What is the risk from wild animals in food-borne pathogen contamination of plants?

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Abstract

Fresh fruits, nuts and vegetables are increasingly linked to food-borne illnesses, outbreaks and recalls. The trend represents a modern-day public health conundrum wherein consumers are encouraged to eat more fresh produce to help prevent chronic health problems such as obesity and heart disease, but at the same time consumption of contaminated produce can lead to potentially life-threatening acute food-borne disease. Identification of environmental sources responsible for the contamination of raw and minimally processed or fresh-cut plant commodities is necessary to develop prevention strategies. Produce-related outbreaks have been caused by faecal contamination of plants or surrounding watersheds following intrusion by wild or feral animals. A wild animal shedding a zoonotic food-borne pathogen could contaminate plants directly through faecal deposition or indirectly via faecal contamination of agriculture water or soil in contact with the plants. Owing to the low infectious dose of zoonotic enteric pathogens and the potential for attachment and possibly ingress into edible parts of plants, even a low level of contamination from faecal pathogens represents a significant public health concern. This review focuses on potential produce food safety risks from wild animals at the pre-harvest level, and downstream processes that may promote pathogen survival and amplification that could lead to human food-borne illnesses, outbreaks, and recalls. Microbe-plant interactions for the major zoonotic food-borne pathogens and higher risk plant commodities are reviewed. Finally, current guidelines and regulations to minimize risks related to wild animal activity in the production environment are summarized.

Keywords: Animals, Wild, Food-borne diseases, Plants, Edible, Risk, Zoonoses

Review Methodology: Databases used in this review included NCBI PubMed and the Center for Produce Safety Global Research Database. References from existing EndNote files and articles obtained from the database searches were also used to identify additional relevant material. Conference proceedings following two special sessions on wildlife and food safety held at the 23rd and 25th Vertebrate Pest Conferences were also reviewed. Produce and plant product safety regulations and guidance documents were found by searching agency/organization websites including the US Food and Drug Administration, Western Growers and the University of California Postharvest Technology online libraries.

Introduction

Fresh fruits, nuts and vegetables are increasingly linked to food-borne illnesses, outbreaks and recalls [1–4]. In the USA, the Centers for Disease Control and Prevention (CDC) estimated that plant commodities caused about 46% of domestically acquired food-borne illnesses from 1998 to 2008 [5]. The majority of these plant-based food-borne illnesses were associated with edible horticultural crops often consumed raw or minimally processed (e.g., fruits, nuts and vegetables) rather than agronomic crops (e.g., cereals, grains, legumes) typically cooked or processed with a pathogen ‘kill step’ such as heat or chemical treatment. The trend represents a modern-day public
health conundrum wherein consumers are encouraged to eat more fresh produce to help prevent chronic health problems such as obesity and heart disease, but at the same time consumption of contaminated produce can lead to potentially life-threatening acute food-borne disease.

Identification of environmental sources of food-borne pathogens and deciphering the key transport processes in the food supply chain are necessary steps to develop targeted intervention strategies. Owing to the complexity of fresh produce production (multiple commodities, different geographic regions, etc.) no single environmental source has been identified as the root cause of microbial contamination of fresh produce. At the farm level, possible environmental sources of enteric food-borne pathogens include runoff or bioaerosols from nearby domestic animal operations, human sewage/septic facilities, infected farmworkers, contaminated agriculture water, untreated manure-based soil amendments, flies or other invertebrates and wild animal intrusion/defecation in the production area [6].

The risk from wild animals in the microbial contamination of leafy greens became an intense area of focus following the highly publicized 2006 *Escherichia coli* O157:H7 outbreak associated with ready-to-eat packaged baby spinach that was traced to one field in the central California coast [7–9]. The outbreak strain was isolated from domestic cattle (*Bos taurus*) and feral swine (*Sus scrofa*) sharing rangeland adjacent to the implicated spinach field. Potential wild animal sources have also been investigated following other outbreaks linked to fresh produce from fields or orchards including dropped apples used in unpasteurized juice, raw shelled peas, fresh strawberries and raw carrots [10–13]. Faecal contamination of plants or surrounding watersheds following intrusion by wild or feral animals is now considered one of the significant risk factors for pre-harvest produce contamination [14–18].

The purpose of this paper is to review the current state of knowledge regarding the risk of zoonotic enteric food-borne pathogen contamination of fresh produce and other edible plant crops by wild animals, and highlight current guidelines and regulations to minimize these risks before and during production and harvest.

The Pathogens

There are over 250 pathogens and toxins that can be transmitted by food and 31 are classified as the major food-borne pathogens [19]. The goal of this section is to highlight the epidemiological features of the major zoonotic bacterial, parasitic and viral food-borne pathogens that have been found in wild animals. Examples of pathogens from wild animals with an emphasis of those found in produce production environments are shown in Table 1. Of note, comprehensive reviews of prevalence surveys of zoonotic enteric pathogens in animal hosts have been published previously and are beyond the scope of this review [20–22].

