the air samples. A comparison of the genomic DNA profiles of *E. coli* strains from animal housing facilities and those isolated from the surrounding environment suggested that the strains were related. In pastures where animals are often not concentrated, bacteria are more stable (i.e., not airborne), and wind may affect bacterial survival differently. In a study of cattle on pasture at three California ranches, wind speed was negatively associated with *E. coli* O157:H7 occurrence in fecal pats. When wind speed was higher, researchers were less likely to detect *E. coli* O157:H7 in fecal pats, most likely due in part to desiccation *(11)*.

Wind speed also plays a crucial role in spreading contamination. According to the Beaufort scale, a moderate breeze with wind speeds of 5.5 to 7.9 m/s (12.3 to 17.7 mph) is associated with dust movement *(11)*. Higher wind speeds are required to move material from the ground (3.0 to 5.4 m/s [6.7 to 12.1 mph]) than are required to move material from plants (0.5 to 2.0 m/s [1.1 to 4.5 mph]) *(71)*. In their study of environmental factors affecting *E. coli* O157:H7 contamination of in-field lettuce in Salinas Valley, Moyne et al. *(94)* recorded wind speeds of ca. 0 to nearly 8 m/s (17.9 mph) over a 24-h period in six trials, with consistently higher wind speeds during the late afternoon and early evening. Dry, windy conditions are the most likely times when bacteria-laden dust is moved from animal sources to crop production areas.

Wind also can stir up surface water and release bacteria sequestered in the underlying sediment, where *E. coli* levels can be 10 to 1,000 times higher than those in the overlying water *(10, 11)*. Falbo et al. *(45)* measured viable *E. coli* levels in sediments from roadside ditches along agricultural and forested land in New York State; the mean was 4,616 most probable number (MPN)/100 ml, and the maximum was >240,000 MPN/100 ml. The highest levels were detected after manure was spread in fields adjacent to ditches. Total suspended solids were 0.51 to 52.2 g/liter. Roadside ditches capture stormwater runoff, which is a source of environmental fecal contamination from such sources as wildlife, pet, septic system, and livestock waste, and transport contamination to watersheds. Crabill et al. *(33)* found that sediment agitation by storm surges was responsible for increased fecal coliform levels in water in Oak Creek, AZ. In Salinas, CA, for every 1 m/s increase in wind speed, Benjamin et al. *(10)* reported a 60.1% increase in *E. coli* levels in irrigation water.

Although wind speed and direction play significant roles in dispersion, they also affect deposition. Deposition of an airborne particle onto a plant involves how many particles are involved and the deposition velocity. Deposition velocity, measured in distance per time (e.g., centimeters per second), is a function of gravitational settling, aerodynamic resistance or drag, and resistance from the surface on which it is being deposited *(66)*. In general, more particles in a particular size class are deposited closer to their source, but this phenomenon deviates when upward currents take particulates higher into the atmosphere, facilitating transport and deposition at long distances *(71)*.

IN THE PRODUCTION ENVIRONMENT: FACTORS THAT INFLUENCE PATHOGEN PREVALENCE, SURVIVAL, AND GROWTH ON SPECIALTY CROPS

Microorganisms do not live in isolation. As for visible living organisms, a microorganism's ability to survive, grow, and reproduce is greatly affected by its surrounding environment and its ability to adapt to that environment *(16, 86)*. When human pathogen cells are deposited on a produce crop in a field, several outcomes are possible but three are more likely: (i) the pathogen may grow, (ii) it may not grow but it may survive in a dormant form, and (iii) it may die. Environmental and plant conditions and crop management practices play critical roles in determining which of these three scenarios occur and provide the subtle distinctions that determine whether a contaminant is eliminated before harvest or remains and results in foodborne illness upon consumption. (Other factors such as pathogen dose also play a critical role in foodborne illness.) Microorganisms, including foodborne pathogens, do not exist in isolation (the planktonic state). They may be in clusters of different sizes, either as a single species or in a mixed species group, attached to particles of different types, in biofilms, internalized in a single cell predator, or in some other unknown form *(16)*. Many laboratory studies of foodborne pathogens on plants have yielded insights into their life cycles, but studies of "naturally" contaminated plants have not been reported due to the difficulty in obtaining samples and analyzing them microscopically, biochemically, or genetically.

