Potential role of wildlife in pathogenic contamination of fresh produce

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Abstract: The safety of fresh produce is an important concern in the United States, especially in the wake of recent national foodborne illness outbreaks. The agricultural industry has implemented steps to enhance food safety along the entire farm-to-fork supply chain. This includes on-farm measures to exclude wildlife and to remove its habitat in and around fields. Farmers and others from across the United States have expressed concern about the ecological consequences and uncertain food safety benefits of such practices. This article reviews the scientific rationale behind management of wildlife and its habitat as part of good agriculture practices for enhancing food safety. The review concludes that, although pathogen prevalence has been documented in wildlife at overall low levels, the potential role that wildlife and its habitat play in pathogenic contamination remains unclear and is interwoven with pathogenic risk from human and domesticated animal sources. The characterization and disruption of potential links between livestock and wildlife is highlighted as a research priority. The findings underscore the importance of appropriate wildlife research and management in the context of food safety and to human–wildlife interactions in general, and they have implications wherever fresh produce is grown in the United States.

Key words: *Escherichia coli* O157:H7, foodborne illness, food safety, human–wildlife conflicts, wildlife pathogens, zoonotic disease

In September 2006, a national outbreak of *Escherichia coli* (*E. coli*) O157:H7 associated with processed, bagged spinach sickened >200 individuals in 26 states and resulted in at least 3 deaths (U.S. Centers for Disease Control 2006). An extensive investigation by federal and state officials ensued, tracing the outbreak to a single field in the Central Coast region of California. The implicated spinach field was fallow at the time of the investigation, and interviews with the grower and harvester did not reveal risk factors for contamination. As such, the official investigation was unable to determine how the contamination occurred, with the final report noting, on page 4, that, "no definitive determination could be made regarding how *E. coli* O157:H7 pathogens contaminated spinach in this outbreak" (California Food Emergency Response Team 2007). The report also noted: "Potential environmental risk factors for *E. coli* O157:H7 contamination identified during this investigation included the presence of feral pigs [*Sus scrofa*] in and around spinach fields and proximity of irrigation wells used for ready-to-eat produce to surface waterways exposed to feces from cattle and wildlife. (California Food Emergency Response Team 2007).

The incident led to increased efforts to promote food safety across the entire farm-to-fork supply chain for leafy greens and other produce. New measures included improvements to processing, shipping, handling, and worker hygiene. In 2007, California and Arizona adopted the Leafy Green Products Handler Marketing Agreement (LGMA), a voluntary compliance program for leafy green food safety practices (Leafy Green Products Handler Marketing Agreement 2012). Although the LGMA food safety practices do not specify the exclusion of wildlife and removal of its habitat in and around fields, reports of buyers requiring practices beyond the LGMA guidelines have sparked serious concerns by growers, as well as government agencies and environmental nonprofit organizations. These concerns were first described by Beretti and Stuart (2008) and have been called a "scorched earth" war on the environment by farmers and the news media (e.g., Lochhead 2009). In 2 large-scale California studies, growers reported yielding to tremendous pressure from auditors, inspectors, and other food safety professionals to change on-farm management practices in ways that not only generate uncertain food safety benefits, but also create serious environmental consequences (Lowell et al. 2010, Stuart 2010). Environmental concerns include reduction

of water quality, removal of wetland, riparian and other habitat, and elimination of wildlife on and near farmland. Speaking on a national radio program in April 2012, a California farmer captured the sentiment of many, stating that it is against nature to have a scorched earth policy (Charles 2012). The concerns expressed underscore the urgent need for collaboration between professionals working in food safety and wildlife management.

While proprietary requirements by individual corporate buyers have received the most attention, government policy also affects on-farm decisions. Current federal standards for food safety certification give incentives to farmers nationwide to remove wildlife. Specifically, farmers must receive a score of >80% to pass the U.S. Department of Agriculture (USDA) audit, and they lose points if they do not demonstrate measures to deter wildlife entering into crop production areas (U.S. Department of Agriculture 2012). During a nationwide series of stakeholder forums attended by one of the authors, farmers from the Midwest, Mid-Atlantic, Northeast, Southeast, and West Coast voiced concerns about implications for wildlife of national food safety regulations being developed by the U.S. Food and Drug Administration. Farmers expressed concerns that aggressive food safety practices to control wildlife are unrealistic, lack a scientific basis, and can contradict laws designed to protect wildlife (Produce Safety Project 2010).

Is wildlife responsible for pathogenic contamination of fresh produce? This article examines the current state of scientific understanding regarding that question. Wildlife has received considerable attention in efforts to identify sources of *E. coli* O157:H7 and other pathogens in fresh produce. Like other situations involving humans, wildlife, and disease, the situation entails ample complexity and presents communication challenges (Decker et al. 2012). In addition, data relevant to food safety risk from wildlife remain scarce and incomplete (e.g., Ilic et al. 2012). Using a systematic methodology for identifying and evaluating research studies, we reviewed studies that examined the role wild animals may play in contamination processes. These studies represented a range of methods, sample sizes, locations, and species. The discussion

focuses on 4 questions: (1) what is known about *E. coli* O157:H7 presence in wildlife? (2) to what extent have wildlife been linked to foodborne illness outbreaks? (3) how does livestock link to wildlife and pathogenic contamination of fresh produce? and (4) what are the priority research gaps? With new federal produce safety regulations under development, answers to these questions should be of interest to a wide variety of agency personnel, researchers, farmers, and others working in the areas of food safety or resource conservation.