Bacteria

**Campylobacter spp.**

*Campylobacter* is a Gram-negative, curved rod-shaped bacterium that lives commensally in the gastrointestinal tract of birds and mammals. *Campylobacter jejuni* is the leading cause of bacterial gastroenteritis worldwide and the second leading cause after *Salmonella* in the USA [19]. Campylobacteriosis is usually self-limiting and deaths are rare; however, antecedent *C. jejuni* gastroenteritis is the leading cause of post-infectious Guillain–Barré syndrome, an autoimmune disease that may lead to permanent paralysis. Campylobacteriosis outbreaks are most often caused by consumption of contaminated raw or undercooked poultry, unpasteurized dairy products and unchlorinated water. Fresh produce-related campylobacteriosis outbreaks are uncommon, probably because of the fastidious growth conditions required by *Campylobacter* compared with other zoonotic enteric pathogens [23]. However, *Campylobacter* has been recovered from fresh vegetables at the retail level [24].

*Campylobacter* is ubiquitous in healthy domestic and wild animal populations, and has been detected in every major vertebrate taxa and flies [25–27]. Bird reservoirs that congregate in flocks are of the most concern for contamination of agricultural fields. For example, Canada geese (*Branta canadensis*) and other waterfowl are natural reservoirs of *Campylobacter* and may contribute to the contamination of crop fields and local watersheds, as well as urban and suburban areas (Figure 1) [28–32]. A notable campylobacteriosis outbreak involving raw shelled peas contaminated with Sandhill crane (*Grus canadensis*) faeces occurred in Alaska in 2008 [11]. The implicated pea farm was located in the Mat-Su Valley near a wildlife refuge where approximately 20 000 Sandhill cranes in the Pacific Flyway migrated. Cranes were observed grazing and defecating in the pea fields and *C. jejuni* strains genetically identical to strains from the patients were cultured from crane faeces and pea–soil mixtures. *Campylobacter* has also been recovered from large game mammals including cervids and wild boar [31, 33]. *Campylobacter* shedding was documented from both gastrointestinal tract and oral cavity samples collected from feral swine captured near spinach fields in California [34]. Small carnivores, wild rodents and rabbits are also potential reservoirs found in and around agricultural fields [26, 31, 35, 36].

*E. coli*

*E. coli* is a Gram-negative, rod-shaped bacterium found commonly in the human and animal gastrointestinal tract.
<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Incubation period</th>
<th>Illness/complications</th>
<th>Infectious dose</th>
<th>Host</th>
<th>Produce-related outbreaks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Bacterial</strong></td>
<td></td>
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</tr>
<tr>
<td>Campylobacter jejuni</td>
<td>2–5 days</td>
<td>Abdominal cramps, diarrhoea (sometimes bloody), vomiting, fever, chills, malaise, nausea, headache; post-infectious Guillain–Barré syndrome (paralysis).</td>
<td>500–10,000 cells</td>
<td>Healthy domestic poultry, ruminants, pigs, dogs; wild birds and mammals</td>
<td>Peas</td>
</tr>
<tr>
<td>E. coli O157 and other pathogenic shiga toxin producing E. coli (STEC)</td>
<td>2–7 days</td>
<td>Abdominal cramps, bloody diarrhoea, vomiting, fever, chills, malaise, nausea, headache; haemolytic uremic syndrome; thrombotic thrombocytopenia purpura</td>
<td>10–100 cells</td>
<td>Healthy domestic ruminants (primarily cattle, sheep, goats), pigs (wild and domestic), deer, wild avian and other vertebrates; flies, slugs</td>
<td>Lettuce, spinach, sprouts, unpasteurized apple juice</td>
</tr>
<tr>
<td>Listeria monocytogenes</td>
<td>3 days – 3 months</td>
<td>Flu-like symptoms; septicemia; meningitis; abortion and still birth (invasive disease)</td>
<td>&lt; 1000 cells for a susceptible person</td>
<td>Primarily soil, water, but may be carried in the gastrointestinal tract of healthy domestic and wild animals; sometimes causes abortion and neurological disease in cattle and small ruminants</td>
<td>Cantaloupe, cabbage, sprouts</td>
</tr>
<tr>
<td><em>Salmonella enterica</em>, non-typhoidal</td>
<td>6–72 h</td>
<td>Abdominal cramps, diarrhoea, vomiting, fever, chills, malaise, nausea, headache; septicemia</td>
<td>1–100,000 cells (depending on individual susceptibility)</td>
<td>Healthy warm- and cold-blooded animals, especially reptiles; some serovars may cause fever and diarrheal illness in domestic livestock and pets</td>
<td>Almonds, arugula, basil, cantaloupe, cilantro, lettuce, mangoes, orange juice, papaya, hot peppers, sprouts, tomatoes</td>
</tr>
<tr>
<td>Yersinia pseudotuberculosis</td>
<td>1–11 days (sometimes much longer)</td>
<td>Fever, acute abdominal pain resembling appendicitis</td>
<td>100,000–1,000,000 cells</td>
<td>Healthy domestic livestock (primarily pigs), wild rodents, fresh water fish</td>
<td>Carrots, lettuce</td>
</tr>
<tr>
<td><strong>Parasitic</strong></td>
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</tr>
<tr>
<td>Cryptosporidium spp.</td>
<td>7–10 days</td>
<td>Mild to profuse diarrhoea, nausea, vomiting, cramps</td>
<td>10–100 oocysts</td>
<td>Many domestic and wild animals; important cause of diarrheal illness in young ruminants</td>
<td>Unpasteurized apple cider</td>
</tr>
<tr>
<td>Angiostrongylus cantonensis</td>
<td>1–3 weeks</td>
<td>Headache, neck stiffness; eosinophilic meningitis, encephalitis</td>
<td>Ingestion of an infected intermediate or transport host</td>
<td>Rats (definitive host); slugs, snails (intermediate host)</td>
<td>Lettuce, unpasteurized orange juice</td>
</tr>
<tr>
<td><strong>Viral</strong></td>
<td></td>
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<tr>
<td>Nipah virus</td>
<td>5–21 days</td>
<td>Encephalitis; respiratory disease</td>
<td>Unknown</td>
<td>Fruit bats</td>
<td>Date palm sap</td>
</tr>
</tbody>
</table>
Most E. coli are harmless, but a subset of strains may cause severe disease. E. coli O157:H7 is the prototype of the shiga toxin-producing E. coli (STEC). Over 200 STEC serotypes have been described, but most human illnesses are caused by E. coli O157 and six other STEC groups (O26, O45, O103, O111, O121 and O145) [37]. The E. coli O157:H7 serotype was first described as a cause of human haemorrhagic colitis in 1982, but remained relatively unknown to the public until 1993 following a highly publicized multistate outbreak linked to undercooked hamburgers served at Jack in the Box fast-food restaurants in the western USA [38]. This outbreak resulted in significant litigation and was the impetus for major policy changes to improve the safety of ground beef in the USA [39].