Scientists are currently trying to answer for the fresh produce industry questions related to contamination. If a pathogen contaminates in-field crops, what happens to it, and how long can it be expected to survive? If, for example, pathogen-contaminated dust or soil particles are deposited onto plants preharvest, can the contamination spread to neighboring plants? If contaminated particles spread, which possible mechanisms are most likely? Which environmental conditions and cropping practices (e.g., bed width, plant density, and tree canopy training scheme) have the most influence on a pathogen's in-field survival? The answers to these questions are dependent on the environmental conditions of a particular growing location, but laboratory and field studies indicate certain conditions seem to consistently influence pathogen survival in various environments.

Relative humidity

When pathogens are deposited in a production area, relative humidity plays a significant role in pathogen survival both on the crops and in the surrounding environment *(94, 127)*. Belias et al. *(9)* investigated how weather and climatic factors affected human pathogen die-off patterns in lettuce and spinach grown in California, New York, and Spain. Die-offs of *Salmonella* and *E. coli* O157:H7 on leafy greens followed a biphasic segmented log-linear pattern, resulting in an initial rapid decline followed by a protracted die-off tailing period. The initial rapid pathogen die-off in the first segment was affected in all three locations by relative humidity. A lower relative humidity was associated with a faster first segment die-off and earlier break point (between the first and second segments). Pathogen die-off, in general, differed significantly among the three locations and among seasons within each location. In addition to being affected by relative humidity, the initial rapid die-off was also affected by the type of leafy green and bacteria: die-off was faster on lettuce than on spinach, and *Salmonella* had a slower die-off than did *E. coli* O157:H7. Due to significant variability in die-off rates, Belias et al. suggested that use of a single die-off rate would be inappropriate across different locations and seasons due to different weather conditions, an important consideration with respect to the Food Safety Modernization Act recommendations. The significant and consistent difference in die-offs between *Salmonella* and *E. coli* indicated that nonpathogenic *E. coli* may not be appropriate as a surrogate for pathogens in field studies.

Moisture

Moist conditions typically promote pathogen survival and redistribution on plant surfaces and may promote growth, whereas rapid desiccation hinders pathogen survival *(94)*. Chase et al. *(24)* researched how heavily contaminated irrigation water would affect the growth of *E. coli* O157:H7 on leafy greens in 10-day field trials during the summer and fall growing seasons in the Salinas Valley, California. After spraying in-field romaine lettuce with animal manure slurries (pig, chicken, and rabbit) at the end of July and in mid-October, the starting *E. coli* O157:H7 levels initially decreased. On day 5 post-spraying, the crop was irrigated, and *E. coli* O157:H7 levels were again measured over 5 days. An initial bump in *E. coli* O157:H7 levels occurred in the days following irrigation; they increased by 1 to 5 log CFU over the starting levels for ca. 24 h before declining to lower than the initial levels by day 10. Moyne et al. *(94)* also reported a positive effect of leaf wetness on *E. coli* O157:H7 survival and growth in their experiments on field-grown romaine lettuce in the Salinas Valley. These studies indicate that the timing of irrigation before harvest could enhance the survival and/or growth of *E. coli* O157:H7, if present, resulting in a substantial increase on lettuce under ideal moisture conditions.

Jones and Harrison *(71)* reviewed the mechanisms by which rainfall may spread pathogens deposited on plants to other plants or from the ground to plants. Studies of bacterial and fungal spores revealed that particles can be released when heavy raindrops first hit a plant before its surface is fully wet. Contaminated particles also may be transferred to other plants in splash droplets. Numerous studies have been conducted to explore transfer of pathogens from the ground (soil, mulch, and plastic coverings) to plants *(22, 78, 139)*. Lee et al. *(78)* measured *Salmonella* coming from the ground. Weller et al. *(139)* found that 39% of lettuce heads within 2 m of *E. coli*-inoculated fecal pellets were positive for *E. coli* following overhead irrigation. Most study findings indicate that pathogen transfer may occur from splashing under certain circumstances, but the effect of splashing on gross in-field contamination is still not completely understood. When the in-field contamination levels are low, pathogen transfer propagated by rainfall or irrigation (i.e., splashing) alone may not pose a major risk of gross in-field contamination but may play a more significant role in spreading contamination during harvest operations or when the product is further trimmed, comingled, or processed during or after harvest.