Methods and methodological challenges

Researchers face several challenges when evaluating the relevance and quality of wildlife–pathogen studies. First, many studies, especially of birds, require careful interpretation because samples are taken from fecal deposits of unknown age, origin, and exposure to contamination after deposition (Craven et al. 2000). Second, some mammal samples are collected without full knowledge of the age, likelihood of contamination (or concentration and survival) post-deposition (Hancock et al. 1998). Third, contamination of fecal material by dust is possible (Varma et al. 2003, Miller et al. 2008). *E. coli* O157:H7 in some fecal material may actually increase after deposition (Feare et al. 1999), and environmental conditions influence the survival of *E. coli* O157:H7 in fecal material (Wang et al. 1996). Fourth, a single study, such as Gray et al. (2007), may be widely cited as justification for a food safety guideline leading practitioners and policy makers to extrapolate research findings beyond the context of the research and conclusions. Finally, studies conducted in laboratory settings must be interpreted with great caution. For example, Kudva et al. (1998) noted that *E. coli* O157:H7 may survive longer in laboratory studies meant to mimic field conditions than it does in actual field conditions.

Perhaps the most important pitfalls of such studies concern the selective identification and interpretation of them. With new federal regulations under development and high economic and environmental stakes nationwide, a risk of biased-advocacy science exists that favors a particular viewpoint or constituency. To minimize this risk, we followed a systematic,

replicable process for identifying and screening wildlife studies. First, we developed a list of wildlife that growers most commonly reported being told by auditors and inspectors were a food safety threat. These included: feral pigs (*Sus scrofa*), deer, birds, rodents, reptiles, and amphibians. Next, we searched the Web of Science/All Databases using key words that combined the term *Escherichia coli* O157 with each of the 6 wildlife terms listed above. We included flies in this search because of growing scientific interest in the ability of filth flies and houseflies (*Musca domestica*) to vector *E. coli* O157. This search identified 550 studies. After duplicate references and studies of domesticated pigs and poultry were deleted, 183 references remained. Third, we ruled out studies based on the following 6 filters: (1) the study focused on wild animals being kept in nonnative settings, such as zoos, pets, or semi-domesticated herds; (2) the study reported results for pooled samples for which the exact species included was not known; (3) the study failed to eliminate risk of cross-contamination of samples (e.g., samples collected from traps, feeding stations, or sites not cleaned between sample collections); (4) the study tested animals in a much different condition than those typically found on a United States produce farm (e.g., stray dogs with diarrhea in Trinidad, pigeons [*Columba livia*] in city parks of Madrid, urban rats [*Ratus ratus*] in Tobago); (5) the study focused on pathogens in meat; and (6) the study reported virulence factors detected in samples tested for *E. coli* O157 without culture confirmation.

We included studies with samples collected from outside the United States because an animal's physical ability to shed *E. coli* O157 and other pathogens in feces is independent of geography. We also included any articles that met the above criteria but did not appear in the results of database searches and were located by other methods. Examples include articles mentioned to us by researchers or found as citations in other works. Although we are aware of several studies in progress—many of them funded by the California-based Center for Produce Safety and involving scientists cited in this paper—we included only those studies that have appeared in the scholarly literature. Finally, we emphasize *E. coli* O157 in the review

but also mention studies with relevant data on wildlife infection with other dangerous *E. coli*, such as non-O157 Shiga toxin-producing *E. coli* (STEC) and enterohemorrhagic *E. coli* (EHEC). Of note, *E. coli* O157:H7 is the most well-characterized pathogen serotype belonging to the STEC serogroup and EHEC pathotype, but other serotypes, such as O26, O45, O103, O111, O121, and O145, are considered emerging foodborne public health threats (Hughes et al. 2009).

Additional challenges

Prevalence studies of pathogens in cattle or other domestic animals typically represent the infection rate in the population as a whole. Population size is known, as is the percentage of the population sampled. However, in most studies investigating pathogen prevalence in wildlife, population size and percentage of the population sampled are not known. For example, if 100 fecal samples are collected after deposition, in many instances it is not known whether this represents fecal samples of 100 animals or fecal samples of 25 animals defecating 4 times each. It is also unclear what percentage of the larger population 100 represents. Also, methods of collection, knowledge of sample age and condition, duration of pathogen shedding from infected animals, magnitude of infection (number colony-forming unit/g fecal matter), and possibility of contamination after deposition, are usually unknown. Because of these uncertainties, studies of scat prevalence rather than population prevalence play an important role in guiding follow-up research but contribute little to characterizing actual risk.

The current state of knowledge regarding the nexus of wildlife, pathogens, and fresh produce is limited. Pathogen prevalence and movement may depend upon the environment in which animals live and coexist. For this reason, full assessment of the risk wildlife and their wastes may pose to food safety must include studies in the relevant growing environment. Relevant research to date addresses primarily whether various species of animals are capable of carrying human pathogens. Contamination processes might include direct transfer of pathogens to fresh produce through fecal deposition directly onto plants. Other contamination processes

Figure 1. Deer and other wildlife enter fresh produce fields and can carry dangerous pathogens.

might involve contamination of the growing environment (e.g., water, soil, dust, bioaerosols), elements of which may subsequently come in contact with crops. The relative importance of these mechanisms in recent contamination processes remains unclear. Without information that describes the contamination process, it is extremely difficult to assess the risk posed by the presence of pathogens in domesticated and wild animals, particularly in wildlife populations where sample collection is more challenging. In the larger context, Ilic et al. (2012) conducted a comprehensive review of 657 studies relevant to microbial contamination of leafy greens, confirming the poor overall state of knowledge about how contamination occurs (including the potential role of wildlife) and determining that nearly 80% of existing data were unsuitable for policy and decision-making due to major deficiencies in study design, execution, and reporting.

