

SURVIVAL OF ENTERIC PATHOGENS ON THE SURFACE OF FRESH PRODUCE
AND INTAKE OF HETEROTROPHIC BACTERIA IN THE UNITED STATES

by

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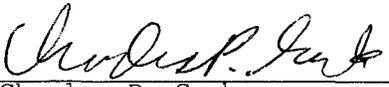
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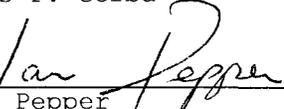
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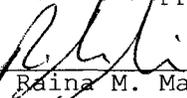
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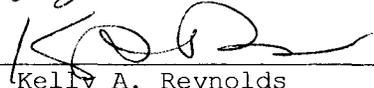
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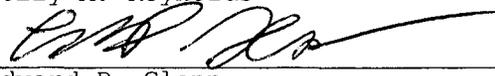
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DEDICATION

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ABSTRACT

Disease due to the consumption of food contaminated with enteric microorganisms has been well established. The first study described in this dissertation was designed to determine the effect of relative humidity on the pre-harvest survival of enteric pathogens on the surfaces of fresh produce. Additionally, *Clostridium perfringens* was evaluated as an indicator of fecal contamination on fresh produce. Pathogenic and surrogate microorganisms, including *Escherichia coli* O157:H7, *Escherichia coli* ATCC 25922, *Salmonella enterica* subsp. *enterica*, *Shigella sonnei*, *C. perfringens*, coliphage PRD1, feline calicivirus (FCV), and hepatitis A virus (HAV), were inoculated onto the surfaces of cantaloupe, iceberg lettuce, and bell peppers. Experiments were conducted in a controlled environment chamber. Survival of microorganisms on the produce surfaces was not uniformly affected by relative humidity. However, due to the survival of all microorganisms at least 14 days in at least one experiment, measures should be taken to lessen the exposure of produce to fecal contamination as harvest time approaches. *C. perfringens* survived longer than all other bacteria and feline calicivirus in all experiments, with the exception of *E. coli* O157:H7 and *S. enterica* subsp. *enterica* on lettuce. This trend suggests that *C. perfringens* may be an acceptable indicator of bacterial contamination and survival in various environments and on different types of crops.

The second study was conducted to determine the intake of heterotrophic bacteria by the average person in the United States from food and water. A literature review was conducted to determine the concentration of heterotrophic plate count (HPC) bacteria in

foods and water from the household tap. Food items from grocery stores and fast food restaurants in Tucson, AZ were also evaluated for HPC bacteria. It was determined that in the United States, 0.048 to 4.5% of the typical consumer's HPC bacteria intake is derived from water consumed from the household tap. Therefore, HPC bacteria in tap water do not represent a significant source of the total HPC bacteria consumed in the average diet of individuals in the United States.

INTRODUCTION

Problem Definition

I. Survival of Pathogenic Microorganism on the Surface of Fresh Produce

Over the past few decades the Center for Disease Control and Prevention has reported an increase in the number of foodborne outbreaks associated with fresh produce (Tauxe, 1997). A combination of factors including changes in trends of produce consumption and importation have led to numerous foodborne outbreaks that are associated with fresh fruits and vegetables (Hedberg et al., 1994; Hedberg, 2000; Meng and Doyle, 2002). Several notable outbreaks associated with fresh produce (Bern et al., 1999; CDC, 2002; CDC, 2003; Naimi et al., 2003) have not only brought with them public attention, but also a realization that gaps exist in our knowledge regarding contamination and survival of pathogenic microorganisms. While much work has been done to gain a better understanding of pathogenic survival on the surface of produce in post-harvest conditions, particularly at refrigeration temperature, pre-harvest survival is often overlooked.

Food crops are grown in a variety of different climates, ranging from the relatively humid regions of South America and the lower Rio Grand Valley in Texas to the drier climates of Arizona and Mexico. Relative humidity is already known to influence the presence of plant pathogens and subsequently the method of post-harvest packing of produce. The link between these methods and post-harvest microbial contamination has been investigated (Gagliardi et al., 2003). However, the direct effect

of relative humidity on the survival of pathogens on the surface of produce in pre-harvest conditions has never been investigated.

Microbial indicators of food safety are often used in food microbiology (Jay, 1996). Unfortunately, an acceptable indicator of fecal contamination for fresh fruits and vegetables is currently unavailable. This is due to the possibility that plant materials may serve as a natural reservoir for traditional indicators such as coliforms (Knittel et al., 1977) and the relatively long survival of hardier pathogens, such as enteric viruses (Gerba, 2000).

II. Intake of Heterotrophic Bacteria in the United States

Heterotrophic bacteria require organic carbon for growth and are aerobic or facultatively anaerobic. All human pathogenic bacteria are heterotrophic. Heterotrophic plate count (HPC) refers to a variety of culture-based tests that are designed for use in the drinking water industry to detect variation in water quality (Gerba, 2000). HPC bacteria can also be used to indicate the ability of pathogenic microorganisms to survive in water. They are found in tapwater and bottled water, as well as other sources of potable water (Rusin 1997). HPC bacteria include some opportunistic pathogens and the public health risk of some members of this group has led to concern by some authors (Leclerc et al., 2002; Pavlov et al., 2002). However, the presence of such bacteria in foods that are minimally processed after purchase by the consumer has suggested that the risk from consumption of water may be minimal (Wadhwa et al., 2002).

Literature Review

A. Introduction

Foodborne illnesses are an important aspect of public health around the globe. The World Health Organization (WHO) has reported that worldwide the number of individuals who suffer from diseases related to contaminated food is in the hundreds of millions (<http://www.who.int/archives/inf-pr-1997/en/pr97-58.html>). Mead et al. (1999) estimated that in the United States the true annual incidence of foodborne illness is 76 million cases, of which 325,000 and 5,000 result in hospitalization and death, respectively. While unknown microbial agents are thought to account for 62 million of these illnesses, it has been estimated that viruses are responsible for 67% of all foodborne illness, bacteria for 30%, and parasites for 3% (Mead et al., 1999). The principle microbial pathogens responsible for foodborne illness are reported in Table 1.

Table 1. Principle microbial pathogens responsible for foodborne infections and the estimated number of cases each caused annually in the United States (as estimated in 1997) (adapted from Mead et al., 1999).

Microorganism	Annual Estimated Number of Cases*
Norovirus	9,200,000
<i>Campylobacter</i>	1,963,000
<i>Salmonella</i> (nontyphoid)	1,342,000
<i>Clostridium perfringens</i>	249,000
<i>Giardia lamblia</i>	200,000
Staphylococcus food poisoning	185,000
<i>Toxoplasma gondii</i>	112,000
<i>Escherichia coli</i> O157:H7 and other Shiga-toxin producing <i>E. coli</i>	92,000
<i>Shigella</i>	90,000
<i>Yersinia enterocolitica</i>	87,000
Enterotoxigenic <i>E. coli</i>	56,000
Streptococci	51,000
Astrovirus	39,000
Rotavirus	39,000
<i>Cryptosporidium parvum</i>	30,000
<i>Bacillus cereus</i>	27,000
Other <i>Escherichia coli</i>	23,000
<i>Cyclospora cayetanensis</i>	14,000
Hepatitis A virus	4,000
<i>Listeria monocytogenes</i>	2,000
<i>Salmonella typhi</i> (typhoid fever)	659
<i>Trichinella</i>	52

* values over 1000 rounded to the nearest 1000

Undercooked meat, poultry, or seafood, as well as unpasteurized milk, are among the foods traditionally implicated in foodborne outbreaks. However, new vehicles of foodborne transmission have been implicated over the past two decades. Foodborne outbreaks of disease such as those associated with the consumption of fresh produce, eggs, apple cider, and oysters harvested from pristine waters have indicated a change in food vehicles of transmission. These new food vehicles share features such as having fewer barriers to microbial growth, such as preservatives and salt, and are typically

contaminated early in the production process (Tauxe, 1997). While the exact number of foodborne diseases caused in association with the consumption of fresh produce is unknown, the Center for Disease Control and Prevention (CDC) reported an increase in the percentage of outbreaks associated with fresh produce from 2% in the period from 1973 to 1987 to 5% in the years between 1988 and 1991 (Tauxe, 1997). Due to the survival and growth of foodborne pathogens on fruits and vegetables, fresh produce is expected to continue to play a role in foodborne illness (Meng and Doyle, 2002).

B. Epidemiology of Foodborne Illness Associated with Fresh Produce

1. Consumption of Fresh Produce

Factors that influence the epidemiology of foodborne diseases associated with fresh produce in the United States have been identified (Hedberg et al., 1994). Traditionally, fresh fruits and vegetables experience greater consumption during the summer months, which might contribute to the summertime increases in foodborne illness. However, increased importation has led to an increase in per capita consumption of such food items year round, which could remove some seasonality from these foodborne diseases. The increased importation of fruits and vegetables may also contribute to the incidence of foodborne illness due to minimal analysis of produce imported from nations with lower sanitation standards for foodborne pathogens.

The consumption of fresh produce has increased in the United States due to the promotion of fresh fruits and vegetables as part of a well-balanced diet over the past two decades. A 13% increase in the annual per capita consumption of such foods in the United States occurred during the 1990s compared to the 1980s. While this trend offers

benefits to the consumer, such as good health and the reduced risk of certain diseases, it has contributed to the rise of fresh produce associated outbreaks over the past decades (Meng and Doyle, 2002).

2. Importation of Fresh Produce

Increased importation of fresh fruits and vegetables into the United States from developing countries illustrates not only the changes in our food supply but also in the type and distribution of foodborne pathogens (Hedberg, 2000). For instance, while *Shigella* and enterotoxigenic *Escherichia coli* (ETEC) are responsible for only 1% of known foodborne illness in the United States, these importation trends have brought with them an increased potential for foodborne illness, such as shigellosis and “traveler’s diarrhea caused by ETEC, that are endemic in developing countries (Mead et al., 1999). Outbreaks of foodborne illness associated with cantaloupe have been caused by several serovars of *Salmonella enterica* over the past decade (Fancis et al., 1991; Mohle-Boetani et al., 1999). In 2002, the U. S. Food and Drug Administration (FDA) issued an import alert on cantaloupes from Mexico after three multi-state outbreaks of *S. enterica* serotype Poona occurred consecutively each spring from 2000-2002 (CDC, 2002). Hepatitis A virus (HAV) was determined to be the cause of a large foodborne outbreak in the United States associated with green onions imported from Mexico in 2003 (CDC, 2003). In 1998, an outbreak of *Shigella sonnei* and enterotoxigenic *E. coli* (ETEC) occurred in the United States and Canada with investigations implicating fresh parsley as the source of illness. A farm in Baja California, Mexico was found to be the source of the parsley (Naimi et al., 2003).

3. Other Factors

The growing population of susceptible individuals, such as the elderly and immunocompromised, is another factor contributing to emerging food safety threats (Rose et al., 2001). It should also be noted that over the last decade antimicrobial resistance of foodborne pathogens, such as *Campylobacter*, *Salmonella*, *Listeria monocytogenes*, Shiga toxin-producing *Escherichia coli*, and *Yersinia enterocolitica*, has increased dramatically (White et al., 2002).

C. Foodborne Pathogens Associated with Fresh Produce

1. Bacteria

a. *Shigella*

S. dysenteriae, *S. boydii*, *S. sonnei*, and *S. flexneri* make up the genus *Shigella* and are small gram-negative rods. All members of the genus are human pathogens and as few as 10 colony forming units (cfu) have been found to initiate infection in susceptible individuals (Jay, 1996). Transmission of shigellosis usually occurs due to person-to-person routes, but several outbreaks have occurred due to the consumption of contaminated water and foods, particularly raw vegetables (Cook et al., 1995; Davies and Wray, 1996; Kapperud et al., 1995; Martin et al., 1986). This is due to the lack of heat treatment of such foods. It has been reported that sliced fresh fruits and vegetables, including watermelon, papaya, and jicama, can support the growth of *S. sonnei*, *S. flexneri*, and *S. dysenteriae* at 22 to 27°C (Escartin et al., 1989).

b. *Escherichia coli*

Escherichia coli is a gram-negative rod that has strains responsible for foodborne gastroenteritis and has been used as an indicator of food safety. Five virulence groups of *E. coli* have been described. These groups are based on disease characteristics and their effect on serologic groupings and include enterohemorrhagic (EHEC), enteroaggregative (EAaggEC), enteropathogenic (EPEC), enteroinvasive (EIEC), and enterotoxigenic (ETEC) (Jay, 1996). Outbreaks of EHEC O157:H7 have occurred in North America, Europe, and Japan and have primarily been associated with the consumption of undercooked beef and dairy products. However, the contamination of raw vegetables, including lettuce, broccoli and alfalfa sprouts, with EHEC O157:H7 has been implicated as the sources of foodborne outbreaks (Barnett et al., 1995; Hilborn et al., 1999). In one outbreak, it was suggested that the source of lettuce contamination originated from one grower who had cattle in the vicinity of the growing and processing areas (Hilborn et al., 1999). EHEC O157:H7 has been found to grow in apple cider at 8°C as well as cantaloupe and watermelon cubes at 25°C (del Rosario and Beuchat, 1995; Zhao et al., 1993). EIEC has also been known to cause foodborne gastroenteritis due to the consumption of contaminated vegetables (Jay, 1996).

c. *Campylobacter*

Campylobacter is gram-negative, spirally curved rod that is microaerobic to anaerobic (Jay, 1996). It was recently estimated that *Campylobacter* is responsible for 2.4 million cases of foodborne infections in the United States, replacing *Salmonella* as the leading cause of such infections (Nachamkin, 2002). While *Campylobacter* enteritis is

most commonly caused by the consumption of contaminated meat, such as poultry, illness has been associated with the consumption of fruits and vegetables (Bean and Griffin, 1990). It has been noted that *Campylobacter jejuni* is capable of surviving long enough on sliced papaya and watermelon to create a risk for the consumer (Castillo and Escartin, 1994).

d. *Salmonella*

The *Salmonella* genus consists of gram-negative enteric bacteria. All members of this genus are considered human pathogens. Surveys of fresh produce have revealed *Salmonella* serotypes capable of causing human infection have been present on vegetables such as lettuce, cabbage, celery, cauliflower, and peppers (Garcia-Villanova Ruiz et al., 1987; Tamminga et al., 1978). While poultry, eggs, and dairy products are the most frequently identified sources of salmonellosis outbreaks, several large outbreaks have occurred in association with the consumption of fresh produce. In the United States, multistate outbreaks have occurred in association with consumption of raw tomatoes (Hedberg et al., 1994; Wood et al., 1991) and cantaloupe (Ries et al., 1990; CDC, 1991; CDC, 2002).

