

Animal and Human Waste as Vehicles for Cross-Contamination of Imported Foods

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Charles P. Gerba and Christopher A. Scott

INTRODUCTION

This chapter focuses on animal and human wastes as potential sources of contamination of imported foods. Global trade of food, in particular, fresh fruit, vegetables, and seafood, has risen dramatically since the 1980s (Huang, 2004); however, not without concern for food safety. Most food exports to the United States ($\approx 80\%$) are regulated by the U.S. Food and Drug Administration. Produce has been a vehicle for the importation of infectious disease into the United States, and the number of outbreaks associated with produce in the United States is on the increase (Sivapalasingam et al., 2004). Public awareness of food safety and potential health risks has increased dramatically, largely based on recent reports of contaminated seafood imported to the United States and pathogen outbreaks associated with domestically produced fresh-cut leafy greens; these public health issues have led to import bans or lawsuits against producers.

The amount of animal and human feces generated on a worldwide basis is enormous. Humans generate 900 billion pounds a year and livestock more than 24 trillion pounds (World Bank, 2005). Exposure to feces may occur by the use of wastes for fertilization of a crop, the use of contaminated water in the production of a product (e.g., irrigation water or water used to produce shellfish), or human handling during harvesting or in the processing of a product. The use of human wastes or wastewater for produce production in developed countries is not allowed or is strictly regulated because of the potential for transmission of enteric pathogens. Although use of highly treated

CHARLES P. GERBA, Department of Soil, Water and Environmental Science, University of Arizona, Tucson, AZ 85721. CHRISTOPHER A. SCOTT, Department of Geography and Regional Development, and Utdall Center for Studies in Public Policy, University of Arizona, Tucson, AZ 85721.

domestic wastewater for food crops to be eaten raw is allowed in some states in the United States, it is seldom practiced because of potential health hazards. The same is true for sewage sludge (biosolids). In the United States, more than half of all biosolids is recycled by application to agricultural land. However, almost the entire amount goes to land where food crops are not grown. Animal wastes have been used for a long time in food crop production; however, such waste is often composted before utilization. In contrast, irrigation with sewage in the developing world is commonly practiced, especially in water-scarce areas (Scott et al., 2004). Direct use of human wastes as a fertilizer is a centuries-old practice in China. Also, much of the world still discharges raw or incompletely treated sewage into rivers and other bodies of water that could be used for irrigation, washing of produce, or growing shellfish.

Contamination of food crops may also occur during human handling of crops before harvest (e.g., turning of cantaloupe to prevent damage to the rind), during harvesting, and during processing. Use of contaminated water during washing or cooling may be an additional source of exposure.

PATHOGENS TRANSMITTED

Domestic animals and humans share many common enteric bacteria, protozoan and worms, which can be easily transmitted between species. In contrast, enteric viruses are usually host specific and are not transmitted between species. The only enteric virus known to be transmitted between nonprimates is hepatitis E virus. The virus infects both man and swine and has been shown to be food-borne (Li et al., 2005). Some respiratory viruses have the potential to be transmitted by water via human or animal waste. For example, influenza virus is present in animal wastes (e.g., it is excreted in the feces of birds) and has the potential to be transmitted by foods but has not yet received serious investigation. Table 1 lists enteric zoonotic pathogens that potentially can be transmitted through the environment. Emerging pathogens continue to be recognized, and new ones are added to the list of food-borne risk factors almost annually.

Only some enteric bacteria, not viruses or protozoa, have the potential to grow in the environment or in/on foods. However, even the enteric bacteria have a limited existence in the environment outside of an animal. Of the enteric bacteria, *Salmonella*, *Campylobacter*, and *Escherichia coli* O157:H7 present the greatest problem because they infect a wide variety of animals besides humans. *Shigella* spp. are only known to infect humans and some primates; thus, they are usually only present in human wastes.

Cryptosporidium and *Giardia* present the greatest problem among the protozoan parasites and are a common cause of gastroenteritis worldwide. Cattle are

Table 1 Enteric bacteria, protozoa, microsporidia, and helminthes that infect animals and humans

Group	Pathogen	Disease or condition
Bacteria	<i>Salmonella</i>	Gastroenteritis, typhoid fever
	<i>Campylobacter</i>	Gastroenteritis
	<i>Vibrio cholerae</i>	Gastroenteritis
	<i>Yersinia enterocolitica</i>	Gastroenteritis
	<i>Escherichia coli</i> (certain strains)	Gastroenteritis
Protozoa	<i>Giardia intestinalis</i>	Gastroenteritis
	<i>Cryptosporidium</i>	Gastroenteritis
	<i>Enterocytozoon bieneusi</i>	Gastroenteritis in immunocompromised person
Microsporidia	<i>Acariis bambusivoides</i>	Ascariasis
	<i>Necator americanus</i>	Hookworm
Helminthes	<i>Tania saginata</i>	Tapeworm (from beef)

a major source of *Cryptosporidium* worldwide, while humans appear to be the major source of *Giardia intestinalis*. Foods imported into the United States and other developed countries have been a source of the protozoan parasite *Cyclospora* (Ho et al., 2002; Hoang et al., 2005). At present, it is not known whether this parasite is limited to humans or has an animal reservoir. *Entamoeba histolytica* is a protozoan parasite that only infects humans (Table 2), while microsporidia only appear to cause serious infections in the immunocompromised; their role in infection of healthy persons is currently unknown.

