Epidemiol. Infect. (2009), **137**, 307–315. © 2009 Cambridge University Press
doi:10.1017/S0950268808001969 Printed in the United Kingdom

REVIEW ARTICLE
The growing burden of foodborne outbreaks due to contaminated fresh produce: risks and opportunities

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(*Accepted 17 December 2008*)

SUMMARY

Foodborne outbreaks from contaminated fresh produce have been increasingly recognized in many parts of the world. This reflects a convergence of increasing consumption of fresh produce, changes in production and distribution, and a growing awareness of the problem on the part of public health officials. The complex biology of pathogen contamination and survival on plant materials is beginning to be explained. Adhesion of pathogens to surfaces and internalization of pathogens limits the usefulness of conventional processing and chemical sanitizing methods in preventing transmission from contaminated produce. Better methods of preventing contamination on the farm, or during packing or processing, or use of a terminal control such as irradiation could reduce the burden of disease transmission from fresh produce. Outbreak investigations represent important opportunities to evaluate contamination at the farm level and along the farm-to-fork continuum. More complete and timely environmental assessments of these events and more research into the biology and ecology of pathogen-produce interactions are needed to identify better prevention strategies.

Key words: Foodborne, outbreaks, produce.

INTRODUCTION

Fresh fruits and vegetables are increasingly recognized as a source of foodborne outbreaks in many parts of the world. In the USA, the proportion of outbreaks linked to fresh produce increased from <1% of all reported outbreaks with known food vehicle in the 1970s to 6% in the 1990s [1]. The median size of produce-related outbreaks also doubled and the proportion of outbreak-associated cases accounted for by fresh produce increased from <1% to 12% of illnesses in that same time period. In Australia, fresh produce accounted for 4% of all foodborne outbreaks reported from 2001 to 2005 [2]. In Europe, recent outbreaks have revealed new and unexplained links between *Shigella* and imported baby corn [3], *Yersinia pseudotuberculosis* and lettuces [4], and noroviruses and raspberries [5], to cite but a few. In the USA, recent outbreaks of *Escherichia coli* O157:H7 infections linked to bagged baby spinach [6], *Salmonella* Saintpaul due to hot peppers and possibly tomatoes [7] and *Salmonella* Poona due to imported cantaloupes [8] underline the challenges related to fresh produce. Several produce-related outbreaks have been multinational in scope (Table 1). In the wake of these outbreaks, research has begun to define the biological

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Table 1. *Selected recent multinational foodborne outbreaks due to contaminated produce items* [*3, 6, 7, 9–11*]

Year	Pathogen	No. of cases	No. of countries	Affected regions	Implicated food
2008	*Salmonella* Saintpaul	1442	2	North America	Fresh peppers, ?tomatoes
2007	*Salmonella* Senftenberg	51	5	Europe, North America	Fresh basil
2007	*Shigella sonnei*	175	2	Australia, Europe	Raw baby corn
2007	*Salmonella* Weltevreden	45	3	Europe	Alfalfa sprouts
2006	*Escherichia coli* O157:H7	206	2	North America	Fresh spinach
2006	*Salmonella* Thompson	20+	3	Europe	Ruccola (arugula)

interactions between microbes and produce, which can be surprisingly complex.

The increase in reported outbreaks related to produce may be the result of several trends. The *per capita* consumption of fresh produce has increased in the USA, and perhaps in other industrialized nations [12]. The desire for fresh produce year round means that in the cold season it is likely to be transported from farther away, either the subtropics or from the other hemisphere. Due to changes in processing, more cutting and coring may be performed in the field at the time of harvest. As agriculture becomes more intensive, produce fields may be next to animal production zones, and the ecological connections between wild animals, farm animals, and produce may be closer.

Reports in this issue of *Epidemiology and Infection* further highlight the challenges, and the need for improved prevention strategies worldwide. The range of vehicles associated with these outbreaks – fresh basil, carrots, and mung bean sprouts – represent three distinct production, storage, and use characteristics. *Salmonella* and other enteric bacterial pathogens in these outbreaks were able to survive extensive transportation or storage for prolonged periods of time. Subsequent handling of the contaminated produce items allowed amplification of the organisms and resulted in the reported outbreaks. Although measures taken at the point of service can reduce the likelihood that contamination will cause outbreaks in commercial food service and institutional settings, primary prevention of contamination is needed to stop widely dispersed outbreaks.