Cut melons have been identified as a food item that meets the model food code criteria for potentially hazardous food established by the U. S. Food and Drug Administration due to the prevalence of *Salmonella* on cantaloupe (Golden et al., 1993). This makes cantaloupe subject to time and temperature requirements set by the code. Recommendations for retail establishments that prepare or sell fresh cantaloupe are displayed in Table 2.

Table 2. U. S. Food and Drug Administration recommendations for retail establishments that prepare or sell fresh cantaloupe (adapted from Golden et al., 1993).

Melons should be washed before cutting.
Surfaces and utensils should be clean and sanitized when preparing cut melons.
Cut melons should be kept at 7°C or less.
Cut melons should not be made available to consumer after 4 h if they are not refrigerated.

S. anatum, *S. chester*, *S. havana*, *S. poona*, and *S. senftenberg* have been found to grow on rind-free watermelon, cantaloupe, and honeydew at 23°C (Golden et al., 1993).

It should be noted that little or no die-off of *Salmonella* was observed at 5°C in this study. It has been reported that *S. Montevideo* can grow on the tomato surface and chopped tomatoes at 20°C (Asplund and Nurmi, 1991) while *S. enteritidis*, *S. infantis*, and *S. typhimurium* can grow in cut tomatoes at 22 and 30°C (Zhuang et al., 1995). The presence of plant pathogens has been found to enhance growth of *Salmonella* on produce. When soft rot causing bacteria are present in tomatoes, *Salmonella* can experience a 10-fold increase in growth rate (Wells and Butterfield, 1997).

e. *Listeria monocytogenes*

Listeria monocytogenes is a gram-positive non-spore forming bacilli that is resistant to cold, heat, salt, pH extremes, and bile (Talaro and Talaro, 1999). *L. monocytogenes* is classified as an opportunistic pathogen. It causes severe illness in the immunocompromised, the elderly, and in the fetus, and 25% of recognized infections lead to death (Slutsker et al., 2000). It is widely distributed on plant vegetation, which can cause direct dissemination of the pathogen via raw produce, or indirect dissemination

by silage contamination in milk (Beuchat, 1995). Lettuce, tomatoes, and cabbage have been linked to outbreaks of listeriosis in the United States (Ho et al., 1986; Schlech et al., 1983). *L. monocytogenes* can grow on fresh produce at refrigeration temperature and in controlled-atmosphere storage (Beuchat, 1995).

f. *Yersinia enterocolitica*

Yersinia enterocolitica is a gram-negative bacilli that causes abdominal pain that mimics appendicitis due to inflammation of the ileum and mesenteric lymph nodes (Talaro and Talaro, 1999). While no outbreak of yersiniosis has been linked to the consumption of raw produce, *Y. enterocolitica* has been found to be present on such foods. It has also been found to grow at refrigeration temperature (Beuchat, 1995).

g. *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive, facultative anaerobic cocci that is considered one of the most resistant of the non-spore-forming bacterial pathogens (Talaro and Talaro, 1999). It has been found on fresh produce (Abbey 1988) as well as ready-to-eat salads (Houang et al., 1991), but does not compete well with native vegetable microflora. *S. aureus* is also known to be carried by food handlers (Beuchat, 1995).

h. *Clostridium perfringens*

Clostridium perfringens is a gram-positive, anaerobic sporeforming rod that has been associated with gastroenteritis since 1895. It is widely distributed in nature, including soil, water, and dust, as well as foods, spices, and intestinal tracts of animals including humans. Five types have been described, including A, B, C, D, and E, which is based on the ability to produce exotoxins. Types A and C are responsible for food-

poisoning syndrome. An enterotoxin that is produced during sporulation is the cause of *C. perfringens* food poisoning (Jay, 1996).

Meat dishes are often the foods involved in *C. perfringens* outbreaks. This may be due to the higher incidence of food-poisoning strains in meats and slower cooling rates in such foods. *C. perfringens* contamination of nonmeat foods occurs due to contact with meat gravy (Jay, 1996). However, due to the presence of *C. perfringens* spores in soil, contamination of fruits and vegetables does occur. A study conducted in the United Kingdom found that almost one-third of the vegetables sampled from retail outlets were contaminated with *C. perfringens* (Roberts et al., 1982). Strong et al. (1963) found the incidence of *C. perfringens* to be 3.8% for fruits and vegetables in the United States. However, it should be noted that for a risk to the consumer to exist fruits and vegetables must be handled in a way that allows both the germination of spores and the growth of vegetative cells (Beuchat, 1995).

2. Viruses

a. Hepatitis A Virus

Hepatitis A virus (HAV) is a picornavirus classified in the hepatovirus group. HAV outbreaks have occurred in association with the consumption of lettuce (Rosenblum et al., 1990), uncooked diced tomatoes (Williams et al., 1995), frozen strawberries (Niu et al., 1992), and frozen raspberries (Reid and Robinson, 1987). In 2003, a large outbreak of HAV associated with green onions occurred in Pennsylvania. The outbreak was associated with the consumption of green onions at a single restaurant. It was determined

that the green onions were contaminated with HAV either during pre-harvest, post-harvest, or distribution and originated from one or more farms in Mexico (CDC, 2003).

b. Norovirus

Norovirus, formerly known as Norwalk-like viruses (NLVs), is a nonenveloped virus with a ssRNA genome. It is believed not only to be the main cause of foodborne illness caused by a known agent, but also the major cause of human gastroenteritis (Gerba and Kayed, 2003). Noroviruses are extremely infectious and have been responsible for outbreaks occurring in hospitals, schools, nursing homes, and cruise ships (Seymour and Appleton, 2001). Outbreaks where noroviruses have been associated with fresh produce have been reported, including fresh cut fruit (Herwaldt et al., 1994), green salads (Griffin et al., 1982), and imported frozen raspberries (Ponka et al., 1999).

3. Parasites

a. *Cyclospora*

Cyclospora is a coccidian protozoan whose round oocyst measures $8 \times 10 \mu\text{m}$. Raspberries imported from Guatemala were the source of *Cyclospora* infections in the United States in 1996 and 1997. It was suggested that contamination occurred after insecticide that had been diluted with surface water contaminated with *Cyclospora* was sprayed onto raspberries (Bern et al., 1999). Salad dishes including lettuce and fresh green leafy herbs were associated with a cyclosporiasis outbreak in Germany (Doller et al., 2002).

b. *Cryptosporidium*

Cryptosporidium is an obligate intracellular coccidian parasite that has an oocyst that ranges in size from 4.5 to 5.0 μm . Cryptosporidiosis has been associated with the consumption of contaminated apples (Hui et al., 2001). Both *Cyclospora* and *Cryptosporidium* oocysts have been recovered from vegetables in markets in Peru (Ortega et al., 1997).

c. *Giardia*

Giardia is a flagellate protozoan which produce pear shaped cysts that are 5 to 12 μm in width and 8 to 20 μm in length. It has a low infectious dose (Gerba, 2000). Lettuce and salad have been associated with *Giardia* infection in England (Stuart et al., 2003) and the United States (Rose and Slifko, 1999), respectively.

D. Other Microorganisms Associated with Food

1. Heterotrophic Plate Count (HPC)

Heterotrophic plate count (HPC) refers to a variety of culture-based tests that are designed to quantify a wide range of microorganisms from water. More specifically, these microorganisms include bacteria that require organic carbon for growth and are aerobic or facultatively anaerobic. Gram-negative bacteria such as *Pseudomonas*, *Aeromonas*, *Enterobacter*, *Citrobacter*, *Klebsiella*, and *Flavobacterium* are included in this group. Some members of the HPC group, such as *Pseudomonas* and *Aeromonas*, are opportunistic pathogens (Gerba, 2000).

It should be noted that while HPC can be used to detect variation in water quality and the ability to of pathogens to survive in drinking water, HPC is not a direct indicator

of fecal contamination (Gerba, 2000). Due to interference of coliform detection, it has been recommended that HPC should not exceed 500/mL tap water (Lechevallier et al., 1980). However, it has been noted that the properties and characteristics of food support higher levels of microbial growth and survival compared to water. Due to this and the greater number of pathogens and their indicators in food, it has been suggested that food is the greater risk to humans in the United States (Wadhwa et al., 2002).

2. Indicator Organisms

Indicator organisms are often used in food microbiology to determine the safety of foods from pathogenic microorganisms. Microbial indicators that have been used for this purpose include coliforms, enterococci, bifidobacteria, and coliphage (Jay, 1996). The characteristics of an ideal food safety indicator organism are reported in Table 3.

Table 3. Criteria for the ideal food safety indicator organism (adapted from Jay, 1996)

<p>The organism should be easily and rapidly detectable.</p> <p>The organism should be present when pathogens are present and absent from foods that are free of pathogens.</p> <p>The organism should possess growth requirements equaling that of pathogens.</p> <p>The organism should have growth and die-off rates that at equal those of pathogens.</p>

3. Microbial Antagonists

Naturally occurring bacterial microbiota on the surface of ready-to-eat salad have been evaluated as microbial antagonists of foodborne pathogens, such as *Staphylococcus aureus*, EHEC O157:H7, *Listeria monocytogenes*, and *Salmonella montedvideo*. In a recent study by Schuenzel and Harrison (2002), of 1,180 isolates found on the surface of ready-to-eat salad, 3.22% showed an inhibitory effect on at least one of these pathogens.

Species of *Pseudomonas*, *Aeromonas*, and *Candida* are among the minimally processed vegetable microbiota that have been found to be microbial antagonists of pathogens (Schuenzel and Harrison, 2002).

E. Sources of Microbial Contamination and Conditions that Influence Them

There are a plethora of sources of microorganisms found in food, including food utensils, food handlers, air, ice, and processing equipment (Beuchat, 1995; Jay, 1996).

Sources of contamination of fresh produce are often divided into categories of pre-harvest and post-harvest and are listed on Table 4.

Table 4. Sources of contamination of fresh produce by pathogenic microorganisms and conditions that influence their survival (adapted from Beuchat, 1995).

Pre-harvest	Post-harvest
Feces	Feces
Soil	Human handling
Irrigation Water	Transport vehicles
Inadequately composted manure	Cross-contamination
Air and dust	Improper storage, handling, and packaging
Wild and domestic animals	Ice
Human handling	Harvesting Equipment
	Processing equipment
	Wash and rinse water

1. Pre-harvest Contamination

a. Domestic and Wild Animals

Fields are more likely to be contaminated with enteric pathogens after they are grazed by livestock or wild animals (Tauxe, 1997). Cattle have been well defined as sources of EHEC O157:H7. It has been shown by numerous studies that EHEC O157:H7 is carried asymptotically and shed in the feces of large numbers of cattle (Borczyk et

al., 1987; Elder et al., 2000). *Cryptosporidium parvum* has also been associated with cattle. An infected calf can excrete concentrations of 10^{10} oocysts in one day (Rusin et al., 2000). In a study of dairy calves in central Mexico, it was found that 29 of the 31 dairies used in the study had one or more calves shedding *C. parvum* oocysts (Maldonado-Camargo et al., 1998).

b. Animal Feeding Operations (AFOs)

Over the past few decades, the livestock industry has experienced several changes in the area of animal production. The number of production facilities has decreased while at the same time the total number of animal units has increased by approximately 3 percent. The amount of animal manure produced in animal feeding operations (AFOs), approximately 128 billion pounds per year in the United States alone, is the biggest risk to the environment and human health created by such operations (USGAO, 1995). Runoff from livestock can enter bodies of water due to overapplication of manure to cropland as well as spills and leaks caused by improper design of storage structures, excessive rainfall events, and the poor maintenance of waste lagoons (USEPA, 2001).

c. Soil

Direct contact with soil has been found to increase the risk of contamination of fruits with pathogens as compared to fruits that grow high above the ground (Roberts, 1990). After flooding experimental plots with wastewater, Teirney et al. (1977) found that poliovirus survived two to three days and two months in summer and winter months, respectively. *Salmonella* and *L. monocytogenes* are capable of surviving for months in agricultural fields treated with sewage sludge (Watkins and Sleath, 1981). While *E. coli*

has an average half-life of 3 days (Bogosian et al., 1996; Temple et al., 1980), *Salmonella* has been found to survive and grow in the soil environment for at least one year (Davies and Wray, 1996; Thomason et al., 1977).

d. Irrigation Water

Worldwide irrigation activity has been estimated to be as much as 95% surface irrigation (Walker, 1989). The United States Department of Agriculture (USDA) estimated that in 1998 approximately 54% of United States farmland is irrigated with surface irrigation systems (<http://www.nass.usda.gov/census/census97/fris/fris.htm>).