Human enteric noroviruses are believed to be the major cause of food-borne illnesses in developed countries (Gerba and Kaye, 2003). Noroviruses cause an estimated 67% of all food-borne illnesses each year in the United States (Mead et al., 1999). Hepatitis A virus has been the cause of shellfish- and

Table 2 Enteric viruses, bacteria, and protozoa that infect only humans

Group	Pathogen(s)	Disease or condition
Viruses	Enteroviruses	Meningitis, paralysis, rash, fever, heart disease, respiratory disease
	Hepatitis A	Hepatitis
	Rotaviruses	Gastroenteritis
	Noroviruses	Gastroenteritis
	Adenoviruses	Gastroenteritis, respiratory illness, eye infections
Bacteria	Astroviruses	Gastroenteritis
	<i>Shigella</i> spp.	Gastroenteritis
Parasites	<i>Entamoeba histolytica</i>	Gastroenteritis

Table 3 Occurrence of enteric pathogens in human feces in the United States*

Pathogen	Incidence (%)	Concn in stool (per gram)
Enteroviruses	10	10 ³ -10 ⁸
Hepatitis A	0.1	10 ⁸
Rotavirus	10-29	10 ⁹ -10 ¹²
<i>Giardia</i>	3.8	10 ⁶
<i>Cryptosporidium</i>	0.6-20	10 ⁶ -10 ⁷

* Source: Gerba, 2000.

imported produce-associated outbreaks in the United States (Sanchez et al., 2007). Rotavirus is the most common cause of childhood gastroenteritis worldwide, but large water-borne (Gerba et al., 1996) and food-borne outbreaks (Centers for Disease Control and Prevention [CDC], 2000) also have been documented in adults. Enteroviruses have been less commonly associated with food- and water-borne outbreaks, probably because the wide variety of illnesses they are capable of causing (Table 2) makes it difficult to identify them as the causative agents.

OCCURRENCE OF PATHOGENS

The occurrence of enteric pathogens in the environment is a reflection of the incidence of infection by the pathogen in a population. In general, the greater the incidence of infection, the greater the occurrence of a particular pathogen in the environment. Enteric pathogens can be excreted in large numbers in the feces of infected humans and animals (Table 3), whether or not the infected individual exhibits the symptoms of clinical illness. Infected individuals may continue to excrete pathogens for many weeks to months after infection—long after clinical signs of illness have passed. The number of asymptomatic infected individuals will vary depending on the virulence of the organism, immune state of the individual, species of host, and age. For example, in the case of hepatitis A virus, only 5% of infected children develop clinical disease, whereas 95% of the nonimmune adults will develop clinical illness (Cuthbert, 2001).

Pathogen concentrations may also vary with the type of wastewater. For example, various studies have indicated that *E. coli* O157:H7 is commonly present in animal and human wastewaters at levels of 10 to 100/100 ml of municipal sewage and 100 to 1,000/100 ml of animal wastewaters and wastewaters from slaughterhouses (Muniesa et al., 2006).

Human Wastes

Feces

Humans excrete between 150 to 400 g of feces per day, depending on diet (Feachem et al., 1983). Infected persons may excrete large numbers of pathogens in their feces (Table 3). Viruses are generally excreted in greater concentrations than bacteria and parasites. Rotaviruses are excreted in concentrations as great as 10¹²/g or more than 10¹⁴/day. The incidence of persons excreting viruses in the United States varies greatly with age group and season, but 10 to 40% of children may be excreting viruses at any point in time (Gerba, 2000). The prevalence of enteric pathogen excretion is much greater in developing countries because of much greater infection rates. Lack of adequate sanitation facilities and poor hygienic practices, in general, in developing countries compound the risk of exposure and reinfection, which are already high because of elevated pathogen concentrations in excreta.

Sewage

Because some persons in a population are always infected, enteric pathogens are always present in raw domestic sewage. The concentration will vary depending on several important factors:

- Incidence of infection within the community
- Public health status within the community
- Season
- Social-economic conditions (in developing countries this may reflect access to pathogen-contaminated water and sanitation, whereas in developed countries this may be a reflection of access to health care)
- Per capita water use

Because of these factors, the concentration of pathogens in raw sewage is much greater in developing countries, a factor that is often overlooked when assessing the risks from the use of wastewater in irrigation of food crops (Blumenthal et al., 2000, 2001; Ensink et al., 2004). The concentrations of pathogens detected in raw sewage are shown in Table 4. In developing countries, the concentration of enteric pathogens is 100 times that reported in developed countries, e.g., 1,000 versus 100,000 per liter (Buras, 1976). In Brazil, the average concentration of rotavirus is 11,300 per liter and as great as 26,600 per liter (Oraguí, 2003). In contrast, the concentration of rotavirus in domestic sewage in the United States ranges from 1 to 321 per liter (Hejkal et al., 1984). This is an important consideration because the ratio of fecal indicator bacteria (e.g., *E. coli*) may be much different in a developing country versus a developed country. Thus, the risk of exposure to enteric pathogens with a given concentration of fecal indicator bacteria is greater in a developing country than in

Table 4 Concentration of enteric pathogens in domestic sewage in developed versus developing countries^a

Microbe	Concn per liter	
	Developed countries	Developing countries
Enteric viruses	10 ³	10 ⁴ -10 ⁵
<i>Giardia</i>	10 ¹ -10 ³	10 ² -10 ⁵
<i>Cryptosporidium</i>	10-10 ²	10-10 ³

^aSource: Buras, 1976; Feachem et al., 1983; Jimenez et al., 2002; Smith and Gimson, 2003.

developed countries. This is true of environmental health conditions, in general, in developing countries, i.e., elevated pathogen concentrations not just in sewage, but in water supplies, on fruits and vegetables, and in soils (Carr et al., 2004)—a factor recognized in the *Stockholm Framework* that informs the 2006 World Health Organization wastewater guidelines (WHO, 2006). The *Stockholm Framework* seeks to manage health risks from all water-related microbial exposures and encourages flexibility to adapt and apply guidelines based on local conditions (social, cultural, economic, and environmental).

