International Food Research Journal 22(5): 1817-1823 (2015)
Journal homepage: *http://www.ifrj.upm.edu.my*

Survival and growth behaviour of *Salmonella enterica* serovar Typhimurium in lettuce leaves and soil at various temperatures

[1]Abd-Elall, A.M.M. and [2*]Maysa, A.I. A.

[1]*Department of Veterinary Public Health, Faculty of Veterinary Medicine, Zagazig University, 44511 Zagazig, Egypt*
[2]*Department of Zoonoses, Faculty of Veterinary Medicine, Zagazig University 44511 Zagazig, Egypt*

Article history

Received: 2 December 2014
Received in revised form: 13 January 2015
Accepted: 20 January 2015

Keywords

S. Typhimurium
Survival
Lettuce
Soil

Abstract

Over the last decades, leafy greens were involved in numerous *Salmonella* outbreaks. In order to control plant contamination, it is necessary to understand pathogen behaviour both on plant and in soil of producing fields. The objectives of this study were to monitor the growth and survival of *Salmonella* Typhimurium on lettuce leaves (uncut and shredded) stored at 4 and 22°C for up to 14 days and in soil (manure–amended and sterile) stored at 5 and 25°C for 42 days. Lettuce samples were inoculated with $1.5x10^6$ CFU g^{-1} of the microorganism and enumerated following storage for 0, 2, 6, 10, 14 days when held at 4°C and after 0, 2, 3 and 7 days when held at 22°C. Soil samples were inoculated with $5x10^7$ CFU g^{-1} of *S. Typhimurium* and enumerated after storage at 5 and 25°C at days 0, 7, 14, 21, 28, 35 and 42.The results showed that at the end of 4°C storage, populations of *S. Typhimurium* on uncut and shredded lettuce declined approximately one log CFU g^{-1}, however, at 22°C populations of the microorganism increased approximately 2 and 3 log CFU g^{-1} on uncut and shredded lettuce, respectively within 3 days. In addition, *S.* Typhimurium counts were found to be significantly higher on shredded than uncut lettuce stored at 22°C. In soil, population density of *S.* Typhimurium was found to decrease over time. The greatest and fastest decline in level of microorganism was recorded in manure-amended soil within 21 days of storage at 25°C, meanwhile, in sterile soil stored at 5°C the pathogen showed the lowest decrease and remained detectable up to end of examination (42 days). Significantly higher levels and survival of *S.* Typhimurium were detected in sterile soil than manure-amended soil and at 5°C than 25°C storage.

Introduction

The incidence of foodborne infection caused by bacterial pathogens continues to be a problem both in developing and industrialized countries (Lampel *et al*., 2000). These infections usually lead to economic losses and health problems particularly in the young, older and immuno-compromised peoples (Bailey, 1998). *Salmonella* is among the most commonly isolated pathogens associated with foodborne infections and its outbreaks were frequent in many countries like Korea (20.7%), Japan (14.2%) (Lee *et al*., 2001) and USA (Sivapalasingam *et al*., 2004). Outbreaks of salmonellosis however caused by numerous *Salmonella* serovars but *Salmonella* serovar Typhimurium and *Salmonella* serovar Enteritidis are the most frequently isolated serovars from foodborne outbreaks throughout the world (Tsen, 2002; Lim *et al*., 2003).

Over the last three decades, there is an increase in numbers of *Salmonella* outbreaks due to consumption of contaminated fresh produce particularly lettuce (Ercolani, 1976), celery (Burnett and Beuchat, 2000) and parsley (CDC, 2000). In addition, since these leafy green produces and their mixed salads are ready to eat food products usually eaten raw or mildly treated, so their contamination is of great health concern and their ingestion poses a serious salmonellosis risk. In the USA, eight lettuce-associated outbreaks were reported with foodborne pathogens including *Salmonella* from 1973 through 1997 (Sivapalasingam *et al*., 2004), whereas, in England and Wales, the national outbreak of multi-resistant *S.* Typhimurium was associated with consumption of lettuce (Horby *et al*., 2003).

Contamination of lettuce and other leafy green produces may occurred either during growth on farm through the use of contaminated soil, manure compost and irrigation water (Islam *et al.,* 2004) or during harvest, transport and further processing and handling (Sanchez *et al.*, 2012). Once contamination of lettuce with *Salmonella* takes place, *Salmonella*

*Corresponding author.
Email: *maysavet@hotmail.com*

cells adhered to each other and /or to the surface and embedded in a matrix of exopolymers forming biofilm (Costerton *et al.*, 1999). Due to the protection afforded to cells enclosed within this biofilm, *Salmonella* could survive and resist treatments and sanitizers (Lapidot *et al*., 2006). Even more, *Salmonella* may grow and reach high populations in such leafy green vegetables depending on storage condition (Sant'Ana *et al.*, 2012). Temperature during transportation and storage of lettuce and leafy vegetable appeared to be the main factor influencing the survival and growth of *Salmonella* (Vandamm *et al.*, 2013; Sant' Ana *et al*., 2014) together with condition of the leafy greens whether cut or uncut (Vandamm *et al*., 2013) damaged or healthy produce (Ansingkar and Kulkarni, 2013).

