Effects of environmental conditions on transfer rates of *Salmonella enterica* and *Escherichia coli* O157:H7 from soil, dust and irrigation water to iceberg lettuce surfaces

Sadhana Ravishankar and Govindaraj Dev Kumar

Report Submitted to the Arizona Iceberg Lettuce Research Council

Introduction

It is estimated that there are 48 million illnesses, 128,000 hospitalizations and 3,000 deaths each year due to foodborne causes in the United States (Scallan et al., 2011). Produce associated outbreaks have increased over the last three decades and leafy greens have been determined as a vehicle of Salmonellosis (Hanning et al., 2009). Outbreaks caused by uncooked produce such as lettuce and other salad greens can result in massive economic losses due to drop in sales and recalled product (Hanning et al., 2009). Understanding the routes of contamination from farm to fork is imperative in developing preventative measures. Leafy greens can get contaminated during contact with soil, irrigation events, flooding and dust storms.

The desert region, while providing the United States and Canada with produce during the winter season, also presents contamination challenges that are unique. Relative Humidity (RH) could play an important role in the cross-transfer of pathogens from soil to leaf surface. More than 80% of the lettuce/spinach supply for the nation in winter is grown in Yuma. Arizona farmers produce about 90,000 acres of lettuce whose value exceeds $304 million. A significant portion of this is iceberg lettuce.

The relative humidity of the desert region usually ranges between very dry (13%) to mildly humid (63%) (weatherspark.com). Changes in humidity could occur suddenly before a rainfall event resulting in faster deposition of dust particles or could also influence the transfer of microorganisms from soil to contact surface. Rainfall and irrigation can also influence the transfer of bacteria to leaf surfaces, as increased soil moisture can result in reduced adhesion of loosely attached bacterial cells. Rain runoff, underground water, surface water currents can all aid in the dissemination of *Salmonella* in agricultural soil and sediment (Chao et al. 1987). Under certain conditions, percolation of contaminated water through soil filters out bacteria that can persist in the field environment. *Salmonella* has been isolated from soils in agricultural areas and adhesion to soil particles might be related to cell surface hydrophobicity (Stenstrom et al., 1989). Soil and sediment could act as reservoirs of organic molecules for bacterial nutrition or a substratum for attachment and hence serve as a niche for pathogenic bacteria (Thomason et al., 1977, Winfield et al., 2003). The potential of wind currents in disseminating bacteria attached to soil and dust to plant surfaces requires to be explored. Understanding attachment strategies to soil particles could help understand how *Salmonella* spp. persist in the field and are transferred from host to host. There was an increased recovery rate of *Salmonella* from bottom sediments than surface water when tested for the presence of the pathogen (Hendricks et al., 1971). The adhesive property of the pathogen could have resulted in better recovery since dissemination is more rapid on the surface (Hendricks et al., 1971). These factors make it imperative to understand the transfer rates of *Salmonella* to leaf surfaces during flooding or irrigation events.

Salmonella serotypes and *Escherichia coli* can associate themselves with particulate matter and can be dispersed from their source. Water, soil, and manure are suspected as sources of contamination on the field. It has been shown that these can be aerosolized and lead to pathogen spread (Brandl et al, 2006, Milner et al., 2009). The potential of airborne or dust associated *Salmonella* serotypes as a contamination threat in agricultural environments requires to be evaluated. Different environments could give rise to aerosols harboring bacteria. Animal

and poultry bird confinement, piles of manure and cattle feedlot operations have been known to increase the overall microbial load in the immediate environment. Agricultural practices such as spray irrigation of waste water and land application of biosolids or manure could lead to the formation and dispersal of bioaerosols (Milner et al., 2009). Biological materials in air could include particulate material containing bacteria that does not necessarily occur as individual particles and could be associated with dust.

In our study, irrigated soil and dust under different temperature and relative humidity combination conditions were evaluated for their cross contamination potential. Two pathogens of concern in produce safety, *E. coli* O157:H7 and *S. enterica* were inoculated into conventional and organic soil as well as dust. These were brought into contact with iceberg lettuce surfaces to study cross-transfer. The objective of this study was to determine cross contamination risks from environmental matrices to iceberg lettuce under conditions simulating the desert region for better risk evaluation in Arizona.