To the surprise of many in public health, in the mid-1990s unpasteurized juices, lettuce and sprouts emerged as E. coli O157 food vehicles [1–4]. Notably, these produce-related outbreaks were also associated with significantly more illness compared with ground beef [40]. The epidemiology of non-O157 STEC is less understood, but beef products, raw milk, lettuce and raw sprouts have been implicated in outbreaks [37]. The reason for the emergence of STEC from plant-based foods is likely multifactorial, but changes in consumer eating habits (consumption of more fresh produce) and the ability to detect geographically widespread illnesses from centralized production facilities are probably contributing factors [41, 42]. For example, two processing firms account for approximately 90% of the entire retail bagged-salad industry in the USA [41].

In 2006, a nationwide outbreak of E. coli O157:H7 associated with ready-to-eat packaged baby spinach grown in California resulted in over 200 illnesses and at least three deaths [7]. Similar to the Jack in the Box outbreak of 1993 [38, 39], the 2006 Dole spinach outbreak riveted the fresh-cut produce industry, and spurred major changes in food safety practices. In 2007, the leafy greens industry implemented voluntary good agricultural practices (GAPs) and auditable metrics through industry marketing agreements in Arizona and California, the primary growing regions for leafy greens in the USA [17, 18]. In 2013, the FDA published ground-breaking proposed ‘Standards for the Growing, Harvesting, Packing, and Holding of Produce for Human Consumption’ under the 2011 Food Safety Modernization Act [43].

It is generally accepted at this time that domestic ruminants (cattle, sheep and goats), pigs (domestic and wild) and deer are the most significant potential sources of E. coli O157:H7 that could be involved in the contamination of leafy greens [17, 18]. Domestic cattle are considered the primary reservoirs of E. coli O157:H7 and possibly some of the other STECs [20]. Deer have been implicated in venison-related STEC illnesses suggesting that cervids may also serve as a reservoir [44–47]. Surveys of deer populations have revealed generally low levels (<2%) of E. coli O157 shedding in faeces regardless of their association with infected cattle [48–55]. Black-tailed deer were investigated as potential sources of two produce-related outbreaks in the western USA. Cody et al. (1999) isolated E. coli O157:H7 from 1 of 11 (11%) of deer droppings collected in an orchard following a multistate outbreak linked to unpasteurized apple juice; however, the isolate was genetically different from the human outbreak strain [10]. In 2011, deer droppings were definitively linked to E. coli O157:H7 contamination of fresh strawberries in Oregon that caused 15 illnesses and two deaths [13]. Other large game mammals have been confirmed as potential reservoirs of STEC including wild boar and their crosses with domestic swine [9, 31, 33, 52, 56]. In 2006, a large population of feral swine was observed on the ranch implicated in a nationwide E. coli O157:H7 outbreak linked to baby spinach [8, 9]. A more detailed analysis of this outbreak is described below (Figure 2).

Reports of E. coli O157 detection in wild birds and small mammals appear sporadically in the literature [21]. The bacterium has been isolated from duck, gull, rat, opossum, pigeon, rabbit, raccoon and starlings [57–63]. European starlings (Sternus vulgaris) have been shown to transport E. coli O157 between cattle herds and could theoretically move the bacteria from infected animal operations to produce fields [64–66]. There is also experimental evidence that filth flies are capable of transferring E. coli O157:H7 to spinach and other leafy greens [67, 68].

Salmonella enterica

Salmonella is a Gram-negative, rod-shaped bacterium that colonizes the gastrointestinal tract of humans and animals. Non-typhoidal Salmonella enterica is the second leading
cause of food-borne illness in the USA following norovirus [19]. Over 2500 serovars have been described, but most human illnesses and outbreaks are from several dominant types (Enteritidis, Heidelberg, Javiana, Newport, Typhimurium) [5]. Poultry and eggs are most often associated with food-borne disease outbreaks and recalls, but plant-based food vehicles of salmonellosis are emerging including raw tomatoes, peppers, melons, salad greens, herbs (cilantro, parsley, basil), unpasteurized juices, tropical fruits (mangoes, papayas) and sprouts [15]. Salmonellosis outbreaks have also been reported from consumption of contaminated low-moisture plant products including nuts (almonds, peanuts, pine nuts), cereals and dried spices. One of the largest food recalls documented in the USA was due to contaminated peanut products from the Peanut Corporation of America in 2009. The outbreak was associated with 714 illnesses and nine deaths in 46 states; 3918 peanut butter-containing products were recalled [69].