Soil is an environmental habitat in which *Salmonella* could persist. This particularly occurs when fresh manure from asymptomatic animal or improperly composted manure is spread on agricultural land (Pell, 1997). Many *Salmonella* outbreaks have been found associated with fruits and vegetables from soil with contaminated manure (CDC, 1991; CDC, 1999; Van Beneden, 1999). Furthermore, pathogens applied directly to plants were found to survive for shorter period of time than those applied to soil (Jones, 1986). In several studies, survival of *Salmonella* species in soil has been investigated and prolonged durations of persistence ranging from 5 weeks (Jensen *et al*., 2006) up to 231 days (Islam *et al.*, 2004) was reported. Numerous factors were found to influence the survivability of *Salmonella* species in soil and among them the incubation temperature (Guan and Holley, 2003; Garcia *et al.*, 2010), manure presence, competition with native soil microorganisms and protozoan predation (Garcia *et al*., 2010) were more important.

The present study was undertaken to investigate the survival and growth behaviour of *Salmonella enterica* serovar Typhimurium inoculated on uncut and shredded lettuce at 4 and 22°C for up to 14 days. Also, to investigate the survival and persistence of *S*. Typhimurium inoculated into sterile and manure-amended soil from leafy green producing fields at 5 and 25°C for 42 days.

Material and Methods

Bacterial strain and inoculum preparation

Salmonella Typhimurium strain resistant to tetracycline and nalidixic acid and originally isolated from lettuce was used in this study (Identified serologically and with PCR in Animal Health Research Institute, Dokki, Giza, Egypt). This strain was cultured on Tryptic soy agar plates containing 25 µg/ml tetracycline and 30 µg/ml nalidixic acid (Sigma- Aldrich) overnight at 37°C preceding the experiments. The obtained colonies were harvested into sterile distilled water and matched with standard McFarland 1.0 and adjusted till the same density (about $3x10^8$ CFU ml^{-1}). From the original suspension, serial dilutions in sterile distilled water were made.

Lettuce samples

Fresh lettuce heads were purchased from a local vegetable market in Zagazig city, Egypt. The outermost leaves of the lettuce heads were aseptically removed and the next 2 layers of leaves were detached and used for the experiment. The specimens of lettuce were examined bacteriologically and those exhibited absence of *Salmonella* species and no CFU of tetracycline/nalidixic acid resistant bacteria were used in this experiment. All lettuces were kept at 2–5°C from the time of purchase to initiation of experiments.

Soil samples

Soil samples were collected from leafy green producing fields in Zankalon village about 5 km from Zagazig city, Egypt. Top soil samples were collected on 2 occasions, before and after application of cattle manure fertilizer (2-3 kg/m^2) by using sterile spoon and sub-samples were taken from scattered locations within a 10 m^2 area and mixed thoroughly to form one pooled sample. The collected samples were packed separately in sterile plastic bags and stored frozen at -20°C. The soil samples were bacteriologically investigated and those found negative for both *Salmonella* species and tetracycline/nalidixic acid resistance bacteria were chosen for the experiment.

Samples preparation and Salmonella *species inoculation*

Lettuce

The selected lettuce leaves were washed three times with deionized water to remove soil and dust, and then were dried. The lettuce leave samples were divided into 2 parts, the first was left uncut, while the second part was shredded into 10-15 mm pieces with a sterile knife. Six replicates of uncut lettuce leaves (each of 100 gm) were spot inoculated, each with 5 ml of $3x10^7$ CFU ml^{-1} of prepared *S*. Typhimurium to achieve a final level of approximately $1.5x10^6$ CFU g^{-1}. The inoculated lettuce was then left to dry at ambient temperature for 1 hour in a laminar flow biosafety cabinet and packed in polyethylene plastic press-to-seal snack bags. Another six replicates of shredded

lettuce (each of 100 gm) were placed individually in sterile beakers and each was inoculated with 5 ml of $3x10^7$ CFU m^{-1} of *S.* Typhimurium suspension to achieve the final level of $1.5x10^6$ CFU g^{-1} of lettuce. Then they left to dry and packed in ten gram amounts in sterile polyethylene plastic press-to-seal snack bags. After packing three replicates from each of the inoculated uncut and shredded lettuce were stored at 4±2°C, meanwhile, the other replicates of uncut and shredded lettuce were stored at 22±2°C. Un-inoculated lettuce samples (uncut and shredded) were used as control.

Soil

In lab, the soil samples collected before application of cattle manure fertilizer were sterilized by autoclaving at 121°C for 15 minutes and then placed in 100 gm divisions (sterile soil) in sterile glass bottles with airtight glass stoppers, however, the soil samples amended with cattle manure (manure–amended soil) were placed directly into their sterile glass containers. Six replicates from each soil scenarios were inoculated separately in their containers, each with 20 ml of $3x10^8$ CFU ml^{-1} of prepared *S.* Typhimurium suspension with good mixing to produce $5x10^7$ CFU g^{-1} of soil. After inoculation, half of replicates from each soil scenarios (triplicate of sterile soil and triplicate of manure–amended soil) were incubated at 5±2°C, meanwhile the other half of replicates were incubated at 25±2°C. Un-inoculated soils of the two different soil scenarios were used as control.

Enumeration of Salmonella *Typhimurium*

S. Typhimurium population on inoculated lettuce and soil samples at different storage temperatures were enumerated on the following days. Lettuce samples incubated at 4±2°C were enumerated at days 0, 2, 6, 10 and 14, lettuce samples incubated at 22±2°C were enumerated at days 0, 2, 3 and 7, whereas soil samples incubated at