Methods

Bacterial culture preparation

Salmonella enterica serovar Newport and *Escherichia coli* O157:H7 were used. Antibiotic resistance has been developed in these isolates in our laboratory by incremental increase in ampicillin and streptomycin exposure, resulting in resistance to both antibiotics. The antibiotic resistance pattern of these isolates provides their efficient traceability in environmental samples such as soil, compost and irrigation water. Growth rate of these isolates is similar to those of non-resistant serotypes. Bioluminescent imaging was performed using bioluminescent *Salmonella* Newport N78 tagged with pAKlux1 plasmid. The pAKlux1 tagged *S.* Newport was cultured and isolated in media containing 25 µg/ml ampicillin to maintain selective pressure. For each experiment, a fresh overnight culture of each of these isolates were washed twice and diluted to the required initial inoculum level of 8 log CFU/ml.

Preparation and inoculation of soil and dust particulates

Conventional and organic soils were obtained from Yuma Arizona. The soil was passed through a No. 20 sieve (U.S.A Standard Testing Sieve) to obtain a uniform grain size for dust. The soil sample was initially screened for the presence of *Salmonella* and *E. coli* O157:H7 by plating on xylose lysine desoxycholate (XLD) agar, sorbitol MacConkey agar (SMAC) and Chromagar O157:H7^{TM} with antibiotics as needed, depending on interference from the background microbiota. Ten gram soil or dust aliquots were stored in sterile centrifuge tubes at 4°C until use. Each soil or dust sample was mixed with a pellet of washed culture of *S.* Newport or *E. coli* O157:H7 by manually mixing it with a sterile hockey stick in a sterile glass container to achieve inoculum levels of 7 log CFU/g. Controls included uninoculated soil or dust.

Soil irrigation conditions

Three different irrigation treatments were used to simulate conditions post irrigation or rainfall- Heavy flood (250% water); Very wet soil- (100% water); and post irrigation soil (25% water). These experiments were performed at 26°C.

Preparation of leaf samples

Organic iceberg lettuce heads were purchased from a local grocery store in Tucson, AZ. The third outer leaf of the lettuce head was used to obtain 8 discs of 1 cm diameter each. The discs were cut from the abaxial portion of the leaf using a sterile cork borer. The leaf was divided into 8 quadrants and 2 discs were obtained from each quadrant. The discs were stored in a sterile petri dish until contact with the soil or dust on the abaxial side was facilitated.

Effect of environmental factors on the transfer of S. *Newport and* E. coli *O157:H7 by soil, dust and soil moistened with contaminated irrigation water*

Environmental conditions evaluated

Each experiment was performed under three combinations of temperature and relative humidity (RH) conditions (26^{o}C, 40% RH; 10^{o}C, 60% RH and 4^{o}C, 60% RH), and the transfer rates of pathogens were compared. The soil or dust samples and leaves were incubated at these conditions for 1 h prior to the initiation of the experiment.

Transfer from organic and conventional soils

Ten grams of inoculated conventional or organic soil was placed in a sterile petri dish. Contact with each of these soils was facilitated by placing iceberg lettuce leaf on the respective soil for 5 or 60 minutes. The lid of the petri dish was left open to expose the samples to the respective temperature-relative humidity combination environment. Post contact, leaf discs was sampled for enumeration of bacteria transferred to the leaf.

Transfer from dust

One hundred milligrams of inoculated dust was placed on the edge of a sterile spatula. A Nalgene aerosol spray bottle was used to produce pressurized air to simulate wind-based dust dispersal. The nozzle of the sprayer and the dust was placed 30 cm away from 10 g of iceberg lettuce leaf sample and pressurized air was released to deliver the dust on to iceberg lettuce leaf surface. Dust-dispersed inoculation of iceberg lettuce leaves was performed in a biosafety cabinet. Appropriate face masks were worn by the lab personnel conducting the experiment.