Growers and food safety auditors regularly find evidence of animal incursion in row crops, such as crop damage, tracks, or fecal material. However, controlled studies examining the frequency of incursion into row crops by these animals and detailed information about how their behavior in the crop might impact contamination processes (e.g., contact of animal or animal feces with the harvested portion of a crop) is not available from the existing literature. Without a better understanding of the contamination process, it is not possible to define risk from these incursions.

Although practice varies widely across food safety programs, the LGMA currently specifies a 1.5-m no-harvest buffer zone if fecal material from "animals of significant risk" is found in the crop production area, and a 0.9 m buffer for areas with evidence of intrusion

but no fecal material (Leafy Green Products Handler Marketing Agreement 2012). The LGMA defines animals of significant risk as those that have been determined by the U.S. Centers for Disease Control to have a higher risk of carrying *E. coli* O157:H7. These include 4 domesticated animals (cattle, sheep, goats, and pigs) and 2 wild animals: deer (*Odocoileus* spp.) and feral pig (Leafy Green Products Handler Marketing Agreement 2012). The 2012 LGMA also recommends co-management for food safety and ecological health using the Lowell et al. (2010) definition of the term (i.e., "an approach to conserving soil, water, air, wildlife, and other natural resources while simultaneously minimizing microbiological hazards associated with food production"). Although the produce industry has made considerable progress in addressing possible conflicts between food safety and conservation practices, approaches still vary widely depending on the commodity, geographic location, and buyer.

Wildlife and *E. coli* O157

Table 1 lists studies by taxonomic group meeting our criteria for identifying and screening wildlife studies. Overall, *E. coli* O157 was detected rarely in the populations studied. Among wildlife testing positive, two (deer, feral pigs) are considered animals of significant risk as defined by the LGMA (2012). Feral pigs received significant attention in the wake of the 2006 national spinach *E. coli* O157 outbreak (California Food Emergency Response Team 2007, Jay et al. 2007), which is discussed in more detail in the next section. Likewise, deer have received much discussion as hosts of pathogens, especially in cases where deer share a common range land with cattle (Figure 1; see the Livestock and wildlife interactions section below). As shown in Table 1, surveys of large mammals in the United States revealed *E. coli* O157 in black-tailed deer (*Odocoileus hemionus columbianus*), white-tailed deer (*Odocoileus virginianus*), and feral pig fecal samples, but the pathogen was not detected in antelope (*Antilocapra americana*), bison (*Bison bison*), bighorn sheep (*Ovis canadensis*), or elk (*Cervus canadensis*) samples. Similar findings occurred in European studies of various deer and wild boar (*Sus scrofa*) populations.

Table 1. *Escherichia coli* O157 surveys in wildlife.

Taxonomic group	Species [a]	Location	Percentage positive [b]	Reference
Avian				
	Canada goose (*Branta canadensis*)	Massachusetts, Virginia, and New Jersey, USA	0/360	Converse et al. 1999
		Colorado, USA	0/397	Kullas et al. 2002
		Sweden	0/105	Wahlstrom et al. 2003
		Washington, USA	0/121	Rice et al. 2003
	Duck, unspecified	Washington, USA	1/20 (5.0%)	Samadpour et al. 2002
		Washington, USA	0/20	Rice et al. 2003
	European starling (*Sturnus vulgaris*)	Washington, USA	0/124	Rice et al. 2003
		Denmark	1/244 (0.4%)	Nielsen et al. 2004
		Kansas, USA	0/434	Gaukler et al. 2009
		Ohio, USA	5/430 (1.2%)	Williams et al. 2011
	Gull (*Larus* spp.)	Sweden	0/50	Palmgreen et al. 1997
		Washington, USA	0/150	Rice et al. 2003
		Sweden	0/111	Wahlstrom et al. 2003
	Gull, other shorebird	England	3/400 (0.8%)	Wallace et al. 1997
	Passerine, unspecified	Sweden	0/101	Palmgreen et al. 1997
	Pigeon (*Columba livia*)	Czech Republic	0/50	Cizek et al. 1999
	Pigeon (*Columba livia*), other wild birds	Wisconsin, USA	1/99 (1%)	Shere et al. 1998
	Sparrow (*Passer* spp.)	Czech Republic	0/20	Cizek et al. 1999
	Swan , trumpeter (*Cygnus buccinator*)	Washington, USA	0/67	Rice et al. 2003
	Swan , tundra (*Cygnus columbianus*)	Alaska	0/100	Milani et al. 2012
	Wild turkey (*Meleagris gallopavo*)	Washington, USA	0/83	Rice et al. 2003
	Wild bird, unspecified	Washington, USA	1/200 (0.5%) [c]	Hancock *et al.* 1998
		Ireland	0/20 [c]	Bolton *et al.* 2011
Large mammals				
	Antelope (*Antilocapra americana*)	Washington, USA	0/1	Rice et al. 2003
	Bison (*Bison bison*)	Washington, USA	0/57	Rice et al. 2003
	Bighorn sheep (*Ovis canadensis*)	Washington, USA	0/32	Rice et al. 2003
	Deer, black-tailed (*Odocoileus hemionus columbianus*)	Oregon, USA	3/32 (9.4%)	Keene et al. 1997
		California, USA	1/9 (11.1%)	Cody et al. 1999
	Deer, fallow (*Dama dama*)	Spain	0/6	Garcia-Sanchez et al. 2007
	Deer , fallow and red (*Dama dama* and *Cervus elaphus*)	Sweden	0/90	Wahlstrom et al. 2003
	Deer, Iberian red deer (*Cervus elaphus*)	Spain	3/264 (1.1%)	Diaz et al. 2011
	Deer, red (*Cervus elaphus*)	Spain	3/206 (1.5%)	Garcia-Sanchez et al. 2007
	Deer, roe (*Capreolus capreolus*)	Sweden	0/195	Wahlstrom et al. 2003
	Deer, red and roe (*Cervus elaphus* and *Capreolus capreolus*)	Belgium	0/133	Bardiau et al. 2010
		Spain	0/20	Garcia-Sanchez et al. 2007
		Spain	1/179 (0.6%)	Mora et al. 2012
	Deer, white-tailed (*Odocoileus virginianus*)	Kansas, USA	5/122 (2.4%)	Sargeant et al. 1999
		Georgia, USA	3/919 (0.3%)	Fischer et al. 2001
		Nebraska, USA	4/1608 (0.2%)	Renter et al. 2001
		Kansas, Nebraska, USA	0/141	Renter et al. 2003
		Washington, USA	5/630 (0.8%)	Rice et al. 2003
		Louisiana, USA	1/338 (0.3%)	Dunn et al. 2004
		Texas, USA	0/26	Branham et al. 2005