Irrigation water has been determined to be the source of several outbreaks associated with the consumption of fresh fruits and vegetables. Several scenarios have been described in determining sources of irrigation water contamination. Animals not only have the ability to contaminate produce directly with feces, but have also been known to contaminate water used as sources of irrigation (Ackers et al., 1998; Hilborn et al., 1999). Wastewater discharge as well as runoff from livestock operations causes agricultural water quality to fluctuate (FDA, 1998).

It is interesting to note that irrigation water has also been found to influence other pathogenic microorganisms not associated with food. The Sub-Saharan African city of Kumasi, Ghana uses urban agriculture to increase the food supply as well as improve nutrition and create employment (UNDP, 1996). Any available water source is used to irrigate these farms and it is feared that breeding sites for malaria vectors could be created, elevating the relatively low prevalence of malaria in the city to levels found in surrounding rural areas (Birley and Lock, 1999; Gardiner et al., 1984; Trape, 1987).

e. Internalization

The internalization of microbial pathogens into plant tissue has traditionally been viewed to occur due to insects, birds, windfall, or other routes that can bruise or wound the surface of the plant. However, it has been suggested that the internalization of pathogens could be due to natural openings in the plant, such as the vascular system.

It has been reported that human pathogens, such as EHEC O157:H7, can enter the internal portion of the leaf via damaged areas of the plant (Seo and Frank, 1999; Takeuchi and Frank, 2001; Wachtel et al., 2002). The internalization of *Salmonella* in tomatoes (Zhuang et al., 1995) and *E. coli* O157:H7 (Buchanan et al., 1999) in apples has been reported to occur via natural openings in the plant. The ability of these human pathogens to become internalized within sprouted seeds has also been confirmed. These include bean sprouts (Warriner et al., 2003), alfalfa (Mahon et al., 1997; Taormina and Beuchat, 1999), and radish (Itoh et al., 1998). Internalization of *E. coli* via the vascular system of the plant has also been described in lettuce (Wachtel et al., 2002), spinach (Warriner et al., 2003), and cabbage (Wachtel et al., 2002), as well as *Salmonella* in tomato plant (Guo et al., 2002). Studies have also shown that when compared to low cell densities of EHEC O157:H7 (10^2 to 10^3 CFU/mL), higher cell densities (10^8 CFU/mL) have a greater extent of colonization on roots and hypocotyls of plants (Solomon et al., 2002; Wachtel et al., 2002).

Warriner et al. (2003) found the stage of spinach development at the time of *E. coli* introduction determined the occurrence of internalization. When inoculated onto spinach seeds, it was observed that *E. coli* experienced growth due to the exudates

released during seed germination. This allowed the *E. coli* to not only become established on the exterior of the roots, but in the interior also. However, the study suggested that *E. coli* was restricted to the root system once the spinach plant has matured. Although there is a low risk that the *E. coli* will be found in the edible portion of the plant, it was suggested that transfer from the root to the inner leaf might be possible during harvest. It should also be noted that 42 days after inoculated seeds were sown, *E. coli* was recovered from the external surface of leaves and roots. However, internalization of *E. coli* into the root tissue did not occur in spinach seedlings from uninoculated seeds. Even though *E. coli* experienced growth in the soil, delaying and prevention of root internalization may have been due to competitive soil microflora.

It should be noted that other studies have found no evidence of microbial internalization into plant tissue via natural openings. Jablasone et al. (2003) found that internalization of *Salmonella* into tomato plant tissues did not occur after contaminated irrigation water was applied to the soil surrounding the plant roots. Viral penetration was not observed in the roots and leaves of cucumber and lettuce plants or the fruit and stem of tomato plants irrigated with reclaimed wastewater in a study by Alum et al. (2001).

f. Climate Variability

Weather and its effects, such as rainfall, runoff, and temperature, can influence the transport, dissemination, and growth/survival of microbial foodborne pathogens. *Cyclospora cayetanensis* oocysts are immature when excreted in fecal waste and require warm temperatures to mature in the environment. The result of this was seen in Lima, Peru where temperatures 5°C above normal caused by El Nino in 1997/1998 led to a two-

fold increase in hospital admissions due to childhood diarrhea. It should also be noted that in the United States, outbreaks associated with cyclosporiasis and fresh produce occur in late spring or early summer (Rose et al., 2001).

2. Post-harvest Contamination

a. Packaging Shed

Melon production sites that experience relatively high humidity (80-90%), such as the lower Rio Grande River Valley, must wash melons prior to packaging and shipping due to plant pathogens that might destroy the melons post-harvest. Arid regions with lower humidity (< 20%), such as Arizona, southern California and Mexico often do not wash melons due to the lower risk of the occurrence of such plant pathogens. In a study conducted by Gagliardi et al. (2003), packaging sheds in the lower Rio Grande River Valley were evaluated to determine if significant bacterial contamination of melons occurred during processing and washing. The study revealed that much of the contamination occurred due to poor water quality of the primary wash tank or hydrocooler used during processing. Rapid loss of chlorine in this water occurred because of chemical reaction with soil and organic matter or evaporation due to warm weather. The authors also suggested that the ready-to-use boxes in which melons are shipped could be contaminated with bird and rodent feces when used in open-air packing sheds.

b. Internalization

Internalization of pathogens into food can occur due to damage to the plant tissue by harvesting equipment and handling practices. While internal microbial contamination

of cantaloupe has not been found to occur if the rind is intact, once the melon is sliced any pathogens on the surface of the rind can be transferred to the nutrient and sugar-rich interior of the fruit (Escartin et al., 1989; Golden et al., 1993). This interior has been found to support significant pathogens growth, which explains outbreak patterns of cut melons that were not consumed quickly (Gagliardi et al., 2003).

F. Conclusions

Changes in food importation and consumption trends, a growing immunocompromised population, and antimicrobial resistance of some foodborne pathogens are changing the face of foodborne diseases. These changes have made foods not traditionally implicated in foodborne outbreaks, such as fresh produce, new vehicles of foodborne disease. A wide range of microbial pathogens including bacteria, viruses, and parasites have been found to survive and grow on fresh fruits and vegetables and are capable of causing foodborne outbreaks if contamination of these foods occurs. Irrigation water, soil, animals, packing sheds, and internalization of pathogens into plant tissue are some of the routes of contamination that have been identified and can occur prior to and/or after harvest. To meet the complex challenge of foodborne disease and prevention, a better understanding of the routes of contamination, survival and growth of pathogens on fresh produce must be obtained.

DISSERTATION FORMAT

This dissertation consists of a literature review followed by two manuscripts as appendices. Appendix A has been submitted for publication in Applied and Environmental Microbiology and discusses the effect of relative humidity on the pre-harvest survival of microbial pathogens on the surfaces of fresh produce. It also evaluates *Clostridium perfringens* as an indicator of contamination and survival of pathogenic microorganisms on produce in pre-harvest conditions. All microbiological aspects of this project were primarily designed, conducted, and analyzed by me. This work was done in collaboration with the Department of Applied and Biosystems Engineering at the University of Arizona. Dr. Christopher Choi, Dr. Jose Pimentel, and Inhong Song were responsible for the design of the engineering aspects of this project and reviewing this manuscript. Dr. Jose Pimentel grew and cared for all crops used in these experiments. Inhong Song assisted in conducting experiments and data analysis. Appendix B is an estimation of the intake of heterotrophic bacteria in the United States from dietary sources, including water and food. It has been submitted for publication in Water Research. Tristen Crenshaw conducted the laboratory activity for the estimation of heterotrophic bacteria in food items from Tucson, AZ. The literature review and data analysis for this project were conducted by myself. Dr. Charles Gerba was instrumental in the design of both projects and the review of the two manuscripts. Appendix C has been included as a complete data set for the project described in Appendix A.

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PRESENT STUDY

The methods, results, and conclusions of two different studies are present in the manuscripts attached to this dissertation. The following is a summary of the most important findings of these manuscripts.

The first manuscript describes an experiment that investigated the effect of relative humidity on the survival of microbial pathogens on the surface of fresh produce. The use of *Clostridium perfringens* as an indicator of bacterial and viral contamination and survival on the surface of produce was also evaluated. The surfaces of cantaloupe, lettuce, and bell peppers were inoculated with microorganisms including *Escherichia coli*, *Escherichia coli* O157:H7, *Shigella sonnei*, *Salmonella enterica* subsp. *enterica*, *Clostridium perfringens*, hepatitis A virus (HAV), feline calicivirus (FCV) and coliphage PRD1 in environmental conditions reflecting low and high relative humidity. Survival of microorganisms on the produce surfaces was not uniformly affected by relative humidity. Each microorganism survived at least 14 days in at least one experiment. *C. perfringens* survived longer than all other bacteria and FCV in all experiments, with the exception of *E. coli* O157:H7 and *S. enterica* subsp. *enterica* on lettuce, suggesting its potential as an indicator organism on crop surfaces.

In the second manuscript, the weekly intake of heterotrophic bacteria in the United States was estimated. An extensive literature was conducted determining the amount of such bacteria present in foods that are traditionally consumed raw, as well as drinking water from the tap and point-of-use (POU) devices. A laboratory experiment

was conducted to have a better understanding of the presence of heterotrophic and coliform bacteria on market vegetables and meats purchased from grocery stores and fast-food restaurants located in Tucson, AZ. Heterotrophic plate counts (HPCs) present on the food items were determined using R2A and SPA media. The main finding of the project was that HPC bacteria in tap water do not represent a significant source of HPC bacteria in the average diet of consumers in the United States.

APPENDIX A

THE EFFECT OF RELATIVE HUMIDITY ON PRE-HARVEST SURVIVAL OF
BACTERIAL AND VIRAL PATHOGENS ON THE SURFACE OF CANTALOUPE,
LETTUCE, AND BELL PEPPER

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ABSTRACT

The purpose of this study was to compare the effects of humidity on the pre-harvest survival of microbial pathogens on cantaloupe, lettuce, and bell peppers. An additional goal was to evaluate the potential of *Clostridium perfringens* as an indicator of fecal contamination on produce. The organisms used in this study included *Escherichia coli*, *Escherichia coli* O157:H7, *Shigella sonnei*, *Salmonella enterica* subsp. *enterica*, *Clostridium perfringens*, hepatitis A virus (HAV), feline calicivirus (FCV) and coliphage PRD1. The study took place in a controlled environment chamber that allowed for the control of temperature and relative humidity. Survival under high and low relative humidity was compared. The edible surface of each plant was inoculated with the study microorganisms. Samples were collected at the beginning of each experiment and at various time intervals over the course of two weeks. Survival of microorganisms on the produce surfaces was not uniformly affected by relative humidity. Each microorganism survived at least 14 days in at least one experiment. Therefore, measures should be taken to lessen the exposure of produce to fecal contamination as harvest time approaches. *C. perfringens* survived longer than all other bacteria and FCV in all experiments, with the exception of *E. coli* O157:H7 and *S. enterica* subsp. *enterica* on lettuce. This trend suggests that *C. perfringens* may be an acceptable indicator of bacterial contamination and survival in various environments and on different types of crops.

INTRODUCTION

It is currently estimated that the incidence of food-borne illness in the United States reaches 76 million cases per year. Of these, 325,000 led to hospitalizations while 5,000 led to death. It has been estimated that viruses and bacteria respectively cause 67% and 30% of the illnesses (40). A growing trend in the consumption of fresh fruit and vegetables has brought with it an increase in food-borne outbreaks associated with such foods (52).

Numerous fruits and vegetables have the potential to be contaminated with pathogenic microorganisms. Outbreaks of foodborne illness associated with cantaloupe have been caused by several serovars of *Salmonella enterica* over the past decade (23, 41). In 2002, the U. S. Food and Drug Administration (FDA) issued an import alert on cantaloupes from Mexico after three multi-state outbreaks of *S. enterica* serotype Poona occurred consecutively each spring from 2000-2002 (15). Both *Shigella sonnei* and *Escherichia coli* O157:H7 outbreaks have been traced to lettuce (18, 32). Hepatitis A virus (HAV) was determined to be the cause of a large foodborne outbreak in the United States associated with green outbreaks in 2003 (13). Iceberg lettuce (45), raw blueberries (12), and frozen strawberries (18) have also been implicated in HAV outbreaks. Norovirus outbreaks have been caused by the contamination of ready-to-eat foods, such as minimally processed and raw fruits and vegetables (4, 17, 22, 35, 38, 42). Many of the foodborne pathogens associated with outbreaks involving produce, such as HAV and *E. coli* O157:H7, have relatively low infectious doses (39, 49).

Potential sources of pre-harvest contamination of fresh produce by pathogenic microorganisms may include feces, soil, wild and domestic animals, human handling, and inadequately composted manure (8). It has also been shown that crops may become contaminated with enteric microorganisms through irrigation with wastewater (44, 47). Contamination of irrigation waters by human sewage pollution is another route of viral contamination (18, 19, 31, 37).