Transfer from soil moistened with contaminated irrigation water

Ten grams of conventional or organic soil (inoculated) were placed in a sterile glass container. Tap water at 250%, 100% and 25% (vol of water/weight of soil) was added to the soil and mixed thoroughly using a sterile hockey stick. The soil sample was then transferred to an open sterile petri dish and exposed to the respective temperature-relative humidity combination environment for 1 h. Contact with each of these soils was facilitated by placing iceberg lettuce leaf discs on the respective soil for 5 or 60 minutes. The lid of the petri dish was left open throughout the course of the experiment. Post contact, leaf discs were sampled for enumeration of bacteria transferred to the leaf.

Microbiological analysis of soil, dust and lettuce leaves

Soil, dust and lettuce disc samples were analyzed for *S.* Newport and *E. coli* O157:H7 populations after each contact event. Buffered peptone water was added to the samples in WhirlPak bags and mixed using a stomacher at normal speed for 1 min. The samples were further serially diluted as needed using 0.1% peptone water and aliquots plated on XLD or SMAC containing ampicillin and streptomycin. Plates were incubated at 37^{o}C for 24-48 hrs and colony forming units were counted.

Determination of Transfer Rates

The transfer rates from soil, compost, compost amended soil and water were calculated based on the following formula (Ravishankar et al., 2010):

$$\% \text{ transfer rate} = \frac{\text{Population of pathogen on destination}}{(\text{Population of pathogen on source} + \text{Population of pathogen on destination})} \times 100$$

where populations of each pathogen were determined by stomaching or mixing and spread plating lettuce samples, dust samples and irrigation water samples with appropriate dilutions on XLD or SMAC agar supplemented with ampicillin and streptomycin.

Imaging the dispersion and distribution of S. *Newport on iceberg lettuce*

The dispersion and distribution of *S.* Newport on the surface of iceberg lettuce was imaged using biophotonic imaging. Inoculation of lettuce surfaces was performed using a similar protocol as described previously. The inoculation was conducted using luminescent *S.* Newport developed in our laboratory. Imaging was conducted using an AMI 1000 imager to determine spatial distribution of the pathogen. The imaging of the iceberg lettuce will help growers understand which areas of the leaf, such as the adaxial surface, stem, veins etc, are most susceptible to contamination by *Salmonella* and will help them to determine if improvement is needed in their sanitization practices.

Study design:

The study was designed as a randomized block factorial treatment arrangement.

Results

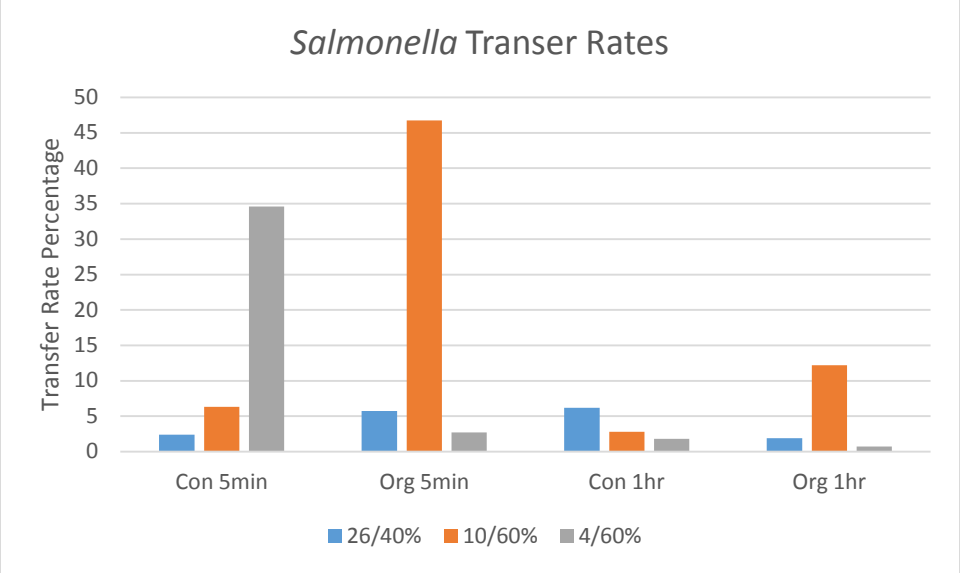
Effect of temperature and humidity on Salmonella Cross Transfer