While treatment of sewage is primarily designed to reduce the amount of biodegradable organic matter, major reductions of pathogens also occur. Because of their large size, helminthes are reduced in large numbers just by settling. Activated sludge is the most common method used for the treatment of domestic sewage in developed countries and is increasing in use in developing countries. In this process, bacteria are most effectively removed, followed by enteric viruses and protozoan parasites (Table 5). Chlorination as commonly practiced can result in significant reduction of bacterial

Table 5 Removal and estimated concentration of enteric pathogens after treatment of domestic sewage^a

Parameter	Enteric viruses	<i>Salmonella</i>	<i>Giardia</i>
Concn in raw sewage per liter	1,000-100,000	5,000-200,000	9,000-200,000
Primary treatment			
% removal ^b	50-98	96-99.8	27-64
No. remaining/ liter	170-50,000	160-3,360	72,000-146,000
Activated sludge			
% removal	53-99.9	98-99.996	45-96.7
No. remaining/ liter	8-47,000	3-1075	6,480-109,500
After chlorination			
% removal	90-96	99.9-99.99	0-20
No. remaining/ liter	1-4,700	3 × 10 ⁻¹ -1	5,184-87,600

^aSource: Maier et al., 2000.

pathogens but has only a nominal effect on the more resistant viruses and protozoa. Ultraviolet light disinfection is very effective against enteric bacteria and protozoa, but some viruses (adenoviruses) are extremely resistant (Meng and Gerba, 1996).

Waste stabilization ponds (oxidation ditches, stabilization lagoons) are often used to treat wastes in the developing world and are still used for small systems in the United States. They are commonly used in tropical and subtropical regions, where the warm climate results in increased performance and lower land area requirements. They have been promoted as a low-cost method of sewage treatment and means to reduce the concentration of pathogens. However, performance is based on residence time, climatic conditions (amount of sunlight), and minimization of short circuiting. Ponds are especially good at reducing the concentrations of helminthes and protozoan pathogens because they are large enough to settle in large numbers (Mara, 2002). In properly operated ponds, reduction of 99.9% or more of the bacteria and viral pathogens can be achieved (Mara, 2002; Oragui, 2003). Even well-operated ponds do not produce pathogen-free wastewater, and the wastewater is of lower quality than that produced by activated sludge sewage treatment plants.

Activated sludge treatment generates large amounts of sludge or biosolids, which must be either disposed of or recycled. Biosolids contain substantial levels of enteric pathogens. The larger helminthes ova settle out during activated sludge treatment and the viruses tend to adsorb to the biological floc that forms during this process. More than half of the biosolids generated in the United States is recycled through application to agricultural land. Sewage sludge is classified into two types, depending on the concentration of pathogens. Class B biosolids contain detectable levels of pathogens; food crops that may be eaten raw cannot be grown on land to which Class B solids have been applied for two years to allow for the die-off of ascaris ova. Class A biosolids are those that receive additional treatment to eliminate pathogens and can be used on food crops. Such processes include thermophilic anaerobic digestion, heating to high temperatures, composting, prolonged storage, and treatment at high pH (pH 12). These processes must reduce the fecal coliform levels to less than 1,000/g of dry solids, and no detectable enteric viruses, *Salmonella*, or viable *Aeraris* ova in 4 g of dry solids (Environmental Protection Agency [USEPA], 1993). Most developed countries have similar restrictions for the use of sewage sludge. Activated sludge treatment is becoming more common in some developing countries, and it has many benefits to agricultural production. However, the concentration of pathogens in sewage sludge generated in developing countries is much greater than in sewage sludge from developed countries (Table 6; Jimenez et al., 2002). Hence, sewage

Table 6 Concentration of enteric pathogens in untreated (raw) sewage sludge (biosolids) in the United States and Mexico^{a,d}

Type	Microbe	United States	Mexico
Virus	Enteric viruses	300	No data
Bacteria	<i>Salmonella</i>	2,800	10 ⁶ -10 ⁸
Protozoa	<i>Giardia</i>	10-1,000	10 ² -10 ⁴
Helminths	<i>Ascaris</i>	<1-9.7	66-136
	<i>Toxocara</i>	1-5	0.3-1.2

^aConcentration per gram of diked solids.^dSource: Straub et al., 1993; Jimenez et al., 2002.

sludge from developing countries will require additional treatment before it can be safely reused.

Animal Wastes

Grazing systems are estimated to cover 50% of all the agricultural land in the world (Gannon et al., 2004b). Hence, it is not surprising that zoonotic pathogens occur in most areas of the world where there is agricultural production. Animal wastes and effluents from farming operations, including manures and slurries, are frequently applied to increase the soil fertility of cropland. Animal manures have been used for centuries as a fertilizer and soil enhancer. Enteric bacterial pathogens have been the microbes most commonly associated with produce outbreaks, although protozoan parasites have also been responsible for produce-associated outbreaks. While hepatitis E virus is shed in feces by pigs (Kasornrorkbua et al., 2005) and can be transmitted through raw or uncooked meats (Li et al., 2005), other human enteric viruses are not currently considered to be zoonotic. The recent finding of human norovirus genotypes in swine and cattle feces, on the other hand, suggests that animal feces could be involved in their transmission (Mattison et al., 2007). Like human feces, animal wastes can contain high concentrations of human enteric pathogens (>10⁶/g, Tables 7 and 8) (Gannon et al., 2004b). Young animals are often more likely than adult animals to shed pathogens, and at higher levels. For example, *E. coli* O157:H7 is shed in calves at concentrations from 10² to 10⁶ per gram of feces, but less commonly isolated from adults (Gannon et al., 2004b). However, recent research suggests that a small number (2 to 7%) of the adult cattle population may be supershedders, excreting large numbers of *E. coli* O157:H7 (Omskin et al., 2003; Mathews et al., 2006). *Cryptosporidium parvum*, which also infects humans, in England was found to infect 50% of farmed calves and was excreted at 10⁵/g (Sturdee et al., 2003). The relative occurrence of human enteric pathogens in the feces of animals in the developing world appears to have received little attention.