Plant characteristics and conditions

Plant characteristics and conditions also affect survival and persistence of pathogens after they are deposited onto specialty crops. Plant wounds and injuries may provide pathogens with access to nutrients that may enhance survival on or in a plant. When plants are wounded or injured by farm equipment, plant pathogens, insects, and/or adverse weather conditions such as frost or intense wind or heat, pathogens on plants may be better able to survive and persist *(5, 6, 17, 57, 95, 121, 123)*. In growth chamber and greenhouse studies, USDA researchers found that the susceptibility of lettuce damaged by downy mildew (caused by the oomycete *Bremia lactucae*) to *E. coli* O157:H7 and *Salmonella* increased 10^5-fold compared with 10^2-fold on plants without downy mildew *(123)*. Plant age and leaf topography (e.g., roughness and stoma density) also affected *E. coli* O157:H7 and *Salmonella* survival on leafy green plants *(18, 39, 61, 82, 85, 142)*.

A plant's genetic traits (genotype) also play a role in susceptibility to human pathogens. Erickson et al. *(43)* tested five lettuce cultivars (Green Star, Muir, New Red Fire, Gabriella, and the romaine lettuce cultivar Coastal Star) in field trials to evaluate differences among these cultivars in vulnerability to *E. coli* O157:H7 and *Salmonella* contamination. Up to 5 days post-inoculation, significant differences were found among the cultivars' responses to *Salmonella* and *E. coli* O157:H7 contamination, but the differences disappeared by day 9. After 5 days post-inoculation, pathogen surface levels were so low that no difference in pathogen survival was measurable, and the study authors concluded that cultivar selection has minimal impact on managing microbiological risk. Jacob and Melotto *(65)* further explored the natural variability in the response of 11 lettuce cultivars to the human pathogens *S. enterica* Typhimurium and *E. coli* O157:H7 and the differences in the immune responses of these lettuce cultivars. In contrast to the findings of Erickson et al. *(43)*, Jacob and Melotto found differences in cultivar susceptibility to pathogen colonization and in the plants' natural defenses against pathogen colonization. Differences in the results of the two studies may be due to the lettuce varieties tested and experimental conditions related to how pathogens were inoculated onto plants. However, similar to Erickson et al., Jacob and Melotto also observed a significant decrease in internalized pathogen populations in 9 of the 11 lettuce cultivars 10 days after surface inoculation.

Other studies have been conducted on the native microbial communities (the microbiota) of specialty crops and the role they may play in pathogen survival by either protecting the plants from adverse conditions or minimizing or excluding pathogens *(30, 108, 110, 142)*. In their 2-year field study, Williams et al. *(142)* identified bacterial communities on romaine lettuce leaves grown in the Salinas Valley, California and reported that lettuce plants with low levels of persistent attenuated *E. coli* O157:H7 also had lower levels of native bacteria. The bacterial communities differed among all four plantings and were strongly associated with the planting season. Bacterial diversity increased as plants grew and was affected by the irrigation method (drip or overhead irrigation). When Poza-Carrion et al. *(108)* studied *S. enterica* survival on romaine lettuce and cilantro leaves, they found that the pathogen survived better on a wet leaf surface when it was associated with precolonized clusters of native bacteria. Cooley et al. *(30)* also reported a differential response of native bacterial species (i.e., some protective and others competitive) to *E. coli* O157:H7 on lettuce. Theofel et al. *(130)* sampled leaves from an almond orchard next to and downwind from a poultry operation. The microbiota of almond trees closest to the poultry operation was different from the microbiota of leaves on trees in the orchard collected further away from the poultry operation.