With 40% RH at 26°C, the transfer rate of *S.* Newport to lettuce surfaces differed between conventional soil and organic soil. After 5 min contact time, the transfer of *S.* Newport from conventional soil to iceberg lettuce surface was 2.5±0.09%. The transfer of *S.* Newport from organic soil to iceberg lettuce surface was 5.75±3.02% after 5 min. After 1 hour of exposure of iceberg lettuce leaf to conventional soil contaminated with *S.* Newport a transfer of 6.17±3.34% was observed. From organic soil, a transfer of 1.89±1.00% was observed on iceberg lettuce discs **(Fig. 1).**

Transfer rates from soil to iceberg lettuce discs were measured at 10°C with 60% humidity. Contact with organic soil resulted in higher transfer rates of *S.* Newport in comparison to conventional soil. Contact of iceberg lettuce discs with organic soil for 5 minutes resulted in a transfer of 46.76±29.20%, while a duration of 1 hour resulted in a transfer rate of 12.18±6.60% **(Fig. 1).** The transfer of *S.* Newport from conventional soil to iceberg lettuce surface was 6.33±3.55% after 5 min. After 1 hour of exposure of iceberg lettuce leaf to conventional soil contaminated with *S.* Newport a transfer of 2.79±2.5% was observed.

At 4°C with 60% RH, transfer from conventional soil to iceberg lettuce discs after 5 min of exposure was 34.6±12.6%. The transfer from organic soil after 5 min of contact with iceberg lettuce discs was 2.72±0.77% after 5 min. After an hour of exposure, the transfer of *S.* Newport from conventional soil to lettuce discs was 1.83±0.53% while the transfer from organic soil to lettuce surface was 0.74+0.29% **(Fig. 1).** At 4°C with 60% RH, transfer rates of *S.* Newport were higher in conventional soil than in organic soil.

Fig. 1. Transfer rate percentages of *S.* Newport to iceberg lettuce leaf from conventional soil (Con) and organic soil (Org) at 26°C/40% humidity, 10°C/60% humidity, and 4°C/60% humidity.



Effect of Irrigation on Salmonella cross transfer to lettuce surfaces

To simulate a flood scenario, 250% irrigation water was added to soil containing *S.* Newport. The transfer rates of *S.* Newport from organic soil and conventional soil to lettuce disc after 5 min of exposure were 33.23±7.99% and 13.73±2.92%, respectively. After one hour contact time the transfer rate percentage from organic soil to lettuce leaf surface was 23.78%. Two other repetitions from the same scenario did not yield detectable transfer to leaf surface from organic soil due to a possible lack of contact with soil surface resulting from floating of discs **(Fig. 2).** Contact of iceberg lettuce disc with conventional soil surface for an hour resulted in 52.8±5.53% transfer rate.

Fig. 2: Iceberg lettuce discs in heavily flooded soil

When 100% (10 ml water to 10g soil) irrigation water was added to the soil to simulate wet soil, post irrigation, the transfer rates of *S.* Newport from conventional soil and organic soil after 5 min of exposure were 34.68±8.80% and 2.19±0.76%, respectively. The transfer of *S.* Newport from conventional and organic soil after 1 hour of exposure was 22.12±1.61% and 19.89±1.99%, respectively.

The addition of 25% (2.5 ml water to 10g soil) irrigation water to conventional and organic farm soil was performed to simulate wet soil, post irrigation. The transfer rates of *S.* Newport from conventional and organic soil after 5 min of exposure were 10.71±5.84% and 7.26±5.75%, respectively. Contact of iceberg lettuce disc with conventional soil surface for an hour resulted in 7.74±2.34% transfer. After one hour contact time, the transfer rate from organic soil to lettuce leaf surface was 5.83±4.87%.