Table 7 Percentage of Great Britain livestock manures contaminated with zoonotic microbes^a

Pathogen	% Contamination							
	Cattle		Swine		Poultry		Sheep	
	Fresh ^b	Stored ^c	Fresh	Stored	Fresh	Stored	Fresh	Stored
<i>E. coli</i> O157:H7	13.2	9.1	11.9	15.5	ND ^d	ND	20.8	22.2
<i>Salmonella</i>	7.7	10.0	7.9	5.2	17.9	11.5	8.3	11.1
<i>Listeria</i>	29.8	31.0	19.8	19.0	19.4	15.4	29.2	44.4
<i>Campylobacter</i>	12.8	9.8	13.5	10.3	19.4	7.7	20.8	11.1

^aSource: Hutchison et al., 2004.^bFresh, collected from location in which deposited^cStored, collected from lagoon or farm yard manure heap^dND, not determined

The closer association of humans with animals in the developing world generates more opportunities for transfer of viruses between livestock and humans with closely related pathogens (Kang et al., 2005).

Properly treated manure is generally a safe fertilizer, but improperly treated manure may contain pathogens that can contaminate fresh produce. The high temperature and production of ammonia play a critical role in the

Table 8 Cell numbers of zoonotic pathogens in British livestock manures^a

Pathogen	No. of cells (CFU/g) of pathogen							
	Cattle		Swine		Poultry		Sheep	
	Fresh ^b	Stored ^c	Fresh	Stored	Fresh	Stored	Fresh	Stored
<i>E. coli</i> O157								
Geo mean	1 × 10 ³	3 × 10 ²	4 × 10 ³	1 × 10 ³	ND ^d	ND	8 × 10 ²	3 × 10 ²
Max	3 × 10 ⁵	8 × 10 ⁴	8 × 10 ⁵	2 × 10 ⁴	ND	ND	5 × 10 ⁴	5 × 10 ³
<i>Salmonella</i>								
Geo mean	2 × 10 ³	3 × 10 ²	6 × 10 ²	6 × 10 ²	2 × 10 ²	4 × 10 ²	7 × 10 ²	6 × 10 ³
Max	6 × 10 ⁵	7 × 10 ⁶	8 × 10 ⁴	2 × 10 ³	2 × 10 ⁴	8 × 10 ³	2 × 10 ³	6 × 10 ³
<i>Listeria</i>								
Geo mean	1 × 10 ³	1 × 10 ³	3 × 10 ³	6 × 10 ²	8 × 10 ²	3 × 10 ²	2 × 10 ²	3 × 10 ²
Max	6 × 10 ⁵	7 × 10 ⁶	8 × 10 ⁴	2 × 10 ³	2 × 10 ⁴	8 × 10 ³	2 × 10 ³	6 × 10 ³
<i>Campylobacter</i>								
Geo mean	3 × 10 ²	5 × 10 ²	3 × 10 ²	2 × 10 ³	3 × 10 ²	6 × 10 ²	4 × 10 ²	1 × 10 ²
Max	2 × 10 ⁵	2 × 10 ⁵	2 × 10 ⁴	1 × 10 ⁵	3 × 10 ⁴	9 × 10 ²	2 × 10 ³	1 × 10 ²

^aSource: Hutchison et al., 2000.^bFresh, collected from location in which deposited.^cStored, collected from lagoon or farm yard manure heap.^dND, not determined.

reduction of enteric pathogens during composting (Haug, 1993). However, proper composting and subsequent storage conditions are critical to the destruction of pathogens and control of regrowth of enteric pathogens (Zaleski et al., 2005).

Composting systems are usually divided into three categories: windrow, static pile, and in-vessel. Usually a bulking agent such as wood chips is added to create air spaces during composting processes (Haug, 1993). In the windrow system, the manure is composted in long rows aerated by convective air movement. The rows are turned periodically by mechanical means (specialized machinery or dump loaders, which may lead to recontamination). Turning exposes the organic matter to oxygen and ensures that all of the material is treated to a high enough temperature to kill pathogens. In a static pile (or forced aeration), piles of manure may be aerated by using a forced aeration system, which is installed under the piles to maintain a minimum oxygen level throughout the compost pile. In-vessel composting (also known as enclosed-reactor composting) takes place in partially or completely enclosed containers under controlled environmental conditions. In windrow composting, it is necessary for temperatures to be at least 131°F (55°C) for 15 days and for the piles to be turned over at least five times to ensure pathogen destruction. In static aerated piles or in-vessel composting, this temperature must be maintained for at least 3 days (USEPA, 1993).

Once composting is completed, there is the potential for regrowth of surviving *Salmonella* and *E. coli* if the moisture increases or contamination occurs from animals (e.g., birds) (Zaleski et al., 2005). Temperatures above 28°C have been favorable to the growth of *Salmonella* in compost. However, once compost was added to soil at typical agronomic rates (3 tons per acre), growth did not occur even when the *Salmonella* was added to the soil/compost in large numbers, probably because of competition or predation from native microflora (Campo et al., 2007).