Salmonella has been recovered from warm- and cold-blooded vertebrates and invertebrates such as flies [31, 33, 35, 70–76]. The food-processing industry has long been aware of the risk of Salmonella from bird, rodent and fly infestations. Good manufacturing practices in processing and retail facilities mandate pest control. Lessons have been learned from the poultry industry with regard to Salmonella risk from rodent infestations. Mice are known carriers of Salmonella Enteritidis on layer farms, which have been linked to human infections from contaminated eggs [77].

Compared with manufacturing plants and intensive poultry operations, less is known about the role rodents and other wild animals may play in Salmonella contamination of open fields and orchards. Wild rodents and birds are common in agricultural areas, and may represent a potential source of Salmonella contamination of plants. Birds aggregating in large numbers may cause heavy faecal contamination of the production environment, especially under roosting areas (powerlines, trees) [78]. In recent studies of the diversity of Salmonella cultured from wild animals captured in the central California coast, Salmonella recovery was generally low in mammals and birds, and...
highest in wild-caught snakes [73, 74]. In a preliminary study of domestic and wild canids, relatively high rates of \textit{Salmonella} were found in coyote scat samples collected near produce fields in the desert southwest, the second largest leafy greens production region in the USA [79].

Only a few examples of a direct link between an animal source and \textit{Salmonella} contamination of a fresh produce commodity have been documented, despite the extensive range of potential animal reservoirs and widespread diversity of fruits, nuts and vegetables that have been associated with numerous salmonellosis outbreaks and recalls (Table 1). This may be explained, in part, by limited investigation into animal sources at the pre-harvest level following investigation into animal sources at the pre-harvest level. In addition, \textit{Salmonella} can persist in the environment for months to years without re-introduction; thus, the original host may no longer be present by the time an outbreak is recognized and investigated. In one example, Parish et al. (1998) tested wild amphibians as a possible source of contamination following an outbreak of salmonellosis linked to unpasteurized orange juice. Different strains of \textit{Salmonella} were cultured from a toad and tree frogs near the processing facility [80]. In 2012, fresh whole cantaloupes grown in Indiana were linked to approximately 261 salmonellosis infections, including three deaths, in 24 states [81]. Wild birds were identified as a potential source of the outbreak as noted in a warning letter from FDA to the company: ‘Bird excrement in the rafters above food contact surfaces (e.g., brush rollers, conveyor belts, grading table) and directly on the processing line itself. Allowing birds to roost in your packing facility could allow them to defecate directly on to food products during conveyance, grading and sorting.’

\textbf{\textit{Listeria monocytogenes}}

\textit{L. monocytogenes} is a Gram-positive, rod-shaped bacterium found commonly in the environment. Most human illnesses are caused by three serotypes (1/2a, 1/2b and 4b). Unlike the enteric pathogens described above, \textit{L. monocytogenes} lives and grows readily outside of the gastrointestinal tract as a saprophyte. \textit{L. monocytogenes} also grows at refrigeration temperature, thus putting consumers at increased risk from ready-to-eat contaminated foods. Invasive listeriosis, the most severe form of disease caused by pathogenic \textit{L. monocytogenes} strains, has the highest percentage of hospitalizations (94%) and number of deaths among all of the reportable bacterial food-borne pathogens [19]. The elderly, pregnant women and immunocompromised persons are most likely to suffer life-threatening illness. Dairy, deli meats and other ready-to-eat foods including packaged salads are most often associated with listeriosis outbreaks and recalls, but raw sprouts and melons have also been implicated [82]. In 2011, fresh whole cantaloupe grown by Jensen Farms in Colorado was the source of the deadliest reported listeriosis outbreak in the USA, to date, with 147 illnesses and 33 deaths reported from 28 states [83]. Multiple outbreak strains were cultured from the packing shed where unsanitary conditions and improper equipment were believed to have caused the contamination. The original source of \textit{L. monocytogenes} introduction into the packing facility was not identified. Although \textit{L. monocytogenes} may be shed in the faeces of healthy domestic and wild animals [83–85], the ability of this pathogen to live outside the animal host in soil, water and biofilms makes wild animals less of a focus during outbreak investigations. Animals could contribute to environmental loading in the produce production and harvesting environment, but have not been implicated in direct contamination of raw produce or other plant-based foods leading to human illnesses or outbreaks.

\textbf{\textit{Yersinia spp.}}

\textit{Yersinia pseudotuberculosis} and \textit{Yersinia enterocolitica} are related Gram-negative, rod-shaped bacteria. \textit{Y. pseudotuberculosis} is also genetically similar to flea-borne \textit{Yersinia pestis}, the causative agent of human plague, but is transmitted through faecal–oral ingestion. Human yersiniosis is characterized by acute gastroenteritis and abdominal pain that may resemble appendicitis and even lead to unnecessary surgery. Most illnesses are associated with raw or undercooked animal-based foods (especially pork) probably following introduction during slaughter and processing of food animals [5]. Wild animal hosts may include beavers and other rodents, birds, wild boars and fresh water fish [86–89]. Produce is rarely associated with yersiniosis, but investigators in Finland documented an unusual outbreak of \textit{Y. pseudotuberculosis} linked to raw carrots [12]. The outbreak strain was cultured from a pooled sample of common shrew intestines from one implicated farm suggesting that wild rodents’ droppings may have contaminated the growing environment.