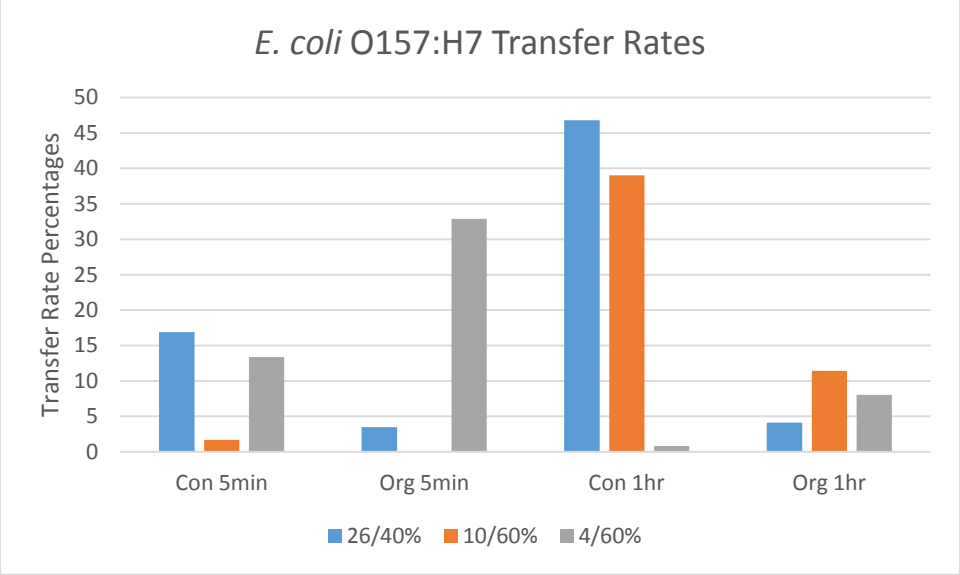
Effect of temperature and relative humidity on E. coli O157:H7 Cross Transfer

With 40% RH at 26°C, the transfer rate of *E. coli* O157:H7 to lettuce surfaces varied between conventional soil and organic soil. After 5 min contact time, the transfer of *E. coli* O157:H7 from conventional soil to iceberg lettuce surface was 16.98±13.58%. The transfer of *E. coli* O157:H7 from organic soil to iceberg lettuce surface was 3.56% after 5 min. After 1 hour of exposure of iceberg lettuce leaf to conventional soil contaminated with *E. coli* O157:H7, a transfer rate of 46.79% was observed. From organic soil, a transfer rate of 4.12±2.28% was observed on iceberg lettuce discs **(Fig. 3).**

Transfer rates from soil to iceberg lettuce discs were measured at 10°C with 60% humidity. Contact of iceberg lettuce discs with organic soil for a duration of 1 hour resulted in a transfer of 11.42±6.99%. The transfer of *E. coli* O157:H7 from conventional soil to iceberg lettuce surface was 1.68±1.44% after 5 min and 39.05% after 1 h **(Fig. 3).**.

At 4°C with 60% RH, transfer rate from conventional soil to iceberg lettuce discs after 5 min of exposure was 13.37±7.83%. The transfer from organic soil after 5 min of contact with iceberg lettuce discs was 32.87±6.08%. After an hour of exposure, the transfer of *S.* Newport from conventional soil to lettuce discs was 0.83±0.53%, while the transfer from organic soil to lettuce surface was 8.05+1.64% **(Fig. 3)**. At 4°C with 60% RH, transfer rates of *S.* Newport were higher in organic soil than in conventional soil. Loss of cell viability was observed in soil at 4°C with 60% RH.

Fig. 3. Transfer rate percentages of *E. coli* O157:H7 to iceberg lettuce leaf from conventional soil (Con) and organic soil (Org) at 26°C/40% humidity, 10°C/60% humidity, and 4°C/60% humidity.



Effect of Irrigation on E. coli O157:H7 cross transfer to lettuce surfaces

To simulate a flood scenario, 250% (25 ml water to 10 g soil) irrigation water was added to soil containing *E. coli* O157:H7. The transfer rates of *E. coli* O157:H7 from conventional soil and organic soil after 5 min of exposure were 43.92±29.39% and 57.08±17.46%, respectively. Contact of iceberg lettuce disc with conventional soil surface for an hour resulted in 60.94±15.92% transfer rate. After one hour contact time, the transfer rate from organic soil to lettuce leaf surface was 12.03±11.81%.