Table 1, continued.

Taxonomic group	Species [a]	Location	Percentage positive [b]	Reference
	Deer, unspecified	Ireland	1/4 (25.0%) [c]	Bolton et al. 2011
	Deer and elk, unspecified	Wyoming, USA	0/5	Olsen et al. 2002
	Elk (*Cervus canadensis*)	Washington, USA	0/244	Rice et al. 2003
	Moose (*Alces alces*)	Sweden	0/84	Wahlstrom et al. 2003
	Mouflon (*Ovis musimon*)	Spain	0/11	Garcia-Sanchez et al. 2007
	Feral pig (*Sus scrofa*)	Sweden	1/68 (1.5%)	Wahlstrom et al. 2003
		California, USA	13/87 (14.9%)	Jay et al. 2007
		Spain	7/212 (3.3%)	Sanchez et al. 2010
		Spain	1/262 (0.4%)	Mora et al. 2012
Small mammals				
	Bat (Chiroptera; 12 species)	Trinidad	0/377	Adesiyun et al. 2009
	Coyote (*Canis latrans*)	Kansas and Nebraska, USA	0/100	Renter et al. 2003
		Washington, USA	0/7	Rice et al. 2003
	Fox (*Vulpes vulpes*)	Ireland	0/124	Nagano et al. 2007
		Spain	0/260	Mora et al. 2012
	Hare (*Lepus timidus* or *Lepus europeaus*)	Sweden	0/125	Wallace et al. 1997
	Norwegian rat (*Rattus novegicus*)	Czech Republic	4/10 (40.0%)	Cizek et al. 1999
	Norwegian rat (*Rattus novegicus*), other wild rodents	Denmark	1/10 (10.0%)	Nielsen et al. 2004
	Opossum (*Didelphis virginianus*)	Kansas and Nebraska, USA	1/25 (4.0%)	Renter et al. 2003
	Rabbit (*Oryctolagus cuniculus*)	England	20/41 (48.8%) [c]	Bailey et al. 2002
		England	8/97 (8.2%)	Scaife et al. 2006
	Raccoon (*Procyon lotor*)	Wisconsin	1 [d]	Shere et al. 1998
		Kansas and Nebraska, USA	0/230	Renter et al. 2003
	Rodent, unspecified	Ireland	0/2 [c]	Bolton et al. 2011
		Washington, USA	0/300	Hancock et al.1998
Invertebrates				
	Fly, unspecified	Wisconsin, USA	1 [d]	Shere et al. 1998
		Washington, USA	2/60 (3.3%) [c]	Hancock et al.1998
		Denmark	0/6 [c]	Nielsen et al. 2004
	House fly (*Musca domestica*)	Kansas	53/1540 (3.4%) [c]	Sanderson et al. 2006
	Slug (*Deroceras reticulatum*)	Scotland	1/33 (3.0%) [c]	Sproston et al. 2006

[a]Species listed alphabetically by common name, then by year of study, oldest to newest.
[b]"Percentage positive" represents the number of positive samples in which researchers cultured *E. coli* O157:H7 divided by the number of samples tested. All sample types are feces, anal and cloacal swabs, or gastrointestinal contents from individual animals unless otherwise noted.
[c]Represents pooled (composite) samples.
[d]Total number of samples not reported.

Studies documenting risk from squirrels, mice, voles, rats, lagamorphs (rabbits [*Oryctolagus cuniculus*)] and hares [*Lepus* spp.), and other small mammals are limited (Table 1). While experimental evidence indicates rodents are capable of carrying various strains of *E. coli*, *Salmonella*, and other infectious bacteria (e.g., Clark 1994, Henzler and Opitz 1992), only a few investigators have sampled these species in their natural habitats. In the United States, *E. coli* O157:H7 was isolated from a single opossum (*Didelphis virginianus*) and a raccoon (*Procyon lotor*) in 2 separate studies in the Midwest (Shere et al. 1998, Renter et al. 2003). Studies in Europe identified *E. coli* O157 in wild rats and rabbits (Cizek et al. 1999, Scaife et al. 2006).

Compared to information regarding mammals as hosts of foodborne pathogens,

a larger body of literature exists for avian species. Most of these avian studies focus on *Salmonella* and *Campylobacter*. In the United States, several large studies of *E. coli* O157:H7 prevalence in Canada geese (*Branta canadensis*) populations failed to identify the pathogen (Table 1). Following a water-borne outbreak of *E. coli* O157:H7 associated with lake water in Washington, Samadpour et al. (2002) found the outbreak strain in a duck (*Anas* sp.) sample. *E. coli* O157 was also isolated from European starlings (*Sturnus vulgaris*) and a pigeon during 2 separate epidemiological studies of dairy farms in Ohio and Wisconsin (Shere et al. 1998, Williams et al. 2011).