Compounding effects

Major changes in environmental conditions such as those caused by wildfires, hurricanes, and other natural disasters also have an impact on the ecosystem in produce-growing regions. For example, wildfires in California destroyed large swaths of insect habitat, which may impact surviving insect populations in various ways including shifting populations from burned areas to nearby crop production areas. Other than the effects of wildfires on insects in forest ecosystems, changes in more widespread insect behavior have not been extensively studied *(106)*. Coupled with any type of crop damage, a higher insect pest burden could result in a higher food safety risk if contamination occurs. These adverse events and weather conditions may not be a high food safety risk on their own, but when they intersect with additional adverse circumstances (e.g., crop damage, increased insect burden, and changes in wildlife populations and habitat), they may create conditions under which crops are more susceptible to pathogen contamination.

PREVENTION: WHAT PRACTICES SHOULD YOU CONSIDER INCLUDING IN YOUR FOOD SAFETY PROGRAM AND WHAT OTHER MITIGATION MEASURES ARE BEING DEVELOPED TO REDUCE THE RISK OF ANIMAL-RELATED CONTAMINATION?

In this section we explore various research-backed practices that minimize the food safety risks associated with growing produce in areas where animal agriculture such as concentrated animal operations are also located. Recent findings may reinforce practices already in place, provide additional details related to industry practices, or introduce new information relevant to reducing contamination risks.

Cattle-focused strategies

Although controlling what happens on the land adjacent to produce fields is generally beyond the produce growers' control, coordinated efforts are being made to find solutions to reduce food safety risks among fresh produce growers and neighboring animal operations in the agricultural community. The Adjacent Lands Subcommittee is a special subcommittee of the Leafy Green Handlers' Marketing Agreement's Technical Committee comprising growers, food safety experts, scientists, landowners, and cattle ranchers. This committee has been examining ways to better assess and mitigate risks that may be present on farms growing leafy greens and on property located near these farms. The Western Center for Food Safety and Security at UC Davis held a "good neighbor" workshop exploring the livestock-produce interface.

Vaccines, diet regiments, probiotics, colicins, and other therapeutics that could decrease pathogenic *E. coli* levels, specifically in cattle, are potential remedies being explored to reduce infection by and transmission of this pathogen from animals to the environment *(83, 91, 115, 125)*. Recently, increased interest and resources have been directed toward targeting vaccines, and studies of various vaccination approaches are currently underway *(119, 122)*.

In a different approach, USDA researchers noted that when contaminated feedlot surface materials were solarized by covering with clear polyethylene, pathogenic *E. coli* was reduced in the cattle feedlot pens, with 2.0-log reductions after 1 week to 3.0-log reductions after 6 weeks *(12)*. Use of materials to increase solarization in pens when they are empty could reduce pathogen spread from contaminated surfaces to uninfected animals.

Protecting crops from dust deposition

Growers, especially those with fields of leafy greens in proximity to animal operations, need to be aware of processes that increase dust production and include monitoring of high levels of dust influx as part of their food safety program. Studies reviewed here provide evidence that pathogens can attach to particulates and dust carried by wind and air currents, and pathogens attached to dust particles can better survive adverse conditions than when not attached to dust. Based on results of the studies reviewed here and recent outbreaks of *E. coli* O157:H7 infection associated with leafy greens *(15, 63)* grown in a region near a massive feedlot or with frequent high winds yielding significant dust, food safety can be improved by an understanding of the mechanisms and trajectories of dust emission from agricultural and livestock production systems.

Growers should be aware of dust that originates from adjacent or nearby animal operations, but the dust from cultivation also can be problematic. Research by Thiel et al. *(131)* points to the risks of releasing pathogens in association with particulates when cultivating contaminated soil. Research of risk factors associated with *Salmonella* and *L. monocytogenes* contamination in produce fields revealed an increased likelihood of finding both pathogens in fields that were cultivated within 7 days of soil sampling *(128)*. In a field adjacent to or near animal operations, restricting the last soil cultivation to within 7 days of harvest may minimize the potential for pathogen contamination.