When 100% (10 ml water to 10g soil) irrigation water was added to the soil, the transfer rates of *E. coli* O157:H7 from conventional soil and organic soil after 5 min of exposure were 43.92±29.39% and 43.92±21.39%, respectively. The transfer rates of *E. coli* O157:H7 from conventional and organic soil after 1 hour of exposure were 60.94±15.12% and 12.03±11.18%, respectively.

The addition of 25% (2.5 ml water to 10g soil) irrigation water to conventional and organic farm soil was performed to simulate wet soil, post irrigation. The transfer rates of *E. coli* O157:H7 from conventional soil and organic soil after 5 min of exposure were 0.4±0.23% and

0.37±0.07%, respectively. Contact of iceberg lettuce disc with conventional soil surface for an hour resulted in 25.38±1.94% transfer rate. After one hour contact time, the transfer rate from organic soil to lettuce leaf surface was 40.06±5.63%.

Transfer of S. Newport to leaf surfaces after dust contact

The transfer of *S.* Newport to lettuce leaf after contact with dust was determined. Brief contact of leaf at 40% RH with conventional and organic dust resulted in populations of 4.08±0.04 and 4.03±0.06 Log CFU/g of *S.* Newport on iceberg lettuce leaves. *S.* Newport was not detected in dust particles indicating a possible loss of viability, as pathogen was observed on leaf surface after contact. Testing soon after inoculation indicated that organic and conventional soil dust had *S.* Newport populations of 7.71 Log CFU/g and 7.43 Log CFU/g, respectively

Biophotonic imaging of pathogen dispersion on lettuce leaf post contact with dust was performed. Contact with organic dust and conventional dust, indicated that transfer occurred immediately after contact. Pressurized air was used as a dispersal aid to simulate a gust of wind. The color schematics of the leaf **(Fig. 4 and Fig. 5)** indicated that certain parts of the leaf hosted higher numbers of pathogen as more photons were emitted from the region (red color- $4x10^7$ photons per pixel; yellow color-3.5 $x10^7$ photons per pixel; and green color 3.0 $x10^7$ photons per pixel).

Fig. 4. Distribution of *S.* Newport on iceberg lettuce leaf post contact with organic soil dust

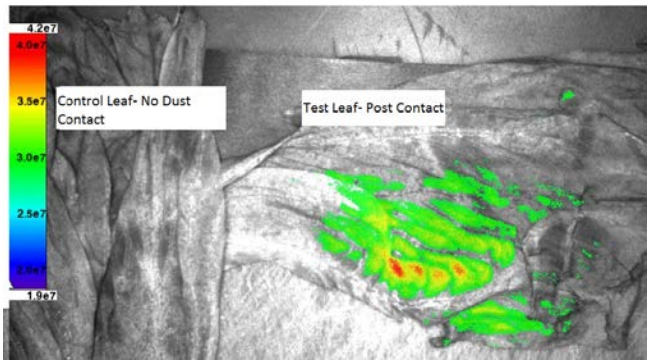
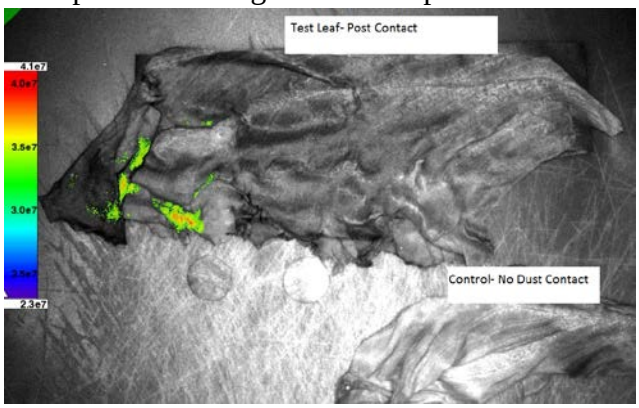


Fig. 5. Distribution of *S.* Newport on iceberg lettuce leaf post contact with conventional soil dust



Transfer of E. coli O157:H7 to leaf surfaces after dust contact

Contact of iceberg lettuce leaf with organic and conventional dust inoculated with *E. coli* O157:H7 resulted in populations of 2.88±0.7 Log CFU/g and 4.66±0.44 Log CFU/g, respectively, on the leaf. No *E. coli* O157:H7 was detected in the dust indicating a possible loss in viability of cells. Immediately after inoculation, the populations of *E. coli* O157:H7 were 6.32 Log CFU/g and 6.44 Log CFU/g, in organic soil and conventional soil dust, respectively.