Captive amphibians and reptiles are well-documented sources of human salmonellosis infections, but the relative significance of these cold-blooded species in their natural habitat, including fresh produce production areas, is unclear. No studies have isolated *E. coli* O157:H7 from wild amphibians or reptiles. Dipineto et al. (2010) described the first isolation of *E. coli* O157 from captive pet frogs (*Anura*). Gray et al. (2007) were able to infect metamorphs (young frogs recently developed from tadpoles) with *E. coli* O157, but the laboratory conditions under which the study occurred limit extrapolation to field settings. Episodic reports of amphibians (typically frogs) found in fresh produce occur in the news media (e.g., Miles 2011) but have primarily represented a food quality issue for the industry.

While not generally classified as wildlife, flies (*Diptera*) and other invertebrates may be important vectors for some pathogens. Filth flies (flies that breed in feces and other organic refuse) have been found to carry *E. coli* O157:H7 and *Campylobacter* at a turkey (*Meliagris gallopavo*)-raising farm (Szalanski et al. 2004) and *E. coli* O157:H7 on cattle farms in Japan (e.g., Iwasa et al. 1999). *E. coli* O157 has been isolated from fly pools collected at feedlots and dairies in the United States (Shere et al. 1998, Rice et al. 2003, Sanderson et al. 2006) and pooled slugs (*Arion aeter*) in Scotland (Sproston et al. 2006). Talley et al. (2009) demonstrated in the laboratory that house flies confined on manure or agar containing *E. coli* O157:H7 were able to transfer the bacteria to spinach plants. Janisiewicz et al. (1999) showed that fruit flies (*Drosophila melanogaster*) can transfer *E. coli*

F-11775, a non-pathogenic strain of *E. coli* found to grow similarly to *E. coli* O157:H7, to wounded apple tissue under laboratory conditions. For a comprehensive review of insects as vectors of foodborne pathogenic bacteria, see Blazar et al. (2011).

Wildlife and foodborne illness outbreaks

On 6 occasions, investigators of foodborne illness outbreaks found reason to suspect a potential wildlife role in contamination of fresh produce (Table 2). Studies indicate that wildlife may play a role in pathogenic contamination of fresh produce, but that any role is likely interwoven with water, livestock, and human factors, such as management practices.

In the Alaska case (Table 2), Gardner et al. (2011) determined that the outbreak "was associated with consumption of commercially grown peas contaminated with crane feces." Investigators observed numerous Sandhill cranes (*Grus canadensis*) foraging in the pea fields, and *Campylobacter jejuni* isolates cultured from crane fecal material collected in the field were genetically the same as strains cultured from human stools and raw peas. This outbreak underscores the importance of wildlife population density in assessing potential risk from wildlife. It also highlights the importance of safe handling practices, in particular, processing deficiencies that public health investigators note may have contributed to the outbreak (Gardner et al. 2011).

In the 2006 *E. coli* O157:H7 spinach outbreak, intensive sampling focused on the area from which the product was harvested (California Food Emergency Response Team 2007, Jay et al. 2007). The investigation focused on detection of *E. coli* O157:H7 in a wide range of sources and attempted to capture important information about how contamination processes involving wildlife and cattle may occur. The study tested water, soil, and wild and domestic animal fecal samples for *E. coli* O157:H7. The outbreak strain of *E. coli* O157:H7 was found in 34% of cattle feces; 0% samples from water troughs; 15% in feral pig feces; 4% in surface water samples; 8% in soil and sediment samples; and 0% in well water samples (Jay et al. 2007). Sightings and tracks documented by investigators indicated that feral pigs on the ranch moved freely

between the cattle pastures and the crop fields and that cattle had direct access to the major surface water source on the ranch.

Cooley et al. (2007) used samples collected in watersheds to trace potential fate and transport of *E. coli* O157:H7 with a source tracking method and compared these isolates with the feral pig strains collected during the 2006 spinach contamination outbreak. Combined with Jay et al. (2007), the 2 studies provide the most comprehensive and relevant data available to address the role of feral pigs in *E. coli* O157:H7 contamination of fresh produce. The influence of high population density of feral pigs and proximity to cattle on prevalence of *E. coli* O157:H7 were identified as important areas for further research. Jay and Wiscomb (2008) subsequently published a review of food safety risks and mitigation strategies for feral swine near agriculture fields that highlights best management practices known at that time.

Black-tailed deer were investigated as potential sources of *E. coli* O157 following 2 produce-related outbreaks in the United States. A small sampling of deer droppings in an apple orchard revealed the presence of *E. coli* O157:H7, but the strain was not genetically related to the human outbreak strain (Cody et al. 1999). In 2011, *E. coli* O157 genetically identical to the human outbreak strain was isolated from deer droppings collected in an implicated strawberry field (W. Keene, Oregon Public Health Division, personal communication).

Livestock and wildlife interactions

A promising area of research relates to understanding how foodborne pathogens may transfer from domestic animals to wildlife (or the reverse). Cattle are widely recognized as the principal reservoir of *E. coli* O157:H7 (Renter et al. 2003; Figure 2). The pathogen has, however, been isolated from other domestic animals and wildlife (Table 1). *E. coli* O157 movement between domestic and wild animals may depend on ecological factors, concentration and persistence in the shared environment, and other variables related to local conditions.