The objective of this study was to evaluate the effect of relative humidity on the survival of enteric pathogens on the surface of fruits and vegetables under pre-harvest conditions. *Escherichia coli* O157:H7, *Escherichia coli* ATCC 25922, *Shigella sonnei* ATCC 9290, and *Salmonella choleraesuis* subsp. *Choleraesuis* serotype Typhimurium ATCC 43971 (formerly known as *Salmonella enterica* subsp. *enterica*), hepatitis A virus (HAV), and coliphage PRD1 were chosen for this study because of their association with foodborne outbreaks and their use as surrogates for pathogens. Due to the fact that human caliciviruses, such as Norwalk virus, cannot be grown under *in vitro* conditions, feline calicivirus (FCV) was selected as a surrogate. FCV has previously been used as a surrogate by other researchers (2, 10, 21).

An additional goal of this research was to evaluate the effectiveness of *Clostridium perfringens* as an indicator of fecal contamination on produce. An acceptable fecal indicator organism is currently unavailable due to the possibility that plant materials may serve as a natural reservoir for traditional indicators such as coliforms (34). It should also be noted that hardier enteric pathogens, such as protozoan

parasites and enteric viruses, experience longer survival than some traditional indicators such as coliforms (27).

C. perfringens is a sulfite-reducing anaerobic spore-forming bacteria that is exclusively fecal in origin. The spores can survive in the environment for extended periods of time, are heat resistant, and are resistant to disinfection (27). They have been found to survive longer on produce and in soil than *E. coli* and coliphage (36). For these reasons *C. perfringens* was proposed as an indicator of fecal contamination of produce. It was first proposed as an indicator of fecal contamination of water in 1899 (11). *C. perfringens* has been found to survive much longer than both coliforms and fecal coliforms in water (29).

MATERIALS AND METHODS

Preparation of microorganisms. Bacteria and viruses used in this experiment were obtained from the American Type Culture Collection (ATCC, Rockville, MD) or from the University of Arizona Department of Soil, Water and Environmental Science culture collection. Media were obtained from the Difco Company (Detroit, MI) unless otherwise stated. *E. coli* O157:H7, *E. coli* ATCC 25922, *S. sonnei* ATCC 9290, and *S. enterica* subsp. *enterica* were grown for 18-24 hours in tryptic soy broth at 37°C. *C. perfringens* ATCC 3624 was grown in cooked meat media for 18-24 hours at 37°C. Duncan-Strong raffinose (Sigma Chemical Co., St. Louis, MO) was used to sporulate *C. perfringens* (24). Coliphage PRD1 was propagated in *Salmonella typhimurium* ATCC 19585 (30). Hepatitis A virus (HAV) strain HM175 and feline calicivirus (FCV) were grown in the

fetal rhesus kidney-derived (Frhk-4) cell line and the Crandell's feline kidney (CRFK) cell line, respectively (20, 28, 51).

Controlled environment chamber. A controlled environment chamber was designed, constructed, and tested for the present study. The experiment was carried out at the Controlled Environmental Agricultural Center (CEAC) of the University of Arizona, Tucson, AZ. The chamber allowed for the control of environmental conditions such as temperature, relative humidity, light intensity, and carbon dioxide levels. The dimensions of the chamber were 12 ft × 10 ft × 8 ft. Prior to inoculation, appropriate time was allowed for the conditions to reach desired levels. Two experiments were conducted with each plant to compare the effect of relative humidity on microbial survival. Two dehumidifiers and two humidifiers were set up in the growth chamber to control relative humidity. High and low levels of relative humidity were used. Environmental conditions are described in Table 1. Environmental data were recorded in a data logger (Datalogger 21X, Campbell Scientific Co., UT) every five minutes.

The 400 W high pressure sodium (PL780N, PL Lighting Systems, Canada) light source for the controlled environment chamber was programmed with a 12 hour photoperiod to simulate natural conditions. The wavelength of the light ranged from 346 to 1003 nm and had an average value of 690 nm. Light intensity over the crop surfaces fluctuated close to 300 W/m².

Plant samples. Cantaloupe (Mission variety hybrid, Willhite Seed Inc., TX), bell peppers (California Wonder, Willhite Seed Inc., TX) and iceberg lettuce (Beacon variety, Paragon Seed Inc., CA), were planted in a greenhouse near the CEAC and irrigated

hydroponically. The plants were grown to maturity and transferred to the controlled environment chamber on the day they were to be inoculated.

Plant inoculation. Prior to inoculation, 3 × 3 cm square boundaries were placed on each plant to identify inoculated portions of the plant. The boundaries were positioned in such a way as to receive the most direct light from the overhead lights. Markers were used to draw the squares on lettuce and bell pepper. Due to the hydrophobicity of the cantaloupe, caulk (#12585, Ace Hardware, Oak Brook, IL) was used to create boundaries that would prevent the inoculum from moving out of the specified area. Leaves were arranged in a way to prevent inoculated areas from being shaded.

Due to limited space on the cantaloupe surface, only four squares were placed on each plant. Each square was inoculated with two microorganisms. The microorganisms were paired as follows: 1) *C. perfringens* and *S. sonnei*, 2) PRD1 and HAV, 3) *E. coli* ATCC 25922 and FCV, 4) *S. enterica* subsp. *enterica* and *E. coli* O157:H7. A micropipetter was used to inoculate the fruit with 50 µL of stock of each organism. Lettuce and bell peppers were inoculated in the same manner.

Light exposure control. A control experiment was conducted to determine the effect of light intensity on microbial survival. Three light conditions were evaluated, including full light exposure, shaded exposure, and no direct exposure. A micropipetter was used to inoculate petri dishes with 100 µL of *E. coli* ATCC 25922. Petri dishes were placed on top of a table for full exposure to the light; for shaded exposure, dishes were placed underneath the shade created by plant leaves. For no direct exposure, the petri dishes

were placed under the plastic containers in which the cantaloupe plants were contained, or under a dark plastic cover in the case of lettuce and bell peppers.

Sample collection and recovery of microorganisms. Background samples were collected before each plant inoculation to determine if select microorganisms were already present on the produce. *E. coli* O157:H7, *E. coli* ATCC 25922, *S. sonnei*, and *S. enterica* subsp. *enterica* were chosen for this purpose. It was assumed that the absence of these microorganisms would indicate the absence of all other microorganisms used in the study.

Plant samples were collected on days 0, 1, 3, 5, 7, 10, and 14, or until microbial numbers were below the detection limits for two consecutive sampling dates. Triplicate samples were collected in plastic bags (Ziploc, S. C. Johnson & Son, Inc., Racine, WI) on each sampling date and stored on ice during transport to the laboratory. Light exposure control samples were collected in an identical manner.

Inoculated areas were removed from each plant sample using a paring knife. Each processed plant sample was placed in a plastic bag with 50 mL of elutant and shaken for 20 minutes. Beef extract (3%) was used as an elutant for PRD1/HAV and *E. coli* ATCC 25922/FCV samples (6), while 0.01 M Phosphate Buffered Saline with a pH of 7.0 was used for *C. perfringens*/*S. sonnei* and *E. coli* O157:H7/ *S. enterica* subsp. *enterica* samples (25). After shaking, the 3% beef extract and Phosphate Buffered Saline were removed and collected. Beef extract pH was adjusted to 7-8.

Microbial analysis. *E. coli* ATCC 25922 was assayed using the Colilert quanti-tray system (IDEXX, Westbrook, MA). *E. coli* O157:H7 and *S. enterica* subsp. *enterica* were

assayed using Hekateon agar while *S. sonnei* was assayed using XLD agar. *C. perfringens* was assayed using m-CP media (5). PRD1 coliphage was assayed using the plaque forming unit method with the bacterial host *Salmonella typhimurium* ATCC 19585 (30). Hepatitis A virus and feline calicivirus were assayed in Frhk-4 and CRFK cells, respectively, using the Reed-Muench TCID₅₀ method (43).

Statistical analysis. Microbial inactivation rates were determined using the equation

$$\frac{N_t}{N_0} = 10^{-k_d t}$$

where N_t is the density of surviving microorganisms (number/cm²) at time t , N_0 is the initial density of microorganisms (number/cm²), t is time (days), and k_d is inactivation rate (1/days). Inactivation rates and their standard deviations were calculated using Minitab Statistical Software release 13.32 (Minitab Inc., State College, PA). Analysis of variance (ANOVA) of inactivation rates using standard deviation was conducted using Microsoft Excel Version 9.00.2720 (Microsoft Corporation, Redmond, WA). Days in which all triplicate samples were below the detection limit were not included in the analysis. Differences between inactivation rates were considered statistically significant if $P < 0.05$.

RESULTS

Bacterial survival on produce surfaces. The amount of time in days for each organism to achieve a three-log reduction is reported in Table 2. Inactivation curves for bacteria on the surface of cantaloupe in dry conditions are shown in Figure 1. Bacterial inactivation rates are reported in Figure 3. Overall, bacterial survival was greater on cantaloupe than

on bell peppers or lettuce, regardless of relative humidity. *E. coli* O157:H7 did not experience a three-log reduction in 14 days on cantaloupe in humid conditions, making this the longest survival for this organism. Under all other conditions it survived less than 5 days. It survived the longest on bell pepper in dry conditions and on cantaloupe in humid conditions compared to other produce assayed. *E. coli* O157:H7 experienced a significantly higher inactivation rate ($P=0.007$) in dry conditions than in humid conditions on cantaloupe. In humid conditions, it had a significantly higher inactivation rate ($P<7.46 \times 10^{-6}$) on lettuce compared to cantaloupe and bell pepper. *E. coli* ATCC 25922 survived at least 7 days longer on cantaloupe than on other produce under both environmental conditions. It experienced a significantly higher inactivation rate ($P<0.036$) in humid conditions on lettuce as opposed to dry conditions. *E. coli* ATCC 25922 had a significantly lower rate of inactivation ($P<0.017$) on cantaloupe in humid conditions than both lettuce and bell peppers. *S. sonnei* survived for 14 days on cantaloupe in humid conditions and on bell peppers in dry conditions. Under both humid and dry conditions on lettuce, and on bell peppers under humid conditions, *S. sonnei* survived 5 days or less. *S. enterica* subsp. *enterica* had the longest survival on cantaloupe under both humid and dry conditions. In all other conditions it survived 3 days or less. *S. enterica* subsp. *enterica* experienced a higher inactivation rate ($P=0.019$) in dry conditions on lettuce. It also had a higher rate of inactivation ($P=0.015$) in humid conditions on cantaloupe, as opposed to dry conditions. Compared to cantaloupe and bell peppers, *S. enterica* subsp. *enterica* experienced a lower inactivation rate ($P<0.05$) on lettuce in humid conditions.

C. perfringens survival ranged from 10 days to greater than 14 days under all conditions. It had higher median and mean $T_{99.9}$ values than all other bacteria and FCV in all experiments. *C. perfringens* experienced a significantly higher rate of inactivation ($P < 0.004$) on cantaloupe in dry conditions compared to lettuce and pepper. On bell peppers *C. perfringens* had a significantly higher inactivation rate ($P = 0.005$) in humid conditions. It experienced a significantly lower inactivation rate ($P < 0.009$) than *E. coli* 25922 on bell peppers in humid conditions and all other bacteria on cantaloupe in dry conditions. *E. coli* ATCC 25922 and *E. coli* O157:H7 experienced higher inactivation rates ($P < 0.043$) than *C. perfringens* on lettuce in both dry and humid conditions. While not statistically significant in all instances, *C. perfringens* had lower rates of inactivation than all other bacteria in all experiments, with the exception of *E. coli* O157:H7 and *S. enterica* subsp. *enterica* on lettuce in dry and humid conditions. *C. perfringens* had lower rates of inactivation ($P < 0.015$) than FCV in all experiments. It experienced lower inactivation rates ($P < 0.008$) than HAV in humid conditions on lettuce and bell peppers. PRD1 experienced significantly higher rates of inactivation ($P < 0.043$) in humid condition on both lettuce and cantaloupe. *C. perfringens* had significantly lower inactivation rates ($P = 0.004$) on cantaloupe in dry conditions compared to all other produce.

Viral survival on produce surfaces. Inactivation curves for viruses on the surface of cantaloupe in dry conditions are shown in Figure 2. Inactivation rates for viruses are reported in Figure 4. PRD1 experienced significantly lower inactivation rates ($P < 0.021$) than FCV and *E. coli* 25922, with the exception of cantaloupe in humid conditions, in all experiments. It had a significantly lower rate of inactivation ($P < 0.013$) than all

organisms with the exception of *C. perfringens* and HAV on cantaloupe in dry conditions. *E. coli* 25922, *E. coli* O157:H7, and FCV had significantly higher inactivation rates ($P < 0.04$) than PRD1 on lettuce in humid and dry conditions. PRD1 experienced a significantly lower inactivation rate ($P < 0.015$) on bell peppers than lettuce and cantaloupe in humid conditions, while in dry conditions PRD1 had a significantly lower rate of inactivation ($P < 0.005$) on cantaloupe. Both PRD1 and HAV experienced higher mean and median $T_{99.9}$ values than all bacteria, with the exception of *C. perfringens*, and FCV. HAV survived in excess of 14 days under all conditions with the exception of lettuce under humid conditions. Of the three plants evaluated, HAV experienced a significantly lower inactivation rate ($P = 0.03$) on cantaloupe in both humid and dry conditions. On lettuce, HAV had a significantly higher inactivation rate ($P = 0.007$) in humid conditions, while FCV experienced significantly higher inactivation rates ($P = 0.012$) in humid conditions on cantaloupe. FCV survived less than 5 days under all conditions with the exception of cantaloupe under dry conditions. When comparing the survival of FCV on each plant surface in dry conditions, it was found to have a significantly lower inactivation rate ($P = 0.04$) on cantaloupe.