PUBLIC HEALTH RECOGNITION

Identifying the source of contamination in any outbreak requires a careful assessment of potential exposures. In outbreaks in defined groups, such as *Y. pseudotuberculosis* infections [13] and enterotoxigenic *E. coli* infections [14] associated with school meals, or shigellosis in airline passengers [15], menus may provide a set of hypotheses that can be directly tested. Outbreaks with cases widespread in the community present a special challenge, as the list of possible exposures includes all foods consumed over a period of several days, as well exposure to other persons, water and other environmental sources. Identifying the source starts with the generation and evaluation of reasonable hypotheses regarding suspected food vehicles [16]. Hypothesis formation is guided by previous experience and biological plausibility, perceptions of which are also guided by previous experience. One caveat to this common approach is that over-reliance on experience and known biology may inhibit recognition of novel or unusual food vehicles, such as certain items of fresh produce.

However, produce-related outbreaks are no longer novel. With increasing awareness of raw produce as a vehicle for foodborne infections, investigators are less likely to dismiss the idea once it has arisen. Thus, when Gupta and colleagues employed open-ended and direct food consumption history-taking to identify foods suspected as the source of *S.* Branderup infections in multiple USA states, they were building on the knowledge that tomatoes had been well documented as a vehicle for *Salmonella* [17, 18].

The growing recognition of raw produce as an important source of foodborne outbreaks may be better understood when compared with other foods that are now well-recognized sources of infection with particular pathogens. Several outbreaks of *Salmonella* Enteritidis (SE) infections caused by duck eggs in the first half of the last century showed that eggs were a possible source of this infection [19], and foreshadowed the SE pandemic due to contaminated hen's eggs in the last decades of the century [20]. Numerous SE outbreaks due to eggs during the 1980s confirmed that eggs were an accepted source, indeed the expected source, of SE outbreaks [21]. This relationship between food and pathogen was recognized by

outbreak investigators even before the complex cycle of vertical transmission in the egg-laying hens and internal contamination of eggs was understood [22]. Even more dramatically, the first recognized outbreak of *E. coli* O157 infections heralded both the newly recognized foodborne pathogen and what turned out to be its predominant food vehicle, ground beef [23]. Although subsequent outbreaks added many other foods to the list, particularly fresh produce items, ground beef continued to be a leading source of foodborne *E. coli* O157 outbreaks [24]. As seen through these examples, the progression of public health system awareness follows a consistent pattern: following initial outbreak investigations that demonstrate that a particular transmission pathway is possible, repeated investigations lead to an acceptance that it occurs, and then to an expectation that it occurs.

The public health system has now reached this same expectation stage with respect to foodborne outbreaks from fresh produce. Fresh produce is routinely considered to be a possible source of foodborne outbreaks caused by a variety of pathogens. In fact, several specific pathogen–food combinations have emerged in recurrent outbreaks – salmonellosis from melons [25], tomatoes [18, 26], and several varieties of sprouts [27]; *E. coli* O157 infections from leafy green vegetables [6]; *Cyclospora* spread by raspberries [28]; hepatitis A infections by green onions [29]. The food vehicle in the first outbreak for each of these produce–pathogen pairs was novel at the time and establishing the link was sometimes a difficult exercise; subsequent similar outbreaks confirmed the food–pathogen pairing. These food–pathogen pairs may yet shed more light on the mechanisms and routes of contamination. Outbreaks due to the same produce item from different growing areas, such as salmonellosis due to melons grown in Mexico [8] and Australia [30] suggest that the problem is probably related to common conditions in the growing environment or undefined peculiarities of plant–pathogen biology. Recurrent outbreaks from produce grown in the same area, such as infections with the same strain of *Salmonella* Newport traced to tomatoes from the same growing region in the USA [31], suggests these ecological conditions may persist over time.

Although fresh produce is now a well-recognized outbreak food vehicle, many challenges remain in the investigation of such outbreaks. Produce in the local market is often globally sourced and can be widely distributed from a central production area. Contamination of these items may lead to widely dispersed cases and outbreaks that are difficult to detect. Pathogen subtyping in routine enteric disease surveillance improves recognition of these outbreaks, as in the recent outbreaks due to contaminated tomatoes in the USA [17, 31]. However, this practice requires an expansion of chronically scarce public health resources and national and international subtyping networks that are still developing [32]. Subtyping methods may similarly illuminate the epidemiology of norovirus, the most common cause of foodborne outbreaks in the USA [33]. Foodborne norovirus outbreaks are often attributed to contamination in the final kitchen, altthough subtyping systematically applied, may in the future connect outbreaks and isolated cases to more remote sources of contamination. The short shelf life, rapid distribution, and consumption of most produce along with the intrinsic time delays in outbreak recognition, investigation, and traceback limit opportunities to prevent further outbreak-related illness. While field investigations of the outbreak source can be daunting, these outbreaks represent major opportunities to learn what went wrong and how to prevent the next outbreak. Harvest is often finished by the time the outbreak is even recognized, much less by the time the harvest site is identified. The multi-disciplinary nature of the problem, limited jurisdiction by food safety regulators, and the lack of established procedures for a non-regulatory, multi-disciplinary investigation further hinders field work that could result in practical control measures. Nevertheless, any insights gained in the field that contribute to control efforts are of high potential yield. Since we eat much produce fresh, without cooking, and the effect of washing contaminated produce appears to be weak [34], prevention of contamination is paramount to control efforts.

BIOLOGY AND ECOLOGY OF CONTAMINATION

While fresh produce can become contaminated at any point in the chain of food production, there are often few intervening steps between farm and table. The likelihood of contamination is highest during three periods: in the field, during initial processing, and during the final preparation in the kitchen. Early contamination may come from wild animals that may contaminate fields or processing sheds, from farm workers without access to latrines or handwashing

stations, and from the water used to irrigate or spray fungicides on the plants. During processing, it may come from contaminated water used for washing, chill tanks or sprays and shipping ice. Late contamination in the restaurant or home kitchen may occur if produce is prepared with unclean implements, if surfaces and hands are also used to prepare raw meat or poultry, through cross contamination during storage, or if an infected foodhandler with poor hygiene is shedding the pathogen as food is prepared.

Recent work by plant pathologists and food microbiologists indicates that the connections between foodborne bacterial pathogens and produce may be more complicated than simple passive transfer [35]. Although these organisms are well adapted to life in the vertebrate gut, they can also survive and flourish on and in plants. *Salmonella* applied to leaves of young coriander (cilantro) plants grow rapidly to take up 80% of the carrying capacity of the leaf surface and then persist indefinitely in greenhouse conditions [36]. Similarly, *Salmonella* can grow to high densities on the surface of tomatoes, and then persist there for weeks [37]. Although *Campylobacter* will not survive on leaves, where exposure to the atmosphere inactivates them, they will persist for at least 4 weeks in the root zone [38]. *Salmonella* and *E. coli* may persist on or in alfalfa and mung seeds indefinitely, and then rapidly grow to high counts in the warm and moist conditions used to convert them to alfalfa or bean sprouts [39, 40].

The bacterial pathogens can also reach the interior of the plants by a variety of routes. Once the pathogen is inside, it is not affected by surface washing or disinfection. The pathways of internalization can be simple. Bacteria can move with water by capillary action from the stem scar or the calyx of an apple into the core [41]. They can enter through wounds or bruises in the surface of a fruit or leaf [42]. They can enter plants through the roots following experimental flooding with contaminated water. For example, in experimental greenhouse settings, *E. coli* O157 present at high levels in irrigation water is taken up by mature lettuce, and *Salmonella* of certain serotypes is taken up by young tomato plants; in both cases the concentrations of the pathogen in above-ground plant tissues can reach 10^3 c.f.u./g. When alfalfa seeds contaminated with *E. coli* O157 or with *Salmonella* are sprouted, the bacteria enter the growing sprout, and appear throughout the deep tissues of the young plant, without causing it harm [43, 44]. Enteric bacteria can also ride along on another important part of the plant life cycle. After they are applied to the stamen of the tomato flower, some strains of *Salmonella* can be recovered from the internal tissues of the mature tomato a month later, suggesting that they can pass via the pollen tube and colonize the new fruit [45]. Although it has not been demonstrated, it is possible that enteric bacteria may in fact be able to persist in the complete plant life cycle, from seed to sprout to mature plant to fruit and seed again. It could also be that the capacity to contaminate the fruit or other edible tissues represents an ecological strategy for gaining access to the gut of another herbivore.

Although virtually all work has been done with bacterial pathogens, it may also be occurring with viruses. In one recent experiment, vaccine viral RNA was detected in the tissues of green onions, after they had been irrigated with killed hepatitis A vaccine virus [46].

The infected human is ultimately presumed to be the source of contamination for infections with norovirus, hepatitis A, *Shigella* and other pathogens with exclusively human reservoirs, and occasionally for other pathogens. Contamination may be direct, via unwashed faecally contaminated hands, or somewhat less direct. In norovirus infections, vomitus can be highly infectious. The persons who vomit may not scrupulously wash their hands, or the surrounding area; they also may not perceive themselves as ill, and thus not exclude themselves from working in the kitchen [33]. Pathogens from human reservoirs may be introduced before produce reaches the kitchen. Contamination of produce by human sewage around the time of harvest has been a suspected cause of several widespread outbreaks of hepatitis A infections [47].

Although the precise mechanism of contamination in most produce-related outbreaks remains unexplained, field research following outbreaks is starting to shed light on the complex ecology of the growing environment. For example, outbreak-related and other strains of *E. coli* O157:H7 have been isolated from water sources in the growing area found to be the likely source of several lettuce-related *E. coli* O157:H7 outbreaks [48]. An environmental investigation following a large spinach-related *E. coli* O157:H7 outbreak traced to the same growing region suggested that feral swine may play a role in contamination in the field [49]. Studies of the interactions of *Salmonella* and tomato plants indicate that some serovars are more likely to persist pre-harvest and to appear in the

fruit than others, suggesting type-specific adaptation to this niche may have occurred [50]. Investigation of the circumstances under which mangos became contaminated with *Salmonella* indicated that water baths used to rid the fruit of fruit-fly larvae were open to the environment and indifferently chlorinated, and thus easily contaminated with *Salmonella* from a variety of sources [51].

Contamination can be amplified by processing steps. Plunging a warm fruit or vegetable into a cold water bath causes the internal airspaces to contract, drawing water and associated contaminants into the fruit. This is the likely mechanism of contamination for the aforementioned outbreak of *Salmonella* infections traced to imported mangos, in which the mangoes were treated with hot water to kill fruit-fly larvae, and then rapidly chilled in cold water that may not have been disinfected; the same potential has been demonstrated for other produce with internal air spaces [52]. Indeed, because of the potential for contaminating tomatoes that way, monitoring temperature and chlorination of the water baths (in which warm tomatoes from the field may be placed) are key control points for the tomato industry [53].

Once the vegetable or fruit is cut, the nutrients in the juices are available to pathogens. This means that after produce is sliced, diced or shredded, contamination can lead to high pathogen counts. After an outbreak of shigellosis was traced to shredded lettuce, rapid growth of *Shigella sonnei* was documented in the lettuce held at room temperature [54]. Similarly, an outbreak of salmonellosis traced to pre-diced tomatoes led to documentation of rapid growth of *Salmonella* on the cut surfaces of tomatoes at room temperature, and ultimately to the United States Food and Drug Administration (USFDA) including cut tomatoes among the foods that require temperature control for safety in retail and food-service operations, as specified in the USFDA Food Code [55–57]. Similar growth has been shown for cut melons, for which the Food Code also specifies temperature control [58]. All of these produce items have a pH $>4{\cdot}0$ that permits the growth of *Salmonella*. In fact, any produce with pH favourable to bacterial multiplication may become inherently more hazardous once cut, and thus need particular care in handling and storing afterwards.

More needs to be learned about the behaviour of enteric pathogens in relation to raw produce. Are some types of tomatoes, lettuce or other produce more susceptible to internal contamination than others? What is the range for plant hosts to which our principal enteric pathogens may be adapted? What are the genetic determinants that permit some strains of *Salmonella* to invade tomatoes, but not others? Are there commensal bacteria or other microorganisms that can inhibit the uptake or survival of enteric bacteria in or on food plants? Can the risk of produce-related foodborne illness be reduced through better understanding of the microbial ecologies in which we produce our food?

KEYS TO PREVENTION

The lessons from numerous outbreaks are clear, despite the uncertainties regarding the biology of pathogens on produce. Contamination cannot be washed off. Produce items that will not be cooked should be considered 'ready to eat'. Prevention of contamination in the first place is vital. In the lexicon of Hazard Analysis and Critical Control Point systems (HACCP), prevention of contamination of fresh produce is a critical control point because once contamination occurs there are at present no points during the processing, distribution and service of fresh produce at which microbiological hazards can be effectively abated [59].

Following the occurrence of numerous fresh juice-associated outbreaks in the USA, the USFDA implemented a juice-HACCP rule. The rule required juice producers to apply interventions capable of producing a 5-log reduction of pathogens such as *Salmonella* or *E. coli* O157:H7, but left open the choice of methods. Implementation of the juice-HACCP rule has reduced the occurrence of juice-associated outbreaks in the USA [60].

A series of guidance documents issued by USFDA deal with the more general problems of production of fresh produce, to Minimize Microbial Food Safety Hazards for Fresh Fruits and Vegetables [61], and to Enhance Safety of Sprouts [62], and Minimize Microbial Food Safety Hazards of Fresh-cut Fruits and Vegetables [63]. These documents promote good agricultural practices for production and good manufacturing practices for processing, using the information that is currently available, but do not include prescriptive regulations and mandatory pathogen reduction steps represented by the juice-HACCP rule.

Privately, major restaurant chains are working with suppliers to implement performance standards to ensure rigorous compliance with good agricultural

practices. These create strong financial incentives for compliance that may compensate for lack of regulatory prescription for that segment of the market. The development of egg quality assurance programmes in both the USA and UK has helped to prevent egg-associated SE outbreaks and reduce the incidence of egg-associated infections [64]. Similarly, when the United States Department of Agriculture (USDA) required all ground-beef producers in the USA to consider *E. coli* O157:H7 a hazard that was reasonably likely to occur and to revise their ground-beef HACCP plans accordingly, the contamination rate of *E. coli* O157:H7 in ground-beef products sampled by USDA fell by 80% and the incidence of *E. coli* O157:H7 infections was cut almost by half [65, 66]. Reducing the burden of produce-associated illnesses will almost certainly require some combination of regulatory oversight and industry incentives. However, a better understanding of the risks and benefits of specific practices are needed to guide the development of these interventions.

To facilitate this, more detailed and timely outbreak investigations are needed to identify production sources for contaminated fresh produce, and to facilitate more thorough environmental and ecological assessments of the contamination events. Whenever possible, outbreak investigations need to include rapid and detailed traceback and exposure assessments so that likely sources of contamination can be identified as far back as the field of production. Assessment of production variables such as field locations and surroundings, use of irrigation and harvesting techniques can improve our understanding of these events, and thus help to develop more effective prevention methods, on-farm and later in the food production chain.

Given our current understanding, improving the prevention of fresh-produce-associated outbreaks will require attention to the five following areas, wherever that produce is grown, processed, transported or prepared for eating:

(1) The quality of water. Water used to apply pesticides to plants, and for post-harvest cooling and processing can transfer microbes directly to the produce, unless the water is treated to drinking-water standards. Even though the expense of water treatment may represent a challenge to many agricultural production areas, the dependence of fresh produce on water and the efficiency with which contaminated water can serve as a vehicle for contaminating fresh produce makes this a critical safety issue for the production of these 'ready-to-eat' foods. Water used for irrigation may also be a source of contamination, particularly if it is contaminated surface water and if during irrigation it comes in contact with the edible portions of the plant.

(2) Protection from faecal contamination. Fresh produce can be easily contaminated in the field by direct and indirect contact with farm animal manure, wild animal faeces and human faeces. The cutting of plant tissues at harvest increases the likelihood of internalization of pathogens from contamination of the cut surfaces.

(3) Washing and sanitizing fresh produce. Currently available washing and sanitizing agents can reduce the levels of surface contamination of raw and processed fresh produce items, and therefore, can help reduce the likelihood that large focal outbreaks may be associated with specific contamination events. Even so, better sanitizing methods are needed to penetrate biological barriers that shelter pathogens in plant materials; the use of irradiation as a pasteurizing method for fresh produce is a possible solution.

(4) Management of the cold storage and supply chain. Refrigeration of cut produce items that are not in the process of being served can reduce the risk of bacterial amplification on the cut surfaces.

(5) Protecting fresh produce items from contamination by foodhandlers who themselves are ill or infected with the pathogen. While infected food workers are a primary source for contamination with norovirus, hepatitis A virus and *Shigella*, they can also be an important source for contamination with *Salmonella* in commercial food-service settings.

ACKNOWLEDGEMENTS

The findings and conclusions in this report have not been formally disseminated by the Centers for Disease Control and Prevention and do not necessarily constitute official policy of the agency (for authors M. Lynch and R. Tauxe).

DECLARATION OF INTEREST

None.

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