Specialty crop growers are familiar with the complexity of balancing food safety and conservation or ecological efforts, often referred to as comanagement. The issue of vegetation attracting wildlife to specialty crop production sites and/or providing cover for physical hazards from human activity is a major part of comanagement discussions *(84)*. A known function of trees that is not often a major part of these discussions is that tree canopies provide protection from airborne particulate deposition *(36, 55)*. In the vast open fields of the western United States, namely California and Arizona, the two states that provide the vast majority of U.S. leafy greens, few greenbelts with large trees have been developed between produce fields, and most fields are not located close to forested land. Unless studies can provide definitive data for assessing risks from dust versus riparian or forest environments, discouraging specialty crop production close to greenbelts and riparian or forested areas to reduce the opportunity for animal intrusion seems at odds with the protection trees provide from dust.

Bacteriophages as mitigators of bacterial pathogens

Bacteriophages are viruses that infect bacteria. Because they typically infect only one type of bacteria, bacteriophages could be used as indicator organisms or surrogates for bacterial presence. Because phages lyse and destroy cells after replicating inside them, phages could also be part of novel pathogen mitigation methods *(81)*. In their study of bacteriophage presence on an organic farm, Lennon et al. *(79)* identified and isolated phages from goat feces, and these phages collectively destroyed *E. coli* O157 and six other non-O157 STEC strains. The authors suggested that these phages may be candidates for biocontrol to reduce STEC presence in the farm environment. Other researchers have isolated lysins, a type of phage enzyme that degrades the walls of the infected bacterial cell *(120)*. Purified lysin can destroy cell walls of sensitive gram-negative pathogenic bacteria such as *E. coli*, *Salmonella*, and *Shigella* on contact, thus acting as an antimicrobial agent. When applied for 10 or 20 min to a romaine leaf model contaminated with *E. coli* O157:H7, 100 ppm of a novel lysin resulted in 2- and 4-log reductions of the pathogen, respectively, with no reported visual or tactile change in lettuce quality *(144)*.

Plant breeding

Although early in the development stage, the breeding of plants that are more resistant to pathogen contamination is gaining momentum. In their 2020 review, Melotto et al. *(90)* discussed the necessity of a multidisciplinary approach to plant breeding for food safety that includes the interactions among factors such as plant genotype, environment, microbe, and management practices. Henriquez et al. *(58)* discussed the potential strategy of screening for varieties and cultivars that are less prone to contamination followed by use of biomarkers to produce varieties that are more resistant to enteric pathogens.

CONCLUSION

In many rural communities around the world, human food crop production and animal agriculture often occur in proximity. Because many animals serve as reservoirs for human pathogens, this closeness presents the potential for these pathogens to spread to food crops that are generally or always consumed raw. After a pathogen leaves its animal host, determination of how it moves through the environment can be challenging. A major part of the challenge is related to the difficulties of conducting experiments in an open environment, which often requires access to privately held land. The frequently transient nature of the suspected microbial contaminant in the affected environment presents a further challenge.

Even with the challenges associated with complex, transient contamination events, our overall understanding continues to improve as the findings of new research and experimentation are published. No two contamination events are the same, and root cause analysis, when conducted, are frequently multifactorial. However, individual study results and outbreak investigations contribute to the ever-expanding knowledge base on how and why zoonotic pathogens are transmitted from their hosts to plants. This information can be used with advanced analytical methods such as modeling to provide an invaluable bridge between research and the industry. These methods are the tools decision makers need to quantify the contamination risk as they observe and measure risk factors in their immediate production environments. In a perfect world of accurate data about pathogen source, transport, levels, physical and biochemical changes, growth, die-offs, etc., and plant physiology and biology, we might change how we grow produce. Alternatively, and more likely, we would be surprised about what we do not know. Animals, including humans, are the source of the major foodborne pathogens of concern; however, the primary source and mechanisms of transport to produce crops in the field remain unknown for most outbreaks. It is time to move from hypotheses and speculation to definitive determination of the root causes of outbreaks followed by strategies for prevention of additional outbreaks.

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