E. coli ATCC 25922 survival in light exposure control. In light exposure control experiments, with the exception of the cantaloupe experiment under humid conditions, samples with full exposure survived less than 1 day on the plastic surface of the petri dishes while those with shaded and no exposure survived less than 3 days. In the cantaloupe experiment under humid conditions, samples with full exposure to light survived less than five days. In the same experiment, samples with no exposure to direct

light and shaded samples were inactivated after 10 days. Overall, no difference was observed between samples with no direct exposure to light and those that were shaded.

DISCUSSION

Light exposure experiments revealed that under the conditions of this experiment samples with no direct exposure to light and those that were shaded survived 2 to 5 days longer than those with full exposure to the light. While microbial survival on plant surfaces is influenced by light exposure, further studies are necessary to determine if shade from plant material such as leaves might afford significantly longer survival to microorganisms.

With the exception of lettuce and bell pepper in humid conditions, *C. perfringens* did not survive significantly ($P < 0.05$) longer than HAV in any experiment. However, while not necessarily statistically significant, *C. perfringens* did survive longer than all other bacteria and FCV in all experiments, with the exception of *E. coli* O157:H7 and *S. enterica* subsp. *enterica* on lettuce. This trend suggests that *C. perfringens* may be an acceptable indicator of bacterial contamination and survival in various environments and on different types of crops.

More microorganisms survived significantly longer ($P < 0.05$) on cantaloupe than lettuce and bell peppers in dry (4/8 organisms) and humid (2/8 organisms) conditions. Previous research suggests that the rougher or more irregular the surface of produce, the longer viruses are able to survive (6). The surface texture and structure of vegetables also plays an important role in the attachment and survival of bacteria (33). Cantaloupes have irregular lenticles, known as netting, on their rind, thus harboring a variety of surfaces

where bacteria can attach (53). It has also been suggested that when open, the lenticles might provide additional sites for microorganisms to colonize and might provide protection from disinfection (26). The complexity of cuticular waxes of vegetables surfaces also plays a role in microbial entrapment (1). The more complex the wax structure, the greater the chance of entrapment (3). However, with the exception of *E. coli* ATCC 25922, the type of produce on which each organism experienced the longest survival changed with relative humidity. This suggests that the ability of the evaluated microorganisms to survive on the surface of cantaloupe, lettuce, and bell peppers is influenced by relative humidity.

Studies have shown that at 20°C, HAV experiences the longest survival at low relative humidity (9, 48). However, our study has shown that survival of microorganisms on the surfaces of different types of produce and under different relative humidity is variable. All of the investigated microorganisms survived at least 14 days in at least one experiment. Due to the potential survival (>14 days) of pathogenic microorganisms on produce surfaces, measures should be taken to lessen the exposure of produce to fecal contamination as harvest time approaches. Even if the produce surfaces are not consumed, as with cantaloupe, risk still exists due to the contamination of the edible flesh during slicing (14, 16). Other potential risks include the transfer of pathogens onto the hands of a consumer or harvester (46) and kitchen contamination during preparation.

Some pathogenic microorganisms, such as HAV, are very stable in the environment (50). Viruses have previously been shown to survive on the surface of vegetables for over 2 months under suitable conditions (7). Future studies should

determine the extent of pre-harvest survival of hardier microorganisms, such as HAV and *C. perfringens*.

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Table 1. Daily Environmental Conditions in the Controlled Environment Chamber during Experiments

Crop	Relative humidity (%)		Air temperature (°C)	
	Dry	Humid	Dry	Humid
Cantaloupe	35 - 60	70 - 99	18 - 26	18 - 26
Iceberg lettuce	30 - 60	70 - 97	21 - 26	22 - 26
Bell pepper	39 - 60	70 - 96	21 - 26	22 - 26

Table 2. Survival of Study Organisms under Different Environmental Conditions

Organisms	$T_{99.9}^{a,b}$					
	Cantaloupe		Lettuce		Bell Pepper	
	Dry ^c	Humid ^d	Dry ^c	Humid ^d	Dry ^c	Humid ^d
<i>E. coli</i> O157:H7	3	>14	1	1	5	1
<i>E. coli</i> ATCC 25922	10	>14	3	1	1	1
<i>S. sonnei</i>	10	14	5 ^e	3	3 ^e	3 ^e
<i>S. enterica</i> subsp. <i>enterica</i>	5	>14	1	>14	1	1
<i>C. perfringens</i>	>14	14	>14	>14	10 ^e	>14
PRD1	>14	10	>14	>14	>14	>14
HAV	>14	>14	>14	10	>14	>14
FCV	14	3	3	3	3	5

^a Days for titer to decline by 99.9%.

^b Air temperature (°C) ranged from 18-26.

^c Relative humidity ranged from 30-60%.

^d Relative humidity ranged from 70-99%.

^e Detection limit was reached before a three-log reduction occurred. The day the detection limit reached is the reported value.

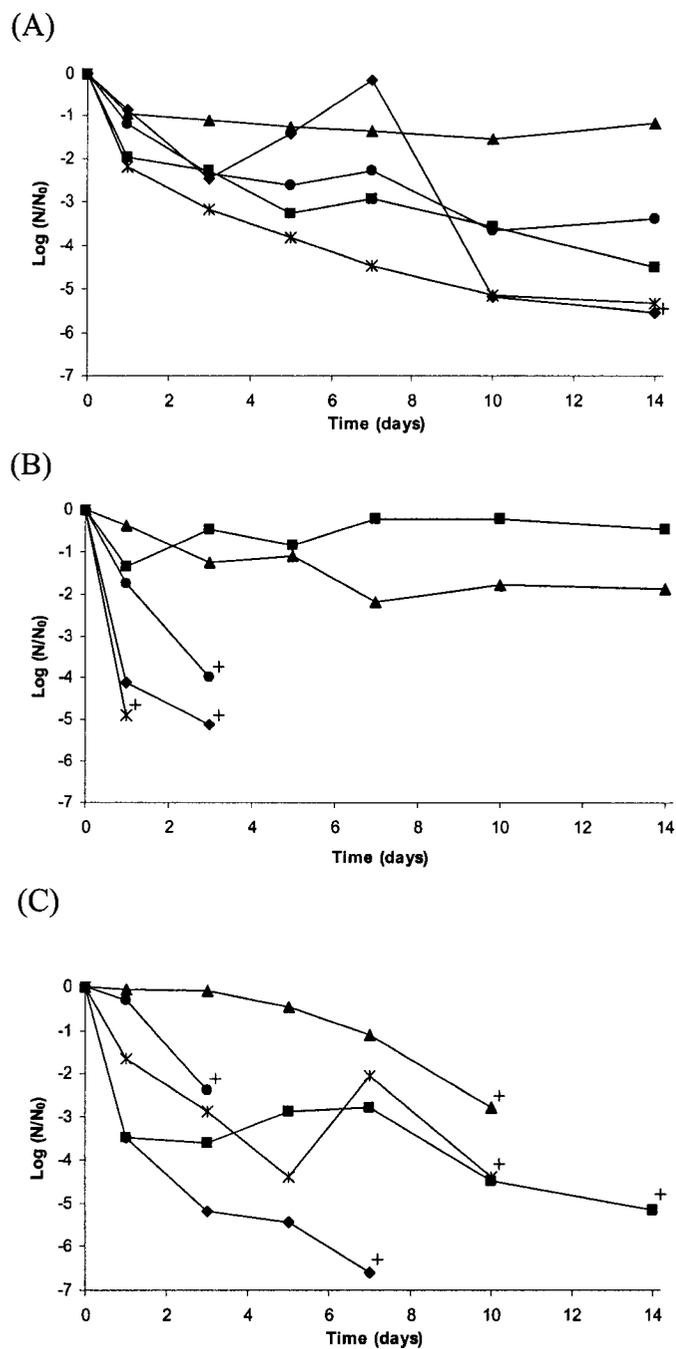
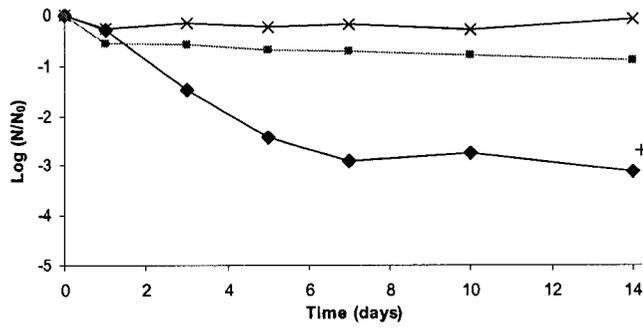


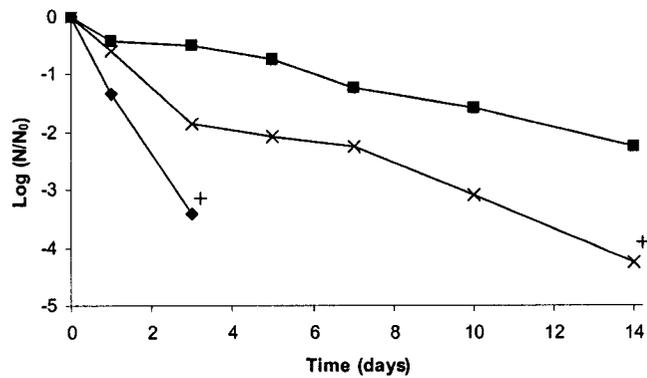
FIG. 1. Inactivation curves for *E. coli* ATCC 25922 (◆), *E. coli* O157:H7 (✱), *S. sonnei* (●), *C. perfringens* (▲), and *S. enterica* subsp. *enterica* (■) on surface of (A) cantaloupe in dry conditions (35-60% relative humidity), (B) lettuce in humid conditions (70-97% relative humidity), and (C) bell pepper in dry conditions (39-60% relative humidity).

+ Detection Limit

(A)



(B)



(C)

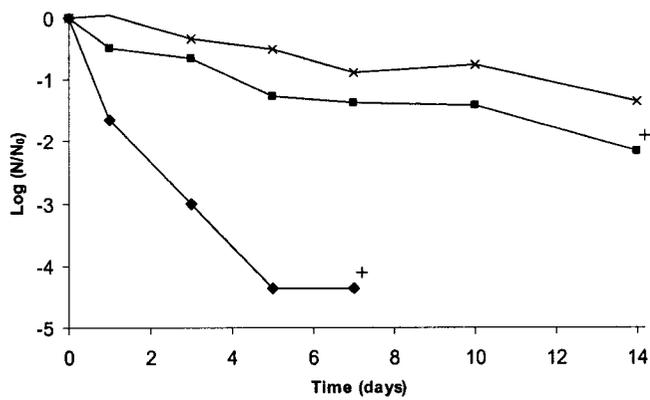


FIG. 2. Inactivation curves for HAV (x), FCV (♦), and PRD1 (■) on surface of (A) cantaloupe in dry conditions (35-60% relative humidity), (B) lettuce in humid conditions (70-97% relative humidity), (C) bell pepper in dry conditions (39-60% relative humidity).
 + Detection Limit

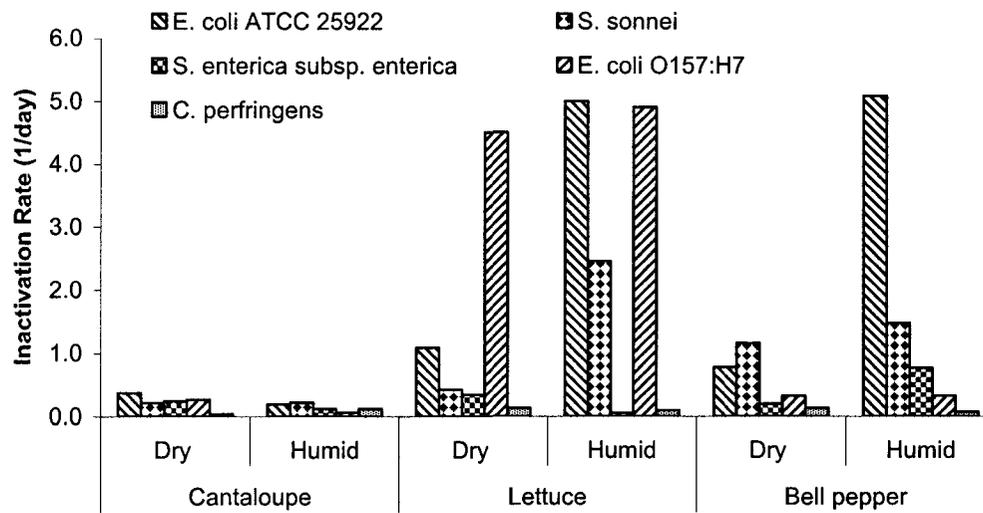


FIG. 3. Inactivation rates for *E. coli* ATCC 25922, *S. sonnei*, *S. enterica* subsp. *enterica*, *E. coli* O157:H7, and *C. perfringens* on surface cantaloupe, lettuce, and bell pepper in dry (30-60% relative humidity) and humid (70-99% relative humidity) conditions.