Surface Waters

Any freshwater surface source is likely to contain enteric pathogens periodically. The risk of pathogen exposure depends on the use of water and the pathogen concentration in the water at the time of utilization. The greatest levels of pathogens occur in water sources receiving discharges of untreated sewage and in watersheds with intensive levels of animal production. In the United States, sewage discharges are usually disinfected. Storm water runoff from agricultural land and septic tanks (referred to as nonpoint sources) is the largest contributor of pathogens to surface water. In Europe and much of the rest of the world, treated sewage discharges are not usually disinfected. Hence, human enteric viruses can be isolated from almost every major river in Europe

(Bosch et al., 2006). Untreated or partially treated sewage is still discharged into surface waters in much of the world, and isolation of enteric pathogens is common in surface waters in developing countries.

The greatest loading of pathogens occurs in surface waters after rainfall events, although concentrations may be elevated during low flow when the proportion of wastewater to natural runoff is highest. During runoff events, accumulated feces are washed into near-by streams or collection systems forcing release of partially or untreated sewage from wastewater treatment plants. Studies of water-borne disease outbreaks in the United States and Canada have correlated above-average rainfall events with drinking water-associated disease outbreaks (Curriero et al., 2001; Thomas et al., 2006). For these reasons, gastroenteritis is usually more common in the rainy season in developing countries.

Use of contaminated water for pesticides may also serve as another mechanism of produce contamination. Guan et al. (2005) found that *Salmonella* and *E. coli* O157:H7 could grow in various pesticide solutions and contaminate tomato plants when they are applied as a spray.

Ground Waters

In general, enteric pathogens are far less common in groundwater because of the natural filtering mechanism of soil. However, every groundwater source is potentially susceptible to contamination. The construction of a well (or location of a spring), nature of the substrata, depth to groundwater, and rainfall can affect the microbiologic quality of the well water. Even in the United States, half of all drinking water-associated disease outbreaks reported annually are caused by contaminated groundwater (Reynolds et al., 2007). Pathogens enter groundwater from latrines, septic tank leach fields, land application of wastewater for irrigation, oxidation ponds, leaking sewer lines, and unlined landfills. Unprotected wells may allow for surface water to run into the well during storm events. In well-structured soils, helminthes ova, protozoa, and bacteria are easily filtered out during transport through the soil. However, in fractured limestone and clay soils, long-distance transport is possible. Viruses are more likely to contaminate groundwater and travel long distances (hundreds of meters) because of their small size. Microorganisms are biocolloids that behave as particulates in their transport in the subsurface; hence, their movement may be much different than solutes, depending on the nature of the subsurface matrix. Their transport in fractured substrata may be 100 times faster than solutes such as nitrates (Mckay et al., 1993). Hence, lack of chemical contamination does not ensure the absence of enteric pathogens. The potential for groundwater contamination by enteric viruses should not be underestimated. In a survey of more than 400 utility drinking water wells across the United

States, enteric viruses were detected in 32%. Moreover, indicator bacteria were not found to be useful indicators of virus contamination (Abbaszadegan et al., 2003).

Irrigation Waters

Irrigation using surface and ground waters mirrors similar water quality and pathogen concentrations, as discussed in the preceding two sections. With growing urban populations that have increased sewerage collection, although often without sewage treatment, irrigation water used downstream of large urban centers has high pathogen loads (Scott et al., 2004). Efforts to undertake a global assessment of irrigation with wastewater (van der Hoek, 2004) have been difficult, given the lack of national-level data and the need to examine a very large number of localities.

Studies are limited on the occurrence of pathogens in irrigation water impacted by nondirect or purposeful reuse of sewage in both developed and developing countries. In a study of irrigation waters in several Central American countries and the United States, the protozoan parasites *Giardia*, *Cryptosporidium*, and microsporidia were detected (Thurston-Enriquez et al., 2002). *Giardia* concentrations were similar in almost all countries (60% of the samples), whereas *Cryptosporidium* concentrations were much greater in Central America. In a study in western Mexico, 48% of the surface irrigation waters were positive for *Cryptosporidium* oocysts and 50% for *Giardia* cysts (Chaidez et al., 2005). Both of these parasites have also been detected in irrigation water used for bean sprout irrigation in Norway (Robertson and Gjerd, 2001). *Salmonella* has been reported in 2 to 14% of the irrigation waters used for produce production in Nigeria (Okafo et al., 2003) and 23.5% of irrigation water used for cantaloupe production in Brazil (Espinoza-Medina et al., 2006). Both *E. coli* O157:H7 and *Salmonella* have been reported in irrigation waters in western Canada (Gannon et al., 2004a).

SURVIVAL OF PATHOGENS

Principal factors controlling the survival of enteric pathogens in the environment are temperature, moisture content, and exposure to sunlight. Knowledge of these factors enables the estimation of pathogen survival in the environment. Other factors influencing survival are type and strain of microbe, pH, organic matter, and antagonism by native microflora. Temperature is the major factor in all environments affecting survival. Moisture and desiccation are important factors in soils and on plant surfaces (Stine et al., 2005a). In surface waters, ultraviolet light in sunlight is a dominating factor. Regrowth of

Table 9 Die-off rates of enteric pathogens in the environment^a

Type	Organism	Die-off (log ₁₀ /day)
Viruses	Enteroviruses	0.01–0.2
	Hepatitis A	0.01–0.2
Bacteria	<i>Salmonella</i> spp.	1–7
	<i>E. coli</i>	0.23–0.46
Protozoa	<i>Giardia</i>	0.023–0.23
	<i>Cryptosporidium</i>	0.0057–0.046

^aSource: Madena et al., 2003.

bacterial pathogens is possible in aquatic sediments, feces, manure, compost, and plant surfaces (Zaleski et al., 2005).