Discussion

The field environment is subject to changes in temperature, humidity and wind currents. The effects of these factors were observed in *E. coli* O157:H7 and *S.* Newport transfer to iceberg lettuce leaves. Among the different levels of relative humidity tested, 60% RH resulted in the highest transfer of *S.* Newport to lettuce leaf surface from organic soil after 5 minutes of contact. It should be noted that contact with both conventional and organic soils resulted in pathogen transfer to leaf tissue. Hence, heavily soiled produce could become contaminated by foodborne pathogens, irrespective of the type of soil. Inspection of leaf surface for soil and soil attachment is important. Transfer rates from organic and conventional soils indicate that humidity could play a role in pathogen attachment and also in the settling of dust. High populations (> 5 Log CFU/10g of leaf) of pathogens were transferred upon dust contact indicating that humidity of 40% might aid in pathogen transfer from dust to soil surface. The dust had a population of 7 Log CFU/g. A previous study performed in our laboratory using dust containing 9 Log CFU/g indicated that without high RH, the transfer rate of *S.* Newport was lower. In the previous study, contact with leaf surface by dust particulates at lower relative humidity (Approx. 15%) resulted in a population of 3.61±0.23 log CFU/g retention of *S.* Newport on the leaf.

Flooding and contact of produce surfaces with freshly irrigated soil could also serve as a source of foodborne pathogen transfer. Higher transfer rates were generally observed in leaves that came in contact with flooded soils containing *E. coli* O157:H7 than *S.* Newport. Animals are the primary hosts of *Salmonella* and the enteric pathogen possesses genes to invade, survive host cells and resist immune defense mechanisms (Wallis, 2000). *Salmonella* also has genes that confer fitness in non-host environments. There are more than 1000 genes in *Salmonella* with unknown functions. Some of these genes could be involved in aiding the pathogen's survival in soil and water. The alternative sigma factor RpoS is expressed both in *E. coli* and *Salmonella* during exposure to sea water. The alternative sigma factor is activated during stress responses such as those encountered in a non-host environment. *E. coli* with the RpoS deleted had a 1000 fold decrease in culturable cells, while *Salmonella* cells had only a 10 fold decrease after both organisms were exposed to seawater for 8 days. This indicates that *Salmonella* might be more adapted to survival in adverse environmental conditions and might have additional genetic machinery aiding it's survival in the environment (Winfield et al., 2003). Though splashing and rainfall events are also associated with higher rates of contamination in produce, contact with irrigated soil could occur more frequently and hence, also be considered as a route of contamination. Rainwater runoff, underground water, surface water currents can all aid in the

dissemination of *Salmonella* in agricultural soil and sediment (Chao et al., 1987). Under certain conditions, percolation of contaminated water through soil filters out bacteria that can persist in the field environment. *Salmonella* has been isolated from soils in agricultural areas and adhesion to soil particles might be related to cell surface hydrophobicity (Stenstrom et al., 1987). Soil and sediment could act as reservoirs of organic molecules for bacterial nutrition or a substratum for attachment and hence, serve as a niche for pathogenic bacteria.

When cross contamination with dust was studied, it was observed that both *S.* Newport and *E. coli* O157:H7 lost viability in dust. This could be because of sudden desiccation in dust. When enteric pathogens such as *E. coli* O157:H7 and *S.* Newport are shed into the environment by infected hosts, they have to survive in soil, manure, or irrigation water. Stresses associated with survival in agricultural waters could include solar radiation, temperature and starvation, which may result in the activation of a unique survival strategy associated with metabolic modulation known as the viable but non culturable (VBNC) state. Presence of VBNC foodborne pathogens in foods and food production environments could directly influence food safety as detection on microbiological media or enrichment using broth before PCR-based detection is not possible. Thus, potential vehicles of crop contamination such as dust, soil and irrigation water could become sources of undetectable pathogens.