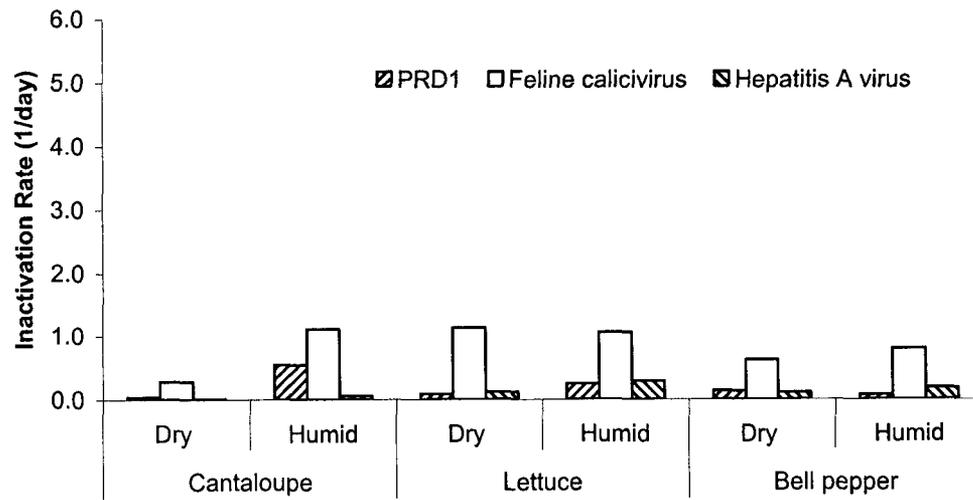


FIG. 4. Inactivation rates for PRD1, FCV, and HAV on surface of cantaloupe, lettuce, and bell pepper in dry (30-60% relative humidity) and humid (70-99% relative humidity) conditions.

APPENDIX B

CONTRIBUTION OF DRINKING WATER TO THE WEEKLY INTAKE OF
HETEROTROPHIC BACTERIA FROM DIET IN THE UNITED STATES

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Abstract

The goal of this study was to assess the relative contribution of heterotrophic bacteria from various sources in the normal diet of an average person in the United States, due to concerns regarding the potential health implications of such bacteria in household tapwater. A literature search was conducted to determine the concentration of heterotrophic plate count (HPC) bacteria in drinking water, as well as foods common to the American diet. Food items were also obtained in Tucson, AZ to further evaluate the consumption of HPC and total coliform bacteria. This was compared to a recent study on HPC bacteria in tapwater with and without POU devices mounted on the tap in Tucson, AZ households. It was determined that only 0.048 to 4.5% of the average consumer's total heterotrophic bacteria intake is derived from drinking water. Thus, HPC bacteria in drinking water do not represent a significant exposure of total HPC bacteria in the average diet of consumers in the United States.

Keywords: Heterotrophic plate count (HPC); Total coliforms; Point-of-use (POU) device; vegetables

1. Introduction

Concern has been generated in the drinking water industry regarding the health effects of heterotrophic plate count bacteria (HPC) that are found in tapwater, bottled water, and other sources of potable water [1]. Heterotrophic bacteria are those that require organic carbon rather than carbon dioxide as a carbon source. All human pathogenic bacteria are heterotrophic. The U. S. Environmental Protection Agency (USEPA) has suggested that the heterotrophic bacterial counts in drinking water should not exceed 500 colony-forming units (CFU/mL), primarily because of the interference of coliform detection [2]. Higher numbers are often the result of bacterial regrowth, particularly in the distribution system [3] and in the water treatment devices mounted at the household tap [4]. Some authors have expressed concern regarding the public health risk of some of these HPC bacteria [5, 6]. It has been suggested that individuals in high-risk categories, such as the immunocompromised, could be at risk from the consumption of drinking water due to the presence of opportunistic pathogens that are members of the HPC group [1]. The goal of this project was to assess the total HPC and coliform bacteria intake of a person in the United States from both food and water to determine what percentage of HPC ingestion is attributable to water.

2. Materials and Methods

2.1 Literature review

An extensive review of the literature was conducted of total bacterial concentrations in food in the United States. Food items were limited to those that are minimally processed after purchase by the consumer. This review also included data on

the concentration of HPC bacterial concentrations in drinking water including, tapwater and tapwater treated by point-of-use (POU) devices, and bottled water. The concentration of HPC bacteria in drinking water from Tucson, AZ households was evaluated using values reported in a previous study [7]. HPC levels from household kitchen taps, sports bottles, commercial bottled water, and a POU device were monitored from seven different households over a period of three months. Tapwater in Tucson, AZ is currently obtained from wells and is disinfected by chlorination. It has an average pH of 7.8, a turbidity of <1.0, total organic carbon value of 0.5 mg/L, and a dissolved solids concentration of 285 mg/L [8, 9].

2.2 Intake of heterotrophic and coliform bacteria in food from Tucson, AZ

To better determine the ingestion rates of HPC and coliform bacteria from food in the diet, a study was conducted in Tucson, AZ on the occurrence of these organisms in market vegetables and meats. Changes in the concentrations of these organisms after food processing and storage were also assessed. Food items were purchased from various local grocery stores and fast food restaurants. To determine changes in the concentration of bacteria after processing and storage, ready-to-eat (RTE) cantaloupe and salad were purchased, then stored at 4-5°C and subsequently sampled after 3 and 5 days storage, respectively. Five different samples of each food item were collected from different stores and assayed. Hamburger meat was sampled both before and after being cooked in a pan.

Food items were individually placed in Stomacher bags along with 0.1% peptone nutrient broth (Difco, Detroit, MI) and pummeled for 30 seconds in a Stomacher model

400 (Dybatech Laboratories, Alexandria, VA). HPC bacterial numbers were determined using R2A media (Becton Dickinson and Company, Cockeysville, MD) and Standard Plate Count (SPA) Media (Difco, Detroit, MI) and were incubated for 5 days at 30°C. Coliform bacteria were assayed on MacConkey agar (Difco, Detroit, MI) and incubated for 24 hours at 35°C. At least two colonies from each food item sampled were confirmed by passage in lactose broth (Becton Dickinson and Company, Cockeysville, MD). API strips (BioMerieux, Hazelwood, MO) were used to identify the isolates.

2.3 Data analysis

Using the data gathered from Tucson, AZ [7], arithmetic mean concentrations of HPC and coliform bacteria in the major food items, including water, in the American diet were determined. Arithmetic means have been shown to be a more appropriate summary descriptor in microbial risk assessment than geometric means [10]. These numbers were multiplied by the average per capita consumptions in the United States provided by the United States Department of Agriculture (USDA) [11]. The daily drinking water ingestion rate was assumed to be 1.15 liters per day and 2 liters per day, based on values reported by the USDA (1995), and the U. S. Environmental Protection Agency (USEPA) [12], respectively. All calculations and analysis of variance (ANOVA) were conducted using Microsoft Excel Version 9.0.2720 (Microsoft Corporation, Redmond, WA). Differences in microbial concentrations were considered statistically significant if $P < 0.05$.

3. Results

The concentrations of HPC bacteria in various water sources determined from the literature review and from Tucson, AZ are reported in Tables 1 and 2, respectively. The literature review revealed that the food items with the highest levels of HPC bacteria included celery and salad vegetables such as lettuce (Table 3). The concentrations of HPC bacteria in food from Tucson, AZ are reported in Table 4. There was no significant difference between HPC counts determined with R2A and SPA. HPC numbers were higher than total coliform levels in all foods (Table 5). The food items with the highest HPC levels were RTE salad (5 days old), bean sprouts, and salad from a salad bar. The food items with the highest amounts of total coliforms were RTE cantaloupe, bean sprouts, and salad from a salad bar. Increases in the number of HPC bacteria were experienced on RTE cantaloupe and RTE salad after 3 and 5 days, respectively. After cooking, HPC bacteria in hamburger meat experienced a significant reduction ($P < 0.05$). The weekly intake of heterotrophic bacteria from consumption of food and water is reported in Tables 6 and Table 7. The total amount of HPC bacteria consumed in one week from food was estimated to be 5.0×10^9 .

4. Discussion

HPC numbers observed in food and water from Tucson, AZ were within the ranges for food [13 - 23] and water [24] reported in the literature. Therefore, HPC levels in food and water from Tucson, AZ were considered to be representative of values found elsewhere in the United States. The lack of a significant difference between heterotrophic bacteria recovered using R2A and SPC media allows for the comparison of

HPC and SPC values from water and food. The results of this study involving food items from Tucson, AZ showed that HPC and total coliform bacteria are found in the greatest numbers in raw fruits and vegetables. The food items with the highest amounts of HPC and coliforms, bean sprouts, cantaloupe, and salad, have all been associated with multiple foodborne outbreaks. Foodborne outbreaks caused by *Salmonella* and *Escherichia coli* O157:H7 have been associated with the consumption of raw bean sprouts [25, 26]. It should be noted that the U. S. Food and Drug Administration (USFDA) has issued health warnings that raw or lightly cooked sprouts should not be consumed by individuals in high-risk categories, including children, the elderly, and the immunocompromised [27]. Cantaloupe have been linked to multi-state outbreaks in the United States [28, 29] with the latest multi-state outbreak leading to an import alert on cantaloupe from Mexico [30]. Multi-state outbreaks associated with fresh vegetables used to prepare salad, such as lettuce and tomatoes, have been linked to *Escherichia coli* O157:H7 [31] and *Salmonella* [32, 33], respectively. Daniels et al. (2002) [34] determined that salads were implicated in 6% of all foodborne disease outbreaks in United States schools from 1973-1997. Some non-*E. coli* coliform bacteria have been found to occur in tap fixtures and POU devices [3, 8]. As was observed in this study, such bacteria are common on many foods. While many coliforms are capable of growth in and on the surface of foods, this is not an indication of fecal contamination. The same is true for the occurrence of coliforms in POU devices and tap fixtures in the household. However, coliforms in raw or treated drinking water are considered a potential indicator of fecal contamination.

The results of this study indicate that foods are the main source of bacteria ingested by the average person in the United States. The average consumer in the United States ingests only 0.048 to 4.5% of the individual's total bacterial intake from household water. Reasons for greater microbial numbers in food compared to drinking water include the physical and physiological characteristics of food [35]. Compared to those of drinking water, these characteristics along with protein, carbohydrates, and ionic strength make food closer to the physiological state of humans and allow for microbial growth.

It should be noted that the types of bacteria that dominate in foods and water might be different in many situations, including the type of food. For example, while members of the lactic acid bacteria group, such as *Lactobacillus* and *Lactococcus*, may be common bacterial species present in some dairy products, they are not found in tap water. However, the species of bacteria found in foods generally reflect the same ones found in tapwater [1]. HPC bacteria commonly found in drinking water, such as *Pseudomonas* and *Xanthomonas*, are also found in and on the surface of foods such as milk, lettuce, tomatoes, cabbage, cauliflower, and cottage cheese [36, 37]. It has been suggested that individuals in high-risk categories, such as the immunocompromised, could be at risk from the consumption of drinking water due to the presence of opportunistic pathogens that are members of the HPC group [1]. However, due to the higher intake of heterotrophic bacteria from food sources a greater risk may exist from the consumption of certain uncooked foods compared to that of water.

5. Conclusion

HPC bacteria in drinking water have previously been identified as a potential health concern, particularly to the immunocompromised. However, HPC bacteria in tap water do not represent a significant source of HPC in the average diet of consumers in the United States. The U. S. Food and Drug Administration recognizes the consumption of foods such as raw or lightly cooked bean sprouts to be a potential health risk to the immunocompromised. Subsequently, HPC bacteria in foods that are minimally processed after purchase by the consumer, such as salad vegetables, represent a much greater risk to such populations than HPC bacteria found in drinking water.