Several researchers have studied *E. coli* O157 occurrence in deer populations sharing range with cattle or other domestic ruminants, and their findings were mixed. Branham et al. (2005) found no *E. coli* O157 in white-

Figure 2. Cattle are the primary reservoir of *E.coli* O157:H7 in the landscape. Approximately 30% of U.S. feedlot cattle shed this pathogen.

tailed deer from Texas grazing in the same rangeland as cattle and sheep that had low (~1%) prevalence of the pathogen. Fischer et al. (2001) compared the genetic relatedness of *E. coli* O157:H7 isolates from cattle and deer using pulsed-field gel electrophoresis and found both different patterns and Shiga toxin genes. In another study, *E. coli* O157:H7 was identified in five of 22 fecal samples of white-tailed deer sharing pasture with cattle in Kansas, but the cattle were not tested (Sargeant et al. 1999). In Ireland, Bolton et al. (2011) isolated *E. coli* O157 from one of 4 deer fecal samples, but did not detect it in domestic ruminants sampled on the same farm. Data from Sanchez et al. (2010) suggests horizontal transmission of genetically similar *E.coli* O157:H7 isolates among sheep, cattle, and deer in the Extremadura region of southwestern Spain. The authors also note that these species can serve as natural sources of phenotypic variants of *E.coli* O157:H7, such as a sorbitol fermenting, beta-glucuronidase positive strain isolated from deer.

Many of the studies examining avian interactions with livestock and foodborne pathogen occurrence were conducted in settings where birds fed in cattle yards or areas of concentrated human waste (e.g., landfills), which the authors noted may increase the incidence of contamination (e.g., Nielsen et al. 2004, Pedersen and Clark 2007, Carlson et al. 2011). For example, in a survey of 150 Ohio dairy farms, Cernicchiaro et al. (2012) did not test for pathogens in birds, but found that the presence of European starlings was one of multiple factors positively associated with *E. coli* O157 in dairy cattle fecal pats. In another Ohio study,

Table 2. Wildlife surveys associated with produce-related foodborne disease outbreaks.

Year, location, reference	Pathogen	Produce vehicle	Wildlife species	Comments[a]
1995, Florida, USA, Parish 1998	*Salmonella* serovars Hartford, Gaminara, and Rubislaw	Orange juice from citrus-processing facility	Frog (*Hyla cinerea*) and toad (*Bufo terrestris*)	1 of 1 toad tested positive for *S.* Hartford, but isolate did not match the outbreak strain; *S.* Newport cultured from 1 of 1 toad and unspecified % of 4 tree frogs near the facility.
1996, Multi-state USA, Cody et al. 1999	*E. coli* O157:H7	Unpasteurized apple juice	Black-tailed deer (*Odocoileus hemionus*)	*E. coli* O157:H7 found in 1/9 (11%) deer droppings near an implicated orchard in California; isolate did not match the outbreak strain.
2004, Finland, Kangas et al. 2008	*Yersinia pseudotuberculosis* O:1	Raw carrots	Common shrew (*Sorex araneus*)	Outbreak strain found in a pooled sample of common shrew intestines from one implicated farm.
2006, Multi-state USA, Jay et al. 2007	*E. coli* O157:H7	Raw bagged, baby spinach	Feral pig (*Sus scrofa*)	*E. coli* O157:H7 11/47 (23%) feral swine fecal samples and 2/40 (5%) necropsy (colon) samples, and 26/77 (34%) of range cattle fecal samples collected at implicated ranch; isolates from 15 cow and 8 feral pig samples contained the outbreak strain.
2008, Alaska, USA, Gardner et al. 2011	*Campylobacter jejuni*	Raw shelled peas	Sandhill crane (*Grus Canadensis*)	*C. jeuni* found in 14/14 (100%) Sandhill crane fecal samples collected at implicated pea farm; isolates from 2 crane and 2 pea/soil samples contained the outbreak strain.[b]
2011, Oregon, USA, W. Keene, Oregon Dept. of Health (personal communication)	*E. coli* O157:H7	Raw strawberries	Black-tailed deer (*Odocoileus hemionus*)	*E. coli* O157:H7 found in 6/34 (18%) deer pellet and 4/24 (17%) mixed deer pellet/soil collected in the implicated fields; 4 deer pellet and 2 deer pellet/soil samples contained the outbreak strain.

[a] Outbreak strain" of *Escherichia coli* 0157:H7 and other foodborne pathogens is defined as the unique clone isolated from human stool samples in an outbreak investigation. "Matching" isolates belong to the same serotype (e.g., *E. coli* O157:H7) and have DNA macrorestriction patterns indistinguishable from each other by using pulsed-field gel electrophoresis analysis.
[b] Multi-strain outbreak.

Kauffman and LeJeune (2011) demonstrated that cattle shedding *E. coli* O157:H7 could transmit the pathogen to previously culture-negative starlings and vice versa. Williams et al. (2011) demonstrated starlings' role in transmitting *E.coli* O157:H7 among Ohio dairy farms. Birds' capacity to serve as reservoirs of the bacteria, coupled with their ability to transport bacteria

long distances, poses complex research and management challenges.

Rodents and rabbits in association with cattle have been examined in a few studies. Hancock et al. (1998) tested a large number of rodents (300) from positive feedlot and dairy herds, but did not recover *E. coli* O157. In contrast, 2 European studies found *E. coli* O157 shedding among rats in close proximity to cattle (Cizek et al. 1999, Nielsen et al. 2004). Likewise, during an outbreak investigation in England, wild rabbits were investigated as a potential transport vector of *E. coli* O157 from a cattle pasture to a picnic area (Bailey et al. 2002, Scaife et al. 2006).