Most enteric pathogens can survive for at least a few days in the environment, but under the right conditions they may persist for years. *Ascaris* ova may survive for as long as two years in soil (Gerba and Smith, 2005). Enteric viruses and protozoan parasites are also capable of prolonged survival, especially at low temperatures (10°C or less), and may survive for many months (Table 9). Because of its resistance to inactivation at elevated temperatures, hepatitis A virus is likely among the longest surviving of the enteric viruses (Stine et al., 2005a). Enteric bacterial pathogens generally have the shortest survival time (days to weeks), although unlike the other enteric pathogens, they do have the ability to persist and increase in number under the right environmental conditions (largely temperature and nutrient dependent). As a general rule, survival of enteric pathogens is in the following order: soil > plants > water (Gerba and Smith, 2005; Stine et al., 2005b).

Soil

Enteric pathogen survival depends on both the type of microbe and the type of soil (Hurst et al., 1980). Die-off will be much greater in warm tropical conditions than in cool damp climates. Enteric viruses generally survive several weeks to months, depending on soil temperature. Helminthes, in particular, can survive up to years in the soil (Gerba and Smith, 2005).

Plants

Few studies exist on the survival of enteric pathogens on produce preharvest. (Farral et al., 2004). *Salmonella* and *E. coli* may be capable of growth on produce surfaces under certain conditions (Stine et al., 2005a). Poliovirus was found to survive on lettuce after flood irrigation in outdoor plots (Tierney et al., 1977). The virus persisted on the lettuce for two months during the winter, but only 2 to 3 days in the summer. Stine et al. (2005a) found that

hepatitis A virus and the feline calicivirus survived better than enteric bacteria on cantaloupe, lettuce, and bell peppers. Little decrease of hepatitis A virus occurred during the two-week study period. Survival of enteric pathogens on produce is influenced by a number of factors, including competition from native microflora, humidity, sunlight, and temperature (Nyeleni et al., 2004; Stine et al., 2005a; Cooley et al., 2006).

Little inactivation of enteric pathogens postharvest is expected if they are chilled or refrigerated. Studies on the survival of enteric viruses on produce postharvest indicate that little virus inactivation occurs because of the low temperatures of storage (Seymour and Appleton, 2001).

ROUTES OF CROSS-CONTAMINATION

Irrigation Water

The largest use of freshwater in the world is in agriculture, with more than 70% being used for irrigation. Approximately 240 million ha, 17% of the world's cropland, are irrigated, producing one-third of the world's food supply (Shannan, 1998). Nearly 70% of this area is in developing countries. Irrigation of food crops with untreated domestic sewage has long been associated with the transmission of infectious diseases. As a result, the use of wastewater for irrigation is forbidden or the wastewater must be highly treated and rigorously monitored in developed countries. Irrigation with sewage or sewage-contaminated surface waters in developing countries is fairly common and usually not regulated. While guidelines for wastewater reuse have been developed by the World Health Organization (WHO, 2006), their application in developing countries will remain difficult, due to inadequate institutional capability and general lack of financial resources.

The likelihood of the edible parts of the plants becoming contaminated during irrigation depends on a number of factors, including:

- growing location of the edible portion of the produce, e.g., distance from the soil or water surface
- frequency of irrigation
- surface of the edible portion, i.e., smooth, rough, or webbed
- type of irrigation method, i.e., furrow or flood irrigation, sprinkler, or drip

If the edible part of the crop grows in or near the soil surface, it is more likely to become contaminated than fruit growing in the aerial parts of the plant. In other cases, some produce surfaces are furrowed or have structures that retain water (e.g., a pepper versus a cantaloupe) and hence are more likely to be contaminated.

Table 10 Irrigated area (1,000 ha) by type of system, 1993 to 1997 (unless otherwise noted)^a

Country	Surface	Sprinkler	Drip, spray, microirrigation
Chile	1,807.32	30.52	62.15
China	50,379.80	611.11	Not reported
India	49,330.00	700.00	71.00
Israel (1991)	Not reported	Not reported	104.30
Mexico	5,802.18	310.80	143.05
Spain (1988-1992)	Not reported	Not reported	160.00
USA (1988-1992)	14,002.14	9,226.84	606.00

^aSource: FAO, 2007.

There are four types of irrigation systems: sprinkler, gravity-flow (furrow), and microirrigation systems (surface drip and subsurface drip irrigation). The type of irrigation system greatly influences the degree of crop contamination that occurs during irrigation. Stine et al. (2005a, 2005b) and others (Gerba and Choi, 2006) have compared coliphage contamination of cantaloupe, iceberg lettuce, and bell peppers by various methods of irrigation. Virus transfer to the lettuce was 4.2%, 0.02%, and 0.00039% for spray, furrow, and drip irrigation, respectively.

Area estimates of the type of irrigation systems used in different countries are inconsistently reported or are not up to date. The period from 1993 to 1997 is the last time that countries with important irrigation and food-export sectors reported their areas by irrigation type, as shown in Table 10 (FAO, 2007). For the majority of those listed countries, sprinkler irrigation is greater than drip, spray, or microirrigation. However, with the exception of the United States, the combined area of nonsurface irrigation (furrow, border, etc.) is less than one percent of total irrigated area, an observation supported by Namara et al. (2007) for India, and Postel et al. (2001) worldwide. Despite the small total area, drip irrigation is growing rapidly and is currently estimated to cover 2.8 million ha worldwide (Postel et al., 2001). Within the United States, California leads the way with a total of 730,500 ha (1.81 million acres) under drip and micro-irrigation (Burt et al., 2001).

Wastewater

Standards for irrigation water quality

Most standards for the microbial quality of irrigation water have been developed for the use of treated wastewater. Few standards have been suggested for water derived from other sources. Based on results of a study of irrigation waters in the western United States in the late 1960s, Geldreich and Bordner (1971) suggested an irrigation standard of 1,000 fecal coliforms per 100 ml

based on the absence of *Salmonella* in irrigation waters, which had values below this level.