\textbf{\textit{Parasites}}

\textbf{\textit{Cryptosporidium spp.}}

\textit{Cryptosporidium} is a protozoal parasite that typically inhabits the gastrointestinal tract of humans and animals. Cryptosporidiosis is an important cause of diarrheal illness among both humans and domestic livestock and pets. Human illnesses are most often associated with waterborne exposure rather than food. Direct contact with sick animals is also a significant source of zoonotic infections. Transmission is via ingestion of \textit{Cryptosporidium} oocysts, which are shed in the faeces of infected humans or animals. \textit{Cryptosporidium} does not replicate outside of the

http://www.cabi.org/cabreviews
host, but oocysts may survive for prolonged periods of time in the environment. Zoonotic Cryptosporidium species could contaminate produce grown near infected animals or exposed to contaminated agriculture water or fertilizer [90]. An outbreak of cryptosporidiosis linked to unpasteurized pressed apple cider occurred in 1993 during a school agricultural fair in central Maine; domestic livestock were suspected as the source of the contamination [91]. The relative importance of wild animals in the contamination of plants is uncertain, but Li et al. (2013) found 26.0 and 24.2% of wild rodents trapped next to produce fields in California were positive for Cryptosporidium spp. and Giardia spp., respectively [92]. Feral swine from the same produce growing region were also shown to harbour these parasites [93].

**Angiostrongylus cantonensis**

A. cantonensis is a tropical parasitic nematode (roundworm) and causative agent of rat lungworm. Natural transmission involves a rat (definitive host) and snail/slug (intermediate host) transmission cycle. Humans are accidental hosts that may be exposed by ingestion of raw snails/slugs or transport hosts such as frogs infected with larvae. Once ingested by a human, the larvae migrate aberrantly and cause neurological disorders characterized by eosinophilic menigitis and encephalitis. Although not among the major food-borne pathogens listed by CDC, lettuce and raw vegetable juice have been suspected as sources of angiostrongyliaisis infections in Hawaii and other tropical regions where consumers may have unknowingly eaten small snails/slugs or been exposed to larvae transported by slime on the plant leaves [94–96].

**Viruses**

Zoonotic viruses comprise the majority of emerging infectious disease agents, and an estimated 75% are of wildlife origin [97]. Zoonotic viruses are frequently transmitted by direct animal–human contact or via an arthropod vector such as a mosquito or tick. Interestingly, very few examples exist of food-borne transmission of zoonotic viruses [98]. This could be because of the limited host range of prevalent human food-borne viruses such as Hepatitis A and norovirus. Underreporting and lack of diagnostic tests may also contribute to under-recognition of emerging food-borne viral zoonoses. Where food-borne transmission of viruses from wild animal sources has been documented, the usual route is by consumption of contaminated meat or direct contact with tissues during handling and preparation. Several exotic viruses have been associated with increased wildlife trade, live-animal markets and consumption of bushmeat and other unusual foods.

**Avian influenza**

Avian influenza viruses are type A and belong to the family Orthomyxoviridae. ‘Bird flu’ is distributed worldwide in wild birds, especially aquatic species (e.g., ducks, geese and gulls) and may also spill-over to domestic poultry and swine [99]. Avian influenza viruses are classified as low or high pathogenicity. Highly pathogenic avian influenza A (H5N1) and H7N9 are considered a significant public health threat due to the potential for interspecies transmission to humans and subsequent human-to-human transmission. The H5N1 virus is endemic in several Asian countries including Bangladesh, China, Egypt, India, Indonesia and Vietnam [99]. Live-animal markets where wild and domestic animals are exposed to crowded conditions contribute to the spread of dangerous subtypes of avian influenza. Food-borne transmission of H5N1 is considered to be extremely rare, but has been linked to eating raw blood-based poultry dishes. Direct contact with infected birds during slaughter and dressing is a major risk factor for human infection. Standard hygienic practices during slaughter and processing, and proper cooking temperatures are recommended to prevent human infections. Although infected birds may shed influenza virus in their faeces, the risk of cross-contamination of produce fields or agriculture water following bird intrusions is unknown.

**Hepatitis E**

Hepatitis E virus is an emerging infectious disease primarily diagnosed in Asia, Africa, the Middle East and Central America. Hepatitis E belongs to the Hepeviridae family, and causes symptoms similar to Hepatitis A virus. The majority of human outbreaks are due to human-to-human fecal–oral transmission via contaminated water, especially following migration of refugees [100]. Zoonotic transmission has been documented for two of four recognized pathogenic genotypes of Hepatitis E and may account for sporadic illnesses seen in developed countries. There is increasing evidence that domestic swine are an important reservoir of zoonotic Hepatitis E. Wildlife are also potential reservoirs and human cases have been documented following consumption of organs from wild boar and undercooked venison in several European countries [101–103]. The risk of faecal shedding and environmental contamination by animal reservoirs is unknown.