Invertebrates, particularly flies, are also considered potential vectors of *E. coli* O157 and other foodborne pathogens that originate from livestock areas (Table 1). The LGMA food safety standards call for an interim guidance distance of 122 m from the edge of a concentrated animal feeding operation and the crop, but research is needed to validate their home range and if this distance would reduce exposure to potentially contaminated flies (LGMA 2012).

Although management of *E. coli* O157 risk lies outside the scope of this review, it is worth noting that pre-harvest strategies to reduce foodborne pathogens in domestic ruminants is an active area of research worldwide. Cattle in both confinement (grain-fed) and pasture (forage-fed) operations often test positive for pathogens, but the most comprehensive review of the literature indicates that *E. coli* populations (including O157:H7) are higher in the feces of cattle that are fed grain diets (Callaway et al. 2009). An average of 30% of U.S. feedlot cattle shed *E.coli* O157:H7 (Callaway et al. 2009). It is unclear if this relationship holds true for other livestock, although preliminary evidence is emerging. For example, Kilonzo et al. (2011) studied 3 northern California sheep farms and isolated *E. coli* O157:H7 in 23% of fecal samples from sheep being raised in a commercial feedlot compared a to 2% rate on a ranch where the sheep grazed native pasture year-round, and 0% on a farm where sheep grazed in open pasture for the summer then ate alfalfa in a dry-lot during the winter rainy season.

A growing number of watershed-scale studies have linked cattle to the presence of pathogens, such as *E. coli* O157:H7, in water, wildlife, and humans. For example, based on surface water sampling at 27 locations across Canada, Edge et al. (2012) found a higher mean concentration of *E. coli* O157:H7 at intensive agricultural sites compared to reference sites. Based on surface water samples from 24 locations in Ontario, Canada, Wilkes et al. (2011) found that *E. coli* O157:H7 detections were related to upstream livestock pasture density. Jokinen et al. (2012) tested surface water in an Alberta, Canada, watershed that was noted for its prominent animal agriculture. The authors found that animal manure unit (AMU) was associated with presence of *Salmonella* spp., *Campylobacter* spp., and *E. coli* O157:H7, and that downstream sites had more of these pathogens than upstream sites, suggesting additive stream inputs. A similar study of surface water in British Columbia, Canada, also demonstrated a link between these 3 pathogens and domesticated animals, such as cattle, horses, and poultry (Jokinen et al. 2010.) Guenther et al. (2010) documented that *E. coli* with multiple antimicrobial resistances were significantly more often detected in wild rodents originating from areas with high livestock density. Friesema et al. (2011) mapped symptomatic cases of *E. coli* O157 in the Netherlands between April 1999 and December 2008 (*n* = 409). The authors found that cattle density >64 cows/km^2 more than doubled the risk of reporting *E. coli* O157 in summer compared to areas with <26 cows/km^2. Beginning in 1999, GIS-based spatial analysis has consistently shown an association between cattle density in a given area and the number of reported *E. coli* O157 infections in humans (Michel et al. 1999, Valcour et al. 2002, Kistemann et al. 2004, Frank et al. 2008). Other authors have found that the concentration of zoonotic pathogens in a given watershed has been found to increase with proximity to and number of animal operations (e.g., Cox et al. 2005, Graham and Nachman 2010).

With cattle's link to microbial pathogens in wildlife and humans well established, future research should focus on understanding and disrupting this link. The next section describes research priorities for this and related topics.

Key research gaps

Whereas previous sections have detailed what is known about wildlife and pathogenic contamination of fresh produce, this section

highlights pressing information gaps with an emphasis on priority research designs and questions. Ample research to date has examined what can potentially happen with regard to pathogens in wildlife. Focus has largely centered on the fact that some species of wildlife can carry pathogens and that they enter crop fields. A fuller assessment of risks posed by these animals, however, would require answering the questions: what does happen, how much risk does it pose, and what can be done to minimize risk? In other words, what are the best practices to promote both public health and ecological health? Answering these questions requires transitioning beyond descriptive studies that dominate the literature (e.g., studies of prevalence rates) and into explanatory studies that test specific hypotheses about causal pathways, with an emphasis on randomized, controlled trials of sample populations that represent target populations.

With respect to priority research questions, researchers should continue the landscape-level studies that help to understand, predict, and minimize risk. Priority topics include wildlife movement patterns and their intersection both with human and livestock sources of pathogens and with crop areas. For example, what are the demonstrable connections among pathogen presence in wildlife and: (1) cattle or other livestock; (2) human sources of pathogens, such as leaching, runoff, and municipal landfills; and (3) crop areas?

Regarding wildlife and crop areas, a vast body of literature on wildlife damage exists, including damages to agricultural productivity (see Conover 2002 for a detailed overview). It is important to note that potential food safety risk from wildlife stems from fecal deposition rather than crop consumption. For example, birds defecating while roosting in trees or utility lines above crops may present risk, as may animals that cross a field but cause no trampling or feeding damage. Overall, wildlife movement patterns into fresh produce fields remain largely unexamined in the scholarly literature. Key research questions include: to what extent does wildlife enter fresh produce crop areas?; which wildlife?; which types of fresh produce are affected?; how do patterns vary by season, crop, location, species, deterrents, and other factors? Answers to these questions can supplement

pathogen prevalence data, offering important pieces of the larger risk puzzle.