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Table 1

Heterotrophic bacterial concentrations in different water sources (adapted from Geldreich, 2002 [24])

Water Source	Heterotrophic Concentration Range per mL
Public water	<1 – 600
Rural well water	10 – 1.9×10^4
Point-of-use device ^a	<10 – 1.7×10^5
Bottled water	<10 – 3.9×10^5
Drinking fountain ^b	35 – 2.7×10^4
Cistern ^c	<10 – 2.3×10^7

^acarbon filter device

^bTaylor and Geldreich (1979) [38]

^ctropical climate

Table 2

Heterotrophic plate counts (cfu/mL)^a from Tucson, AZ households (adapted from Pepper et al., 2004 [7])

	Kitchen tap	Commercial bottled water	Sports bottle	Point-of-use device ^b
Range	$4 - 7 \times 10^7$	$0 - 9 \times 10^4$	$240 - 3.4 \times 10^4$	$4 - 1 \times 10^7$
Arithmetic Average	300	1,750	1.7×10^4	4,000

^acfu – colony forming units

^bcarbon filter device

Table 3
Concentrations of HPC bacteria in foods

Food Items	HPC (cfu/g) ^a	Reference
Celery	$1.0 \times 10^4 - 1.0 \times 10^{10}$	[13, 14]
Cheese	$100 - 7.9 \times 10^6$	[15, 16]
Cucumber	$1.0 \times 10^6 - 1.0 \times 10^7$	[17, 18]
Lettuce	$1.0 \times 10^4 - 4.0 \times 10^8$	[17 - 21]
Milk ^b	$1.0 \times 10^4 - 9.7 \times 10^6$	[22, 23]
Spinach	$1.0 \times 10^4 - 1.0 \times 10^8$	[13, 17]
Salad ^c	$3.2 \times 10^6 - 5.8 \times 10^8$	[17]

^acfu – colony forming units

^bcfu/mL

^cprepared by restaurant

Table 4
Concentrations of HPC bacteria in food items from Tucson, AZ

Food Items ^a	HPC – R2A (cfu/g) ^b		HPC – SPA (cfu/g) ^b	
	Mean	Range	Mean	Range
Bean sprouts	1.21×10^8	8.96×10^7 – 1.45×10^8	8.44×10^7	9.12×10^7 – 1.14×10^8
Cabbage	5.43×10^6	3.25×10^4 – 1.35×10^7	2.79×10^6	6000 – 6.80×10^6
Cantaloupe, RTE ^c	3.39×10^6	8.65×10^4 – 9.68×10^6	3.69×10^6	8.00×10^4 – 9.28×10^6
Cantaloupe, RTE ^{c,d}	5.08×10^6	1.10×10^6 – 1.53×10^7	6.33×10^6	7.90×10^5 – 1.57×10^7
Celery	9.48×10^5	3.40×10^5 – 1.84×10^6	4.78×10^5	1.80×10^5 – 1.02×10^6
Hamburger, fast food	3.67×10^4	1100 – 7.6×10^4	2080	300 – 5500
Hamburger, raw	5.31×10^6	1.20×10^6 – 8.24×10^6	3.10×10^6	3.90×10^4 – 5.60×10^6
Hamburger, cooked	1590	600 – 5,550	8	<1 – 200
Lettuce, iceberg	1.35×10^6	7.15×10^4 – 2.56×10^6	9.60×10^5	3.00×10^5 – 2.03×10^6
Milk, whole ^e	1.54×10^5	1000 – 6.20×10^5	4.13×10^5	1.25×10^4 – 2.00×10^6
Salad, RTE ^c	3.36×10^6	1.70×10^4 – 9.80×10^6	1.04×10^6	6,000 – 4.45×10^6
Salad, RTE ^{c,f}	3.06×10^8	7.90×10^5 – 9.20×10^8	5.92×10^7	3.00×10^5 – 2.84×10^8
Salad, salad bar	3.74×10^8	5.88×10^6 – 6.80×10^8	6.21×10^6	5.45×10^5 – 2.20×10^7

^afive samples assayed per food item

^bcfu – colony forming units

^cready-to-eat

^dsampled after 3 days at 4-5°C

^ecfu/mL

^fsampled after 5 days at 4-5°C

Table 5
Concentration of coliform bacteria in food items from Tucson, AZ

Food Items ^a	Total Coliforms (cfu/g) ^b	
	Mean	Range
Bean Sprouts	7.41×10^4	$<1 - 3.70 \times 10^5$
Cabbage	6.40×10^2	$<1 - 3,200$
Cantaloupe, RTE ^c	1.00×10^4	$200 - 3.70 \times 10^4$
Cantaloupe, RTE ^{c,d}	8.69×10^4	$2450 - 2.60 \times 10^5$
Celery	6.40×10^3	$500 - 1.10 \times 10^4$
Hamburger, fast food	7.00×10^2	$200 - 1,800$
Hamburger, raw	3.10×10^3	$<1 - 8,350$
Hamburger, cooked	<1	–
Lettuce, iceberg	7.60×10^2	$<1 - 2,900$
Milk, whole ^c	<1	–
Salad, RTE ^c	2.78×10^3	$<1 - 1.3 \times 10^4$
Salad, RTE ^{c,f}	1.40×10^2	$<1 - 500$
Salad, salad bar	1.90×10^4	$<1 - 9.40 \times 10^4$

^afive samples assayed per food item

^bcfu – colony forming units

^cready-to-eat

^dsampled after 3 days at 4-5°C

^ecfu/mL

^fsampled after 5 days at 4-5°C

Table 6
HPC Bacteria Consumed from drinking water on a weekly basis in Tucson, AZ

Water source	Weekly consumption (mL)	HPC Bacteria Consumed (cfu/g) ^c
Kitchen tap ^d	8,050 ^a	2.4×10^6
Kitchen tap ^d	14,000 ^b	4.2×10^6
POU treated water ^d	8,050 ^a	3.2×10^7
POU treated water ^d	14,000 ^b	5.6×10^7
Sports bottle ^d	8,050 ^a	1.7×10^8
Sports bottle ^d	14,000 ^b	2.4×10^8
Commercial bottled water ^d	8,050 ^a	1.4×10^7
Commercial bottled water ^d	14,000 ^b	2.5×10^7

^aUSEPA, 2001 [12]

^bUSDA, 1995 [11]

^ccfu – colony forming units

^dPepper et al., 2003 [7]

Table 7

HPC Bacteria Consumed from food on a weekly basis in Tucson, AZ

Food item ^a	Weekly consumption (g)	HPC bacteria consumed (cfu/g) ^b
Cabbage	78.67 ^c	4.3×10^8
Cantaloupe, RTE ^d	89.85 ^e	9.0×10^5
Celery	55.07 ^c	5.2×10^7
Cooked red meats	1,686 ^c	2.7×10^6
Lettuce, iceberg	182.7 ^c	2.5×10^8
Milk, whole ^f	1,862 ^c	2.9×10^8
Total	–	5.0×10^9

^afive samples assayed per food item^bcfu – colony forming units^cUSDA, 1995 [11]^dready-to-eat^eUSDA, 2002 [39]^fcfu/mL

APPENDIX C

BACTERIAL AND VIRAL SURVIVAL DATA FROM FRESH PRODUCE
SURFACES

Table 1. Concentrations of Microorganisms Inoculated on the Surface of Fresh Produce

Organism	Cantaloupe Dry ^a	Cantaloupe Humid ^b	Lettuce Dry ^c	Lettuce Humid ^d	Bell Pepper Dry ^e	Bell Pepper Humid ^f
<i>E. coli</i> ATCC 25922 ^g	1.51E+08	1.25E+08	1.63E+08	3.15E+08	2.78E+08	1.71E+07
<i>Shigella sonnei</i> ^g	5.85E+07	7.50E+07	8.73E+05	2.03E+06	1.40E+04	6.38E+04
<i>Salmonella enterica</i> ^g	1.19E+08	2.15E+09	1.60E+08	7.70E+07	6.55E+07	9.85E+07
<i>Clostridium perfringens</i> ^g	2.75E+06	2.75E+06	3.38E+06	3.38E+06	1.08E+06	1.08E+06
<i>E. coli</i> O157:H7 ^g	8.30E+07	1.45E+08	7.28E+07	1.10E+08	4.78E+07	1.18E+07
PRD1 ^h	6.00E+09	6.00E+09	1.85E+09	1.85E+09	4.50E+10	4.5E+10
Feline calicivirus ⁱ	8.00E+08	8.00E+08	8.00E+08	8.00E+08	8.00E+08	8.00E+08
Hepatitis A virus ⁱ	9.00E+05	9.00E+05	9.00E+05	9.00E+05	9.00E+05	1.85E+05

^a 35-60% relative humidity, 18-26°C

^b 70-99% relative humidity, 18-26°C

^c 30-60% relative humidity, 21-26°C

^d 70-97% relative humidity, 22-26°C

^e 39-60% relative humidity, 21-26°C

^f 70-96% relative humidity, 22-26°C

^g colony forming units/mL

^h plaque forming units/mL

ⁱ most probable number/mL

Table 2. Inactivation of Microorganisms on the Surface of Cantaloupe in Dry Conditions^a

Time (days)	<i>E. coli</i> ATCC 25922 ^b	<i>Shigella sonnei</i> ^b	<i>Salmonella enterica</i> ^b	<i>Clostridium perfringens</i> ^b	<i>E. coli</i> O157:H7 ^b	PRD1 ^c	Feline calicivirus ^d	Hepatitis A virus ^d
0	1.98E+05	7.56E+06	4.06E+07	5.28E+04	1.58E+07	3.14E+08	1.99E+04	1.58E+03
1	3.50E+03	6.58E+05	4.42E+05	4.94E+03	1.28E+05	9.08E+07	3.88E+03	6.19E+02
1	8.89E+03	1.19E+05	7.11E+05	5.72E+03	9.17E+04	1.19E+08	3.88E+03	1.11E+03
1	6.81E+04	6.33E+05	1.40E+05	7.11E+03	9.81E+04	4.56E+07	2.36E+04	6.19E+01
3	1.11E+01	3.36E+04	1.09E+05	2.22E+03	5.28E+02	9.39E+07	4.03E+02	3.51E+02
3	<5.56E-01	2.31E+04	5.39E+05	5.22E+03	2.67E+04	7.97E+07	5.16E+01	3.06E+02
3	2.03E+03	4.50E+04	8.11E+03	4.83E+03	5.44E+03	7.94E+07	1.53E+03	6.19E+02
5	1.34E+04	1.40E+04	3.00E+04	4.11E+03	6.58E+03	6.97E+07	6.19E+01	3.88E+02
5	9.63E+03	1.38E+04	3.44E+03	7.22E+02	<5.56E-01	6.75E+07	1.11E+02	NS
5	1.97E+02	2.56E+04	3.19E+04	3.50E+03	5.83E+02	6.03E+07	4.38E+01	6.19E+02
7	4.28E+05	9.36E+04	1.01E+05	3.00E+03	5.56E+01	6.08E+07	4.45E+01	3.88E+01
7	<5.56E-01	2.47E+04	2.19E+04	2.17E+03	4.17E+02	4.42E+07	<1.53E+01	3.88E+02
7	3.93E+01	4.28E+03	1.75E+04	1.78E+03	1.08E+03	7.81E+07	<1.53E+01	NS
10	2.28E+00	3.14E+03	4.97E+03	2.67E+03	8.33E+01	4.31E+07	4.45E+01	3.51E+01
10	1.11E+00	1.28E+03	2.67E+04	9.78E+02	1.94E+02	6.42E+07	4.45E+01	4.45E+02
10	<5.56E-01	3.61E+02	1.44E+03	8.67E+02	<5.56E-01	4.33E+07	<1.53E+01	4.45E+02
14	<5.56E-01	7.69E+03	3.42E+03	4.94E+03	1.11E+02	4.14E+07	<1.53E+01	4.45E+02
14	<5.56E-01	1.11E+03	5.56E+01	3.33E+03	<5.56E-01	2.89E+07	<1.53E+01	6.19E+02
14	5.56E-01	8.33E+01	2.22E+02	2.56E+03	<5.56E-01	5.17E+07	<1.53E+01	4.45E+02

NS – No Sample

^a 35-60% relative humidity, 18-26°C^b colony forming units/mL^c plaque forming units/mL^d most probable number/mL

Table 3. Inactivation of Microorganisms on the Surface of Cantaloupe in Humid Conditions^a

Time (days)	<i>E. coli</i> ATCC 25922 ^b	<i>Shigella sonnei</i> ^b	<i>Salmonella enterica</i> ^b	<i>Clostridium perfringens</i> ^b	<i>E. coli</i> O157:H7 ^b	PRD1 ^c	Feline calicivirus ^d	Hepatitis A virus ^d
0	3.93E+04	4.33E+06	4.97E+06	1.94E+04	4.08E+06	6.83E+07	4.43E+04	3.06E+02
1	7.52E+04	5.78E+05	3.06E+06	3.19E+02	1.13E+06	2.36E+08	1.94E+04	2.43E+03
1	4.28E+05	6.00E+04	4.61E+06	6.42E+03	9.25E+04	4.69E+08	2.16E+05	6.19E+01
3	3.41E+05	3.22E+05	1.64E+06	4.78E+02	6.89E+05	8.31E+05	<1.53E+01	1.11E+02
3	4.54E+05	1.69E+05	4.78E+06	4.56E+02	1.46E+05	4.31E+07	1.61E+01	4.45E+02
5	4.04E+04	1.44E+05	6.31E+06	1.33E+03	4.58E+05	7.39E+07	1.53E+01	6.19E+02
5	8.63E+04	5.89E+03	3.17E+05	2.67E+01	2.56E+03	5.19E+07	<1.53E+01	3.88E+02
7	2.28E+02	2.42E+04	8.67E+06	2.75E+02	5.03E+04	1.50E+02	<1.53E+01	6.36E+02
7	3.50E+02	<5.56E+01	8.64E+05	2.17E+01	6.33E+04	3.11E+05	<1.53E+01	7.09E+02
10	1.34E+04	6.44E+03	7.67E+04	7.53E+02	9.56E+04	5.56E+00	<1.53E+01	3.51E+01
10	9.21E+02	7.50E+04	6.22E+05	9.44E+03	1.39E+06	3.58E+04	<1.53E+01	3.88E+01
14	3.22E+03	<5.56E+01	8.17E+04	2.22E+01	4.14E+04	1.39E+02	<1.53E+01	1.11E+02
14	1.57E+02	<5.56E+01	1.04E+05	8.33E+00	6.08E+04	<5.56E+00	<1.53E+01	1.11E+02

^a 70-99% relative humidity, 18-26°C

^b colony forming units/mL

^c plague forming units/mL

^d most probable number/mL

Table 4. Inactivation of Microorganisms on the Surface of Lettuce in Dry Conditions^a