**Noroviruses**

In the USA, human norovirus is the leading cause of viral gastroenteritis including among produce-related food-borne disease outbreaks [5, 19]. Noroviruses belong to Caliciviridae, a diverse family characterized by host-specificity. Human norovirus is spread by faecal–oral transmission and generally causes a self-limiting
gastroenteritis. Prevention efforts are focused on proper waste management, good hygiene and removal of sick food handlers from the food production chain. The lack of interspecies transmission probably explains why zoonotic transmission does not appear to be important in the ecology and epidemiology of noroviruses. However, bioaccumulation of human enteric viruses by shellfish following exposure to sewage has resulted in illnesses and outbreaks from consumption of raw shellfish [104]. In addition, animal enteric viruses such as murine norovirus have proven to be useful surrogates to study the behaviour of human enteric viruses in plant production and processing environments [105, 106].

### Exotic viruses

Exotic viruses are defined as rare viruses with a limited geographic distribution and infections are often associated with high case-fatality rates. Many of the zoonotic exotic viruses occur in tropical countries and have wild animal reservoirs. Nipah and Hendra (Henipaviruses) are examples of emerging zoonotic viruses in the family Paramyxoviridae. Henipaviruses are found in parts of Asia and Australia where fruit bat reservoirs in the Pteropodidae family occur. These viruses cause potentially fatal encephalitis or respiratory disease in humans. Direct contact with infected fruit bat reservoirs or domestic animals with the virus is the usual mode of transmission. Interestingly, in Bangladesh food-borne Nipah virus has been associated with consumption of raw date palm sap pots contaminated with fruit bat excreta [107, 108].

Lassa virus (family Arenaviridae) and severe acute respiratory syndrome (SARS; family Coronaviridae) are zoonotic emerging infectious diseases. Lassa virus is an important cause of haemorrhagic fever in Western Africa; it is shed in the faeces and urine of peridomestic multimammate rats inhabiting villages. Food-borne transmission has been documented following consumption of infected rats [109]. The SARS virus was first recognized in 2002–2003 following an outbreak that originated in Asia and spread around the world via infected travellers. The SARS virus is primarily transmitted human-to-human by respiratory droplets. The virus is believed to have been introduced by horseshoe bats and civets in live-animal markets in Asia. Food-borne transmission is theoretically possible through consumption of contaminated animal products (bats, civets), but has never been documented [98]. Theoretically, fresh produce could be contaminated with faeces and urine from infected wild animal hosts, but this mode of transmission has not been documented.

### Transmission and Survival on Plants

Zoonotic food-borne pathogens shed by wild animals can be spread to plants by direct deposition of faecal material onto the plant, or by indirect contamination of agriculture water, soil, compost, farm equipment and other fomites such as worker boots and clothing. Incidental transmission from contaminated fur, feathers or the oral cavity of a colonized animal or insect (regurgitation) may also represent a route of transmission to plants [21]. The plant types most vulnerable to microbial contamination are those consumed raw or minimally processed since there is no 'kill step' to remove pathogens. Fresh-cut, ready-to-eat packaged salads and other produce have unique concerns with regard to microbial contamination as described below [110]. Crops grown close to the ground such as leafy greens (lettuce, spinach), edible herbs (basil, cilantro), strawberries and tree nuts (almonds) harvested on the ground are at risk of faecal contamination by wild animals. Bird droppings can be a concern particularly when plants are grown under roosting areas such as trees and utility lines. Irrigation canals and ponds may also serve as wild animal habitat, thus could require additional microbiological quality monitoring compared with well or municipal agricultural water sources.

Food-borne pathogens are generally not part of the normal microflora of agricultural crops. Thus, once introduced onto the plant surface, most of these microbes face a hostile environment and die-off within hours to days [111, 112]. However, there are recurring plant–pathogen combinations associated with produce-related outbreaks and recalls that suggest some degree of pathogen adaptation to the plant environment (Table 1). For example, Mandrell in 2011 reviewed produce-related outbreaks from 1995 to 2008 where pre-harvest contamination was suspected and identified E. coli O157 as the cause of 29 outbreaks linked to lettuce or spinach in four countries [15]. In contrast, all of the tomato-associated outbreaks were due to Salmonella spp. in the same period.

Brandl in 2006 reviewed the major factors that influence the ability of zoonotic enteric pathogens to survive and grow in the plant environment and identified epiphytic fitness, physiochemical nature of the plant surface, biofilm formation, microbe-microbe and plant-microbe interactions [111]. There is evidence that cut surfaces, injury and plant diseases may promote attachment and growth of E. coli O157 and Salmonella. It has been speculated that the disproportionate number of outbreaks due to fresh-cut produce may relate to the increased availability of attachment sites and nutrients for the enteric pathogen to utilize [111–113]. Other plant physical characteristics that may promote bacterial survival include uneven surfaces such as netted melons (versus a smooth or waxed surface), hairs (raspberries) and the presence of a stem. Organic material (soil, faeces) on the plant may serve to protect more fragile bacterial species such as Campylobacter from unfavourable temperature, atmosphere or UV conditions. For example, it was suspected that a pea–soil–bird faecal mixture brought into the shelling/processing area after mechanical harvest was a

Since many of the zoonotic enteric pathogens have a low infectious dose (Table 1), attachment and internalization of even a few cells is of concern because consumers cannot simply wash the fresh produce to protect themselves from exposure. Internalization of fresh produce by E. coli O157 and Salmonella root uptake has been demonstrated experimentally, but its importance under natural conditions is still unclear [114–117]. Some organisms also have the ability to enter and attach to plant leaf stomata (pores) where the cells are then protected from rigorous washing and chemical sanitizing agents used by the fresh-cut produce industry [111].