Another clear priority is to research transfer pathways between cattle and wildlife, such as shared range (including pastures and feedlots), waterways, and migratory routes. For example, it would be intriguing to conduct molecular epidemiological studies comparing foodborne pathogens isolated from migratory bird populations to strains found in domestic livestock. Gardner et al. (2011) noted that the cranes associated with the Alaska pea outbreak (Table 2) belong to the migratory Pacific Flyway population, which spends most of the year in the Central Valley of California. According to government regulators, the Central Valley is home to 81% of the state's confined animal feeding operations, including 1,600 operations that average >800 cows each (e.g., Albright 2009). Molecular comparison of the genetic relatedness of strains from cranes and cattle in this geographic region could elucidate potential transfer mechanisms.

Similarly, during winter months most of the nation's leafy greens are grown in the Yuma, Arizona, desert region, an area that is also an important migration and wintering stop for waterfowl in the Pacific Flyway. This critical growing area also supports large livestock feeding operations in close proximity to irrigation canals and fresh produce fields. Foodborne pathogen movement between livestock, birds, and water in this important region has not yet been studied.

Researchers should determine if wildlife and waterways tend to have less *E. coli* O157:H7 in areas where cattle are absent, have low infection rates, or have been vaccinated. An *E. coli* O157:H7 cattle vaccine recently was licensed in the United States; but its efficacy remains uncertain (Snedeker et al. 2012), and its use has been limited primarily to feedlots and stockyards to protect the beef supply from contamination (Cull et al. 2012). The role of cattle vaccination to prevent environmental dissemination to watershed, bioaerosols, and wildlife has not yet been evaluated. The vaccine approach appears promising, but notably controls only 1 foodborne pathogen, and, thus, represents just 1 potential intervention in a comprehensive best practices program to control food safety risks associated with cattle operations.

In addition to well-designed experiments, post-outbreak and post-recall investigations play a key role in discovering the sources. Such investigations rarely extend to the immediate farm environment, let alone to the larger watershed that may be the source of the pathogens. Significant scientific advances will occur when such investigations are conducted with standardized approaches that include collection of appropriate sample types and numbers based on findings from the investigation in consultation with experts in disciplines potentially not directly related to food safety (e.g., wildlife biology, water quality, livestock management). An interdisciplinary approach is needed to track pathogens to their root source.

Another important research topic is the potential role of biodiversity in strengthening food safety. Certain food safety practices, such as removing wildlife habitat from around fields, could increase food safety risk rather than reduce it. Numerous studies have shown that non-crop vegetation in and around fields can significantly reduce pollution and the survival and movement of pathogens (see Lowell et al. 2010 for a review). Vanderzaag et al. (2010) found that acid resistant *E. coli* survived at least twice as long in soil samples collected from an agricultural field than in soil from an adjacent riparian area. Ragosta et al. (2011) found that each 1% decrease in riparian forest canopy cover was associated with a 3.6 most probable number/100-ml increase in *Enterococcus* in stream water. These findings highlight the role of biological diversity near fields. They also raise important questions about the science behind requiring removal of wildlife habitat from around fields.

Finally, a fertile research area lies with wildlife and other pathogens, including new ones. Concerns over *Salmonella*, non-O157 Shiga toxin-producing *E. coli*, and *Campylobacter* in fresh produce continue to rise. A growing body of literature has documented these pathogens in wildlife (e.g., Parsons et al. 2010, Gorski et al. 2011, Jay-Russell et al. 2012). A variety of new pathogen serotypes continues to emerge. For example, the 2011 foodborne illness outbreak in Germany that was linked to fenugreek sprouts and that killed 35 people was caused by *E. coli* O104:H4, which was little known to scientists prior to the outbreak (Muniesa et al. 2012).

Conclusion

Farmers nationwide are concerned that pressure to eliminate wildlife and habitat for food safety purposes may be unrealistic and scientifically unjustified. Sufficient information to define and effectively manage potential risk that wildlife may pose is currently lacking. The literature regarding pathogen presence in wildlife reflects a wide range of geographies, conditions, methods, and findings. Many studies occurred before development of improved detection methodologies. In particular, pathogen movement, persistence, and other attributes can depend on local conditions that vary by geographical region. Thus, caution is required when applying research findings beyond where the studies occurred.

It is beyond the scope of this review to consider all the factors likely to contribute to contamination processes. This review focused on wildlife and food safety risks because wildlife featured prominently in investigations of a high-profile outbreaks linked to fresh produce (i.e., apple juice, spinach, peas, strawberries), and because growers report taking actions to reduce wildlife for food safety reasons. Several other potential sources of farm-based contamination exist, including domesticated animals, water, soil amendments, and workers. Additionally, the role of modern post-harvest processes, such as mechanized and a centralized production system (e.g., bagged salads) needs to be addressed (Stuart 2011). Ongoing research is examining pathogen presence in bagged, ready-to-eat products (e.g., Kase et al. 2012).

Overall, the evidence herein builds upon the findings by Ferens and Hovde (2011) that wildlife does not constitute a significant source of EHEC O157, but that sporadic isolation of the bacteria likely reflects environment-mediated transmission from humans and animal reservoirs. The authors further conclude that wildlife and other vectors are unlikely to support the continuous existence of the pathogen in the absence of cattle or sheep. Results from a few outbreak investigations suggest that a "perfect storm" may result in contamination of fresh produce, with wildlife species and livestock potentially playing a role in environmental dissemination. We have identified the characterization and disruption of links between wildlife and livestock as a top

research priority, along with a general research shift toward landscape studies and risk assessments that test specific hypotheses about causal pathways using randomized, controlled trials of sample populations that represent target populations. Such research can play a key role in understanding and addressing wildlife's potential role in pathogenic contamination of fresh produce.

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