Conclusions

- Contact of iceberg lettuce with pathogen contaminated soils that are irrigated or flooded could result in cross-contamination.
- Humidity can increase cross-contamination of lettuce leaves when these come in contact with contaminated soil (conventional or organic soil).
- Conventional farm soil contaminated with *E. coli* O157:H7 resulted in the highest % transfer rate of the pathogen to lettuce leaf surface after one hour exposure at 26°C with 40% humidity.
- Organic farm soil contaminated with *S.* Newport resulted in the highest % transfer rate of the pathogen to lettuce leaf surface after 5 min exposure at 10°C with 60% humidity.
- Humidity can increase cross-contamination of produce due to contact with conventional or organic dust. This is important because, dust from compost piles, manure piles, poultry facilities, and feed lots can contain foodborne pathogenic bacteria.
- Both *E. coli* O157:H7 and *Salmonella* can lose their ability to be detected in media when present in dust, possibly due to desiccation.
- Irrespective of the type of soil (conventional or organic), it would be important to test the produce after irrigation, flooding, dust storms and on days with higher than normal humidity, such as before a rainfall event.

Roadblocks and New Alternate Approaches

Traceability of foodborne pathogens in environmental matrices is challenging because of background microbiota. Leafy greens and soils can have 3-6 Log CFU/g of bacteria. To account for *Salmonella* and *E. coli* O157:H7 in the presence of native microbiota, antibiotic resistance is conferred to the organism, so that they can be isolated on microbiological media while

eliminating background microbiota. The development of antibiotic resistance in foodborne pathogens is performed by exposure to incremental concentrations of antibiotics. While development of dual antibiotic resistance in *S.* Newport was possible in 2 months, the development of dual antibiotic resistance in *E. coli* O157:H7 took a longer duration (4 months) because of physiological differences and antibiotic resistance mechanisms in species. Antibiotic resistance was developed by reducing increments and multiple subcultures, which necessitated testing additional antibiotic concentrations at smaller intervals.

References

Scallan, E., R. M. Hoekstra, F. J. Angulo, R. V. Tauxe, M. A. Widdowson, S. L. Roy, J. L. Jones, and P. M. Griffin. 2011. Foodborne illness acquired in the United States--major pathogens. *Emerg Infect Dis*. 17:7-15.

Chao, W., R. Ding, and R. Chen. (1987). Survival of pathogenic bacteria in environmental microcosms. *Chinese J. Microbial Immunol. (Taipei), 20*, 339-348.

Millner, Patricia D. (2009). Bioaerosols associated with animal production operations. *Bioresource Technology, 100*(22), 5379-5385. doi: 10.1016/j.biortech.2009.03.026

Stenstrom, T A. (1989). Bacterial hydrophobicity, an overall parameter for the measurement of adhesion potential to soil particles. *Appl. Environ. Microbiol., 55*(1), 142-147.

Thomason, B. M., D. J. Dodd, and W. B. Cherry. 1977. Increased recovery of salmonellae from environmental samples enriched with buffered peptone water. *Appl Environ Microbiol*. 34:270-3.

Brandl, M. T. (2006). Fitness of human enteric pathogens on plants and implications for food safety. *Annu Rev Phytopathol, 44*, 367-392. doi: 10.1146/annurev.phyto.44.070505.143359

Winfield, M. D., and E. A. Groisman. 2003. Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Appl Environ Microbiol*. 69:3687-94.

Hendricks, C. W**.** 1971. Increased recovery rate of salmonellae from stream bottom sediments versus surface waters. *Appl. Microbiol. 21:379-380.*

Ravishankar, S., Zhu, L., & Jaroni, D. (2010). Assessing the cross contamination and transfer rates of *Salmonella enterica* from chicken to lettuce under different food-handling scenarios. *Food microbiology*, *27*(6), 791-794.