The World Health Organization recently revised its recommendations for the safe reuse of wastewater and greywater (WHO, 2006) by using a risk-based approach for development of treatment and microbial standards. The WHO guidelines for the safe use of wastewater in agriculture are based on a risk analysis approach and recommend treatment requirements for pathogen reduction, monitoring (verification of treatment performance), and management strategies. The level-of-protection goal is defined in terms of disability-adjusted life years (DALY), a measure which combines years of life lost by premature mortality with years lived with a disability, standardized or weighted by severity of illness (Pruss and Havelaar, 2001). The acceptable level of risk from consumption of pathogens on food is defined as 10⁻⁶ DALY (WHO, 2006). Based on this analysis, the minimum requirements for irrigation water for use on root crops are equal or less than 1,000 *E. coli* per 100 ml and zero helminth eggs per liter. This guideline is based on a wastewater treatment process that provides a 4-log (99.99%) reduction in pathogens (approximately equivalent to an *E. coli* of 1,000/100 ml in unchlorinated effluents), a 2-log pathogen reduction due to die-off between the last irrigation and consumption, and a 1-log reduction by washing of the salad crops or vegetables with water prior to consumption. The WHO believes this option provides the needed 7-log pathogen reduction for crops eaten uncooked. For options totally dependent on the treatment to remove pathogens (again a 7-log reduction) to the required level of acceptable risk, the *E. coli* level in irrigation water for crops eaten uncooked should be equal to or less than one *E. coli* per 100 ml.

Unfortunately, many assumptions on the ratio of *E. coli* to pathogens were made in the risk analysis because of the lack of data on pathogens in wastewater in developing countries. In addition, the reliability of the treatment has to be considered in the assessment of risks. Even treatment plants designed to produce high-quality reclaimed wastewater in developed countries like the United States do not produce pathogen-free effluents at all times, because of the occurrence of suboptimal operation of various processes in the treatment train. Even short-term (hours), suboptimal operation can result in the occurrence of pathogens in the treated wastewater. In a study of the quality of reclaimed wastewater in California, Tanaka et al. (1998) found that the processes in use were not reliable enough to recommend the treated wastewater for unrestricted irrigation of food crops to be eaten raw. Finally, some wastewater treatment processes may be very effective at removing *E. coli*, but very inefficient in removing other groups of pathogens. For example, chlorine disinfection is very good at reducing the levels of *E. coli* but has

little effect on *Cryptosporidium* oocysts when chlorination is applied at conventional levels. Management of risks from wastewater irrigation must take all of these factors into consideration when developing guidance for wastewater used in agriculture.

Use of wastewater irrigation in the developing world

How common wastewater irrigation is in the developing world is not known with certainty, but it has been estimated that 20 million ha of crops are irrigated with raw or diluted sewage (Scott et al., 2004). The World Health Organization estimates that 10% of the world's population consumes food produced by irrigation with untreated wastewater (WHO, 2006). The percentage is considerably higher among populations in low-income countries with arid and semiarid climates. Irrigation of sewage-contaminated surface waters is common, although it may not occur as end-of-pipe wastewater for crop production. Ideally, restrictions are in place for the treatment of sewage before reuse and irrigation is limited to crops that pose the least risk to consumers. Van der Hoek (2004) gathered available data on the occurrence of wastewater reuse across the globe. The bulk of the untreated sewage from Mexico City is used for non-food crop irrigation. This accounts for half of the known 500,000 ha irrigated with wastewater in Latin America. Significant wastewater irrigation of food crops near the major cities occurs in Peru and Bolivia. Wastewater is directly used for irrigation in almost all towns in Pakistan, where vegetables are the most commonly irrigated crops, because they bring the highest prices. It has been estimated that at least 32,500 ha of land are irrigated directly with wastewater (Emsink et al., 2004). In China 20 billion cubic meters (5,280 billion gallons) of municipal wastewater was discharged in 1998 (Wei et al., 2000) with only 5% of it being treated. In Africa, Cornish and Kielen (2004) compared the use of untreated wastewaters in Ghana, where wastewaters are an important source of reliable irrigation water. In this study, microbial quality of irrigation water varied greatly (in terms of fecal coliforms) depending on location, dilution, and effects of natural remediation (e.g., antimicrobial effects of sunlight). Wastewater in these areas provides an important source of financial gain for many growers operating small plots across a wide area, pointing to the real challenges in regulating wastewater irrigation.

Compost and Night Soil

Night soil refers to human feces collected in buckets, pit latrines, or composting systems without a collection system. In developing countries, especially in rural areas, where collection systems are not available, this is the common method of waste disposal/treatment. Night soil is still commonly

used in parts of eastern Asia, as it has been used for centuries as a fertilizer. In Asia, especially in China and Vietnam, the use of human excreta for crop production is still widespread. In one region of central Vietnam, more than 75% of the farmers studied used fresh or partly composted human excreta to fertilize their farms or gardens (Jensen et al., 2005). This practice likely accounts for the high prevalence of intestinal parasites in Vietnam. In some farming communities in north and central Vietnam, hookworm rates with human populations are 70% or more (Verle et al., 2003). It was estimated in 1993 that China produced 109 million tons of night soil per day by 200 million people in 450 cities lacking any treatment facilities (Bo et al., 1993). The untreated night soil is transported to rural areas where it is used in agriculture. Night soil generated in the rural areas has also been commonly used for garden crops. In areas where untreated night soil is used, the incidence of intestinal parasites has been as high as 93% compared with as low as 28% in areas where treated (stored) night soil was used.