Time (days)	<i>E. coli</i> ATCC 25922 ^b	<i>Shigella sonnei</i> ^b	<i>Salmonella enterica</i> ^b	<i>Clostridium perfringens</i> ^b	<i>E. coli</i> O157:H7 ^b	PRD1 ^c	Feline calicivirus ^d	Hepatitis A virus ^d
0	2.96E+04	4.58E+04	2.78E+06	4.75E+05	2.39E+06	2.03E+08	1.11E+05	8.09E+02
0	2.43E+04	1.44E+04	2.17E+06	4.94E+05	2.50E+06	1.21E+08	1.61E+05	2.39E+03
1	<5.56E-01	<5.56E+01	2.78E+01	1.75E+05	2.78E+01	6.81E+07	3.51E+02	1.94E+03
1	8.33E+01	<5.56E+01	6.39E+02	1.69E+05	<5.56E+01	7.03E+07	3.88E+02	1.53E+03
1	<5.56E-01	7.78E+02	5.56E+02	1.67E+05	2.78E+02	8.56E+07	1.53E+03	1.94E+03
3	1.28E+01	<5.56E+01	<5.56E+01	3.28E+04	<5.56E+01	2.17E+07	<1.53E+01	2.78E+02
3	<5.56E-01	9.92E+03	5.56E+01	3.06E+04	<5.56E+01	9.97E+07	<1.53E+01	8.09E+02
3	<5.56E-01	<5.56E+01	<5.56E+01	6.56E+04	5.56E+01	5.11E+07	3.88E+01	6.19E+01
5	<5.56E-01	<5.56E+01	<5.56E+01	3.33E+03	<5.56E+01	4.69E+06	<1.53E+01	1.53E+02
5	<5.56E-01	<5.56E+01	<5.56E+01	4.69E+04	<5.56E+01	2.61E+07	<1.53E+01	2.15E+01
5	<5.56E-01	<5.56E+01	2.78E+02	3.19E+04	<5.56E+01	2.75E+07	<1.53E+01	3.35E+01
7	<5.56E-01	<5.56E+01	1.03E+03	6.03E+03	<5.56E+01	2.47E+07	<1.53E+01	4.45E+01
7	<5.56E-01	<5.56E+01	<5.56E+01	6.50E+03	<5.56E+01	1.06E+07	<1.53E+01	1.53E+02
7	<5.56E-01	<5.56E+01	<5.56E+01	4.11E+03	<5.56E+01	6.61E+06	<1.53E+01	3.06E+01
10	NS	NS	<5.56E+01	1.06E+04	NS	1.75E+07	<1.53E+01	1.94E+01
10	NS	NS	<5.56E+01	6.39E+03	NS	5.42E+06	<1.53E+01	1.61E+01
10	NS	NS	<5.56E+01	2.17E+03	NS	6.36E+06	<1.53E+01	5.16E+01
14	NS	NS	<5.56E+01	3.97E+03	NS	2.94E+06	<1.53E+01	<1.92E+00
14	NS	NS	<5.56E+01	2.89E+03	NS	5.56E+06	<1.53E+01	5.72E+01
14	NS	NS	<5.56E+01	5.75E+03	NS	1.69E+07	<1.53E+01	8.09E+01

NS – No Sample

^a 30-60% relative humidity, 21-26°C^b colony forming units/mL^c plaque forming units/mL^d most probable number/mL

Table 5. Inactivation of Microorganisms on the Surface of Lettuce in Humid Conditions^a

Time (days)	<i>E. coli</i> ATCC 25922 ^b	<i>Shigella sonnei</i> ^b	<i>Salmonella enterica</i> ^b	<i>Clostridium perfringens</i> ^b	<i>E. coli</i> O157:H7 ^b	PRD1 ^c	Feline calicivirus ^d	Hepatitis A virus ^d
0	7.22E+06	8.64E+05	4.11E+06	2.83E+05	7.19E+06	1.94E+08	2.78E+05	3.51E+04
0	7.85E+06	2.07E+05	4.11E+06	3.06E+05	2.89E+06	1.78E+08	8.09E+04	3.51E+04
1	7.85E+02	<5.56E+01	5.28E+04	2.03E+04	<5.56E+01	6.33E+07	2.39E+04	1.53E+04
1	5.56E-01	2.31E+04	2.22E+04	3.06E+05	5.56E+01	8.36E+07	1.99E+02	1.11E+04
1	9.63E+02	4.89E+03	4.89E+05	4.53E+04	8.33E+01	7.08E+07	1.11E+03	2.16E+02
3	<5.56E-01	<5.56E+01	1.10E+06	1.75E+04	<5.56E+01	3.58E+07	<1.53E+01	1.61E+02
3	<5.56E-01	<5.56E+01	4.67E+05	1.50E+04	<5.56E+01	4.25E+07	1.74E+02	5.16E+02
3	<5.56E-01	<5.56E+01	2.63E+06	1.53E+04	<5.56E+01	9.75E+07	<1.53E+01	8.09E+02
5	<5.56E-01	<5.56E+01	1.44E+06	3.19E+04	<5.56E+01	4.75E+07	<1.53E+01	5.16E+02
5	<5.56E-01	<5.56E+01	1.17E+05	3.75E+04	<5.56E+01	3.39E+07	<1.53E+01	8.09E+01
5	<5.56E-01	<5.56E+01	1.61E+05	0.00E+00	<5.56E+01	2.14E+07	<1.53E+01	3.06E+02
7	<5.56E-01	<5.56E+01	2.54E+06	2.19E+03	NS	1.05E+07	<1.53E+01	2.16E+02
7	<5.56E-01	<5.56E+01	2.00E+06	3.31E+03	NS	1.35E+07	<1.53E+01	3.00E+02
7	<5.56E-01	<5.56E+01	2.94E+06	0.00E+00	NS	8.06E+06	<1.53E+01	6.19E+01
10	NS	NS	1.80E+06	4.17E+03	NS	<2.78E+00	<1.53E+01	<1.92E+00
10	NS	NS	2.46E+06	5.00E+03	NS	9.31E+06	<1.53E+01	8.09E+01
10	NS	NS	3.00E+06	5.00E+03	NS	5.33E+06	<1.53E+01	<1.92E+00
14	NS	NS	8.33E+05	4.17E+03	NS	5.78E+03	<1.53E+01	<1.92E+00
14	NS	NS	1.74E+06	2.78E+03	NS	2.67E+06	<1.53E+01	<1.92E+00
14	NS	NS	1.46E+06	4.50E+03	NS	5.11E+05	<1.53E+01	<1.92E+00

NS – No Sample

^a 70-97% relative humidity, 22-26°C^b colony forming units/mL^c plaque forming units/mL^d most probable number/mL

Table 6. Inactivation of Microorganisms on the Surface of Pepper in Dry Conditions^a

Time (days)	<i>E. coli</i> ATCC 25922 ^b	<i>Shigella sonnei</i> ^b	<i>Salmonella enterica</i> ^b	<i>Clostridium perfringens</i> ^b	<i>E. coli</i> O157:H7 ^b	PRD1 ^c	Feline calicivirus ^d	Hepatitis A virus ^d
0	1.11E+05	9.44E+03	1.18E+07	3.28E+04	9.44E+05	2.42E+09	2.16E+05	3.51E+03
0	2.28E+06	1.67E+04	4.78E+06	3.44E+04	1.94E+06	3.11E+09	5.08E+05	4.12E+03
1	2.13E+03	1.81E+04	4.22E+03	2.69E+04	3.06E+04	1.13E+09	NS	3.51E+03
1	5.56E+01	<5.56E+01	2.42E+03	2.72E+04	4.22E+04	7.36E+08	5.16E+03	2.62E+03
1	5.56E+01	7.22E+02	1.47E+03	3.53E+04	2.28E+04	8.14E+08	1.11E+04	5.72E+03
3	1.11E+00	<5.56E+01	5.83E+03	2.08E+04	5.83E+03	8.19E+08	3.18E+02	2.16E+03
3	9.72E+00	<5.56E+01	<5.56E+01	1.86E+04	5.56E+01	5.97E+08	NS	NS
3	3.26E+01	<5.56E+01	<5.56E+01	4.00E+04	5.56E+01	4.25E+08	4.03E+02	1.11E+03
5	<5.56E-01	1.94E+02	<5.56E+01	1.75E+04	5.56E+01	6.42E+07	<1.53E+01	1.11E+03
5	2.44E+01	<5.56E+01	3.28E+04	1.47E+04	5.56E+01	1.67E+08	NS	NS
5	<5.56E-01	<5.56E+01	<5.56E+01	2.33E+03	5.56E+01	2.19E+08	<1.53E+01	1.11E+03
7	<5.56E-01	1.11E+02	<5.56E+01	2.06E+03	4.72E+03	9.58E+07	<1.53E+01	6.19E+02
7	<5.56E-01	<5.56E+01	4.11E+04	2.56E+03	3.39E+04	2.22E+08	NS	NS
7	<5.56E-01	<5.56E+01	<5.56E+01	3.33E+03	5.56E+01	2.28E+07	<1.53E+01	3.00E+02
10	<5.56E-01	<5.56E+01	6.39E+02	2.94E+03	5.56E+01	1.72E+08	<1.53E+01	6.19E+02
10	<5.56E-01	<5.56E+01	<5.56E+01	4.56E+02	5.56E+01	1.05E+08	<1.53E+01	6.19E+02
10	<5.56E-01	<5.56E+01	1.11E+02	1.36E+03	5.56E+01	4.06E+07	<1.53E+01	NS
14	NS	NS	2.11E+04	2.22E+03	2.53E+04	2.14E+07	<1.53E+01	1.99E+02
14	NS	NS	<5.56E+01	1.92E+02	5.56E+01	2.17E+05	<1.53E+01	1.11E+02
14	NS	NS	<5.56E+01	2.58E+03	5.56E+01	3.47E+07	<1.53E+01	NS

NS – No Sample

^a 39-60% relative humidity, 21-26°C^b colony forming units/mL^c plaque forming units/mL^d most probable number/mL

Table 7. Inactivation of Microorganisms on the Surface of Pepper in Humid Conditions^a

Time (days)	<i>E. coli</i> ATCC 25922 ^b	<i>Shigella sonnei</i> ^b	<i>Salmonella enterica</i> ^b	<i>Clostridium perfringens</i> ^b	<i>E. coli</i> O157:H7 ^b	PRD1 ^c	Feline calicivirus ^d	Hepatitis A virus ^d
0	2.42E+05	4.89E+04	1.04E+06	3.08E+04	1.54E+06	2.06E+09	4.03E+05	6.19E+02
0	1.25E+05	5.47E+04	4.86E+06	1.97E+04	2.18E+06	1.08E+09	6.19E+04	4.45E+02
1	5.56E-01	1.11E+02	5.56E+01	2.19E+04	5.56E+01	1.92E+09	4.03E+03	3.51E+02
1	4.72E+01	2.81E+03	5.56E+01	2.28E+04	5.56E+01	4.31E+09	8.09E+04	6.19E+02
1	5.56E+00	1.60E+04	1.14E+03	2.81E+04	2.78E+02	2.42E+09	6.19E+04	1.74E+03
3	<5.56E-01	<5.56E+01	1.22E+03	1.81E+04	6.67E+02	7.03E+08	1.11E+03	1.11E+03
3	<5.56E-01	<5.56E+01	<5.56E+01	1.78E+04	<5.56E+01	1.69E+09	<1.53E+01	5.16E+01
3	<5.56E-01	<5.56E+01	<5.56E+01	1.86E+04	<5.56E+01	3.00E+08	<1.53E+01	2.32E+01
5	<5.56E-01	<5.56E+01	<5.56E+01	1.39E+04	<5.56E+01	7.19E+08	<1.53E+01	6.19E+01
5	<5.56E-01	<5.56E+01	<5.56E+01	2.33E+04	<5.56E+01	2.19E+09	8.09E+01	1.11E+02
5	<5.56E-01	<5.56E+01	<5.56E+01	1.64E+04	6.39E+02	1.86E+09	<1.53E+01	1.11E+02
7	<5.56E-01	<5.56E+01	<5.56E+01	2.69E+04	<5.56E+01	1.42E+09	<1.53E+01	5.16E+01
7	<5.56E-01	<5.56E+01	<5.56E+01	5.00E+03	<5.56E+01	8.14E+08	<1.53E+01	4.42E+00
7	<5.56E-01	<5.56E+01	<5.56E+01	1.19E+04	<5.56E+01	1.16E+09	<1.53E+01	5.60E+00
10	NS	NS	<5.56E+01	1.56E+04	<5.56E+01	6.28E+08	<1.53E+01	5.60E+00
10	NS	NS	4.44E+02	3.92E+03	2.39E+03	9.08E+08	<1.53E+01	5.60E+00
10	NS	NS	<5.56E+01	2.11E+04	<5.56E+01	2.42E+08	<1.53E+01	4.42E+00
14	NS	NS	<5.56E+01	2.81E+03	<5.56E+01	2.25E+07	<1.53E+01	4.42E+00
14	NS	NS	<5.56E+01	2.58E+03	5.56E+01	2.06E+08	<1.53E+01	2.51E+00
14	NS	NS	<5.56E+01	2.33E+03	5.56E+01	8.11E+08	<1.53E+01	2.51E+00

NS – No Sample

^a 70-96% relative humidity, 22-26°C^b colony forming units/mL^c plaque forming units/mL^d most probable number/mL