Assessing Risk from Field to Fork

The level of risk from a particular domestic or wild animal population in the microbial contamination of plant crops is dependent on multiple factors including pathogen prevalence in the population, concentration of the pathogen (number of cells shed per gram of faeces), volume of faecal material produced per defecation and the population density [79]. Indeed, given the few examples of contamination events linked to wild animal carriers, the risk could be characterized as a low probability, high consequence event. However, lessons from notable outbreaks described in this review suggest that a ‘perfect storm’ can tip the odds to a higher probability event, especially if crops are exposed to large numbers of infected animals shedding food-borne pathogens in the field. In-field microbial contamination in combination with downstream opportunities for survival and amplification during processing, distribution and storage of ready-to-eat and raw agricultural commodities enhances this risk.

Danyluk and Schaffner in 2011 published a quantitative microbial risk assessment explaining the plausibility of an in-field contamination event leading to the 2006 E. coli O157:H7 spinach outbreak [118]. The model predicted that pathogen concentration in the field as low as −1 log CFU/g at 0.1% prevalence of plant contamination could have caused an outbreak of the magnitude of the spinach outbreak (205 reported illnesses, 4112 estimated illnesses multiplying by the CDC 26.1 underreporting factor). The model also predicted that with this starting level, the bacteria could have increased by as much as 1 log CFU/day under optimal temperature conditions, and 99.2% of the illnesses could be attributed to cross-contamination of cut spinach pieces during washing. In addition, bacterial attachment to the cut spinach pieces and stomata plus utilization of nutrients released from the injured (cut) plant leaves could have contributed to the survival and growth of the pathogen [99].

Considering this model, Figure 2 illustrates a hypothetical scenario of an in-field faecal deposition source of the 2006 E. coli O157:H7 spinach outbreak. Alternative scenarios to explain the in-field contamination include faecal contamination of the agricultural well water [119] or unreported use of surface water or untreated animal manure as fertilizer. In the faecal deposition scenario, it is hypothesized that equipment used to mechanically harvest the baby spinach could have been contaminated with dirt and animal faecal material (Figure 2). Once introduced into the processing plant, post-harvest factors likely contributed to the majority of illnesses regardless of whether the original in-field source of E. coli O157:H7 was faeces or contaminated water/soil [118]. Specifically, using lot codes from patients’ leftover bags of spinach, the packaged baby spinach traceback implicated a single lot and shift at a San Juan Bautista processing plant [8]. The plant’s records showed that the spinach originated from four fields in two counties of the central California coast. The outbreak strain was found in environmental samples (cattle and feral swine faeces, river water/sediment, and pasture soil) at one of the four ranches [8–9]. The implicated field was located in San Benito County and supplied only 1002 pounds of spinach from about 2 acres of a 50 acre field. Spinach harvested from this field was then mixed with 14 658 pounds of spinach from the other three fields. The washed, cut spinach was packaged in 41 760 six-ounce bags and distributed throughout the USA and Ontario, Canada. As perspective, it is worth noting that an estimated 680 million pounds of fresh spinach were consumed in 2005, compared with the total volume of 15 660 pounds implicated in this outbreak [41].

Prevention and Control

Since fresh fruits, nuts and vegetables are not grown in a sterile environment the ideal approach to pathogen control would be minimizing in-field contamination followed by a post-harvest processing step such as heat or a chemical treatment that inactivates enteric pathogens on plants. After several food-borne disease outbreaks, the almond and processed juice industries implemented mandatory treatment to control pathogens. Unfortunately, many fresh-cut fruits and vegetables are not readily amendable to pasteurization or another type of treatment step to achieve an adequate log reduction of enteric pathogens during processing. Likewise, some raw agricultural commodities are field-packed and not subject to a processing step. Irradiation is one approach that could potentially be effective in reducing or eliminating pathogens in fresh produce, especially if attached to pores or internalized in the plant tissue [120]. However, this technology is not currently utilized by the fresh produce industry due to potential quality concerns, limited availability of irradiation facilities, regulatory constraints and opposition by some consumer groups.

Because there is no ‘kill step’ for most fresh produce commodities, preventing in-field contamination of edible plants becomes critically important to protect the
<table>
<thead>
<tr>
<th>Commodity</th>
<th>Enforcement</th>
<th>Food safety practices</th>
<th>Date (reference)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culinary herbs (basil, chives, cilantro, dill, parsley, etc.)</td>
<td>Industry, voluntary</td>
<td>Environmental assessment of wildlife activity and food safety risk; metrics and decision tree with focus on evidence of faecal contamination and plant damage by animals</td>
<td>2013 [121]</td>
</tr>
<tr>
<td>Green onions</td>
<td>Industry, voluntary</td>
<td>Similar to culinary herbs</td>
<td>2010 [122]</td>
</tr>
<tr>
<td>Fruits, vegetables, tree-nuts¹</td>
<td>FDA, mandatory (proposed rules)</td>
<td>Requires monitoring for evidence of animal intrusion; efforts to prevent contamination of covered produce with animal excreta</td>
<td>2013 [43]</td>
</tr>
<tr>
<td>Fruits and vegetables (multiple)²</td>
<td>FDA, voluntary</td>
<td>Recommends to the extent possible, where high concentrations of wildlife are a concern, consider establishing GAPs to deter or redirect wildlife</td>
<td>1998 [123]</td>
</tr>
<tr>
<td>Leafy greens (arugula, lettuce, chard, spinach, cabbage, kale, spring mix, etc.)</td>
<td>FDA, voluntary</td>
<td>Site location to minimize potential access by wildlife (considering proximity to water, wildlife harbourage); monitoring and reducing potential harbourage and standing water; utilizing repellents/attractants; considering no-harvest zones when evidence of heavy wildlife activity or faeces; training harvest employees</td>
<td>2009 [124]</td>
</tr>
<tr>
<td>Lettuce and leafy greens³</td>
<td>Voluntary marketing agreement (Arizona, California)</td>
<td>Metrics for animal intrusions, no harvest buffer zones in field; decision tree for conducting pre-harvest and harvest assessments</td>
<td>2012 [17–18]</td>
</tr>
<tr>
<td>Melons</td>
<td>Industry, voluntary</td>
<td>Environmental assessments; considering no-harvest zones where there is evidence of unusually heavy wildlife pest infestations (faeces, large areas of animal tracks or burrowing); training harvest</td>
<td>2005 [125]</td>
</tr>
<tr>
<td>Melons</td>
<td>FDA, voluntary</td>
<td>Similar to industry guidelines</td>
<td>2009 [126]</td>
</tr>
<tr>
<td>Strawberries</td>
<td>Industry, voluntary</td>
<td>Assessments and good agriculture practices for wildlife intrusion prior to and during planting, growing, and harvesting</td>
<td>2011 [127]</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>Mandatory (Florida)</td>
<td>Minimize wild animal presence in fields to the degree possible by methods identified by wildlife experts.</td>
<td>2008 [128]</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>Industry, voluntary</td>
<td>Environmental assessments; minimizing wildlife presence (barriers, deterrents), minimizing attractants, harbourage; redirecting wildlife to non-sensitive areas and/or by other methods identified by wildlife experts; removal of potentially contaminated product</td>
<td>2008 [129]</td>
</tr>
<tr>
<td>Tomatoes</td>
<td>FDA, voluntary</td>
<td>Similar to industry guidelines</td>
<td>2009 [130]</td>
</tr>
</tbody>
</table>

¹Covered produce in the proposed rule does not include grains, cereals, and produce commodities that are rarely consumed raw (e.g., artichokes, brussels sprouts, kidney beans).

²GAPs = good agricultural practices.

³The metrics currently specify a 1.5 m no-harvest buffer zone if faecal material from animals considered of significant risk for carrying E. coli O157 (cattle, sheep, goats, pigs-domestic and wild, deer) is found in the crop production area, and a 0.9 m buffer for areas with evidence of intrusion, but no faecal material.
public health. A number of GAP guidance documents for higher risk commodities have been published by industry groups and regulatory agencies [17, 18, 121–130]. Table 2 shows examples of regulations and GAP guidelines that specifically address wild animal activity and/or faecal material in or around crop fields. Management of produce food safety risks from potential wild animal sources is particularly challenging in open crop fields and orchards. Unlike agricultural water and soil amendment metrics that can be quantified and audited using microbiological testing criteria, for example, best practices related to wild animals tend to be non-specific and difficult to measure or enforce. The wildlife component of GAP programmes generally involves conducting pre-season and pre-harvest environmental risk assessments; monitoring for animal intrusion and faecal contamination of the production environment during growth and harvest; establishment of no-harvest zones where product may be contaminated from animal activity/faeces; and training of farm workers to recognize, report and mitigate these risks.

Although there is a large body of literature that addresses wildlife damage control related to agricultural crop loss [131], a paucity of species-specific, targeted approaches that consider wild animals in the context of food safety risks exist in the literature [132–134]. The US Department of Agriculture estimates that wildlife damage to fruit, nut and vegetable crops causes over 146 million dollars of damage per year in the USA with deer, rodents, crows, raccoons and rabbits being the most frequently reported species causing the damage [135]. The costs due to food safety-related damages from wild animals have not been quantified.

Perhaps due in part to the limited understanding of best management practices for potential wild animal risks, some food safety practices have resulted in conflicts with conservation and water quality programmes in agricultural areas [136]. For example, poison bait stations to control rodent and bird populations, fences and habitat modification near produce fields to purportedly reduce wildlife attraction, are practices that have been cited as detrimental to environmental stewardship goals (Figure 3). Co-management is a concept that has emerged to resolve potential conflicts between food safety and conservation goals. Co-management is defined as an approach to conserving soil, water, air, wildlife and other natural resources while simultaneously minimizing microbiological hazards associated with food production [136]. Several industry guidelines have incorporated the co-management concept into their best practices [17, 18, 121].

Conclusions

In summary, wild animals are one of several potential sources of zoonotic food-borne pathogens that could contaminate fresh and minimally processed or fresh-cut fruits, nuts, vegetables and other edible plants grown in open fields and orchards. Pre-harvest microbial contamination from wild animal activity in the production environment represents a public health risk because of the low infectious dose of many of these zoonotic enteric pathogens, and the potential for downstream survival and amplification of pathogens during harvest, processing, transportation and storage. There is a need to better understand the predisposing factors that contribute to microbial contamination of plants from wild animals in comparison with other sources in the growing environment. The goal should be to develop species-specific, targeted mitigation strategies for risks from wild animals, while also promoting co-management of food safety and environmental stewardship in the agricultural landscape.

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