Often night soil is stored in vaults or composted before use on agriculture land; however, prolonged storage and composting (10 months or longer may be required to kill intestinal parasites) are essential to reduce the risk from enteric pathogens (Feachem et al., 1983; Jensen et al., 2005). Vietnam allows the use of composted hygienic safe human excreta as a fertilizer in horticulture but has only recently defined the minimum required composting times (Jensen et al., 2005).

Shellfish-Growing Waters

Shellfish consumed raw have long been known to be associated with the transmission of infectious disease because these filter feeders have the ability to concentrate microorganisms, including pathogens, from water. In its quest for food, an oyster may filter as much as 1,500 liters of water per day (Gerba and Goyal, 1978). The use of strict standards in the United States for shellfish-harvesting waters and processing has virtually eliminated outbreaks from enteric pathogens (FDA, 2005), although occasional outbreaks from norovirus still occur from shellfish harvested in the United States (Berg et al., 2000). Enteric viruses in shellfish are the most common enteric pathogen of concern in shellfish and have been responsible for many outbreaks (Gerba and Goyal, 1978; Richards, 2006). Detection of enteric viruses in shellfish from around the world indicates that virus in shellfish is a common occurrence (Bosch et al., 2001; Kingsley et al., 2002; Beuret et al., 2003). Although the protozoan parasite *Cryptosporidium* has been detected in shellfish, no outbreaks have been documented (Gómez-Couso et al., 2003). Guidance is provided for bacteria standards for shellfish-growing waters and shellfish meat (FDA, 2005); however, the longer survival of enteric viruses in

marine waters and slower rate of depuration can limit their ability to assess the safety of the shellfish for consumption. That is why a sanitary survey of growing waters, harvesting, and processing is critical to reduce risks from enteric viruses.

According to the FDA (2005), approved shellfish-harvesting areas can average 14 fecal coliforms per 100 ml (using a five-tube most probable number method). These same standards apply to shellfish imported into the United States that are intended to be consumed raw. In contrast, the European Union regulates on the basis of fecal coliform levels in shellfish meats (Richards, 2006).

Outbreaks of disease associated with imported shellfish occur in many European countries (Bosch et al., 2001), Japan (Richards, 2006), and recently in the United States (Kingsley et al., 2002). Clams imported from China into the United States were associated with an outbreak of norovirus. Although import regulations require that clams from China be cooked, these clams were labeled as cooked but had the appearance of raw product (Kingsley et al., 2002).

Strict control of shellfish harvest waters is essential in any country where shellfish harvesting is practiced because of the ability of these organisms to concentrate pathogens. To ensure product safety, development of practical routine methods for virus detection and monitoring in growing waters and shellfish meats are needed.

Other Routes of Cross-Contamination

Fecally contaminated hands are another potential source of produce contamination during production, harvesting, and processing. Such contamination may result not only from ill individuals but also from the presence of asymptomatic excretors, which is likely to be greater in developing countries. Espinoza-Medina et al. (2006) found by using polymerase chain reaction (PCR) methodology evidence of *Salmonella* on the hands of 16.7% of the packers of cantaloupes in Mexico. More on this topic of hygienic deficiencies may be found in Chapter 5.

Use of any fecally contaminated water postharvest can result in contamination of produce. For example, Keraira and Drechsel (2004) found in Ghana that wash water was an important source of bacterial contamination of fresh produce sold in urban markets. The use of ice made from contaminated water may also be a factor (Cannon et al., 1991).

Use of human and animal feces and/or sewage for fish aquaculture is common in much of the world, especially in Asia, where much of the production takes place (Scholtssek and Naylor, 1988). Carp and tilapia are especially suited for growth in sewage/aquaculture systems. Fortunately, numerous

studies suggest that while the gills, skin, and intestine of fish growing in these systems become contaminated with enteric bacteria and viruses, little contamination of the edible tissues occurs (Hejkal et al., 1983; Buras et al., 1985; Easa et al., 1995; Khalil and Hussein, 1997; Lan et al., 2007). As long as the harvested fish are handled and processed (e.g., cooked to proper temperatures) in a hygienic manner, there appears to be little risk to the consumer (Lan et al., 2007).

Use of municipal water in the developing world does not necessarily ensure that the water is safe for use in processing. Contamination of drinking water distribution systems is a major problem in the developing world, where an intermittent water supply is often the norm (Lee and Schwab, 2005). This results in loss of pressure and contamination of distribution systems by polluted water infiltrating the distribution system because of the negative pressure that occurs. This type of breakdown in the water supply system has been responsible for several drinking water-associated outbreaks in developing countries (Lee and Schwab, 2005). Other factors, such as poor infrastructure maintenance and lack of cross-connection control programs, exacerbate the problem.

THE WAY FORWARD

Multiple contamination sources and pathways pose risks to food safety, with solid and liquid waste handling and irrigation of produce and shellfish production representing major risk factors. A multiple-barrier approach is needed that identifies (and attempts to contain) the source of contamination, while at the same time it minimizes the transmission of contaminants to food, particularly through irrigation and postharvest handling. The WHO guidelines (WHO, 2006) for the safe use of wastewater include valid recommendations to manage these risks.

Clearly more information is needed on the microbiologic quality of water used in the production of food, especially for export so that the potential risks from contamination can be assessed and meaningful standards developed that protect human health. Fecal contamination of surface water will always be a problem. The use of animal manure and human solids wastes in the form of biosolids applied to soil are essential to the sustainability of agriculture and the recycling of nutrients. Management strategies are essential in the minimization of pathogen risks during agricultural production. These must reflect the types of crops, means of irrigation, harvesting, and processing in association with the level of environmental sanitation.

Finally importers and exporters of food (that is potentially contaminated) and national authorities must establish mechanisms to share information

regarding food contamination with potentially harmful agents as well as disseminate and provide resources for safe production and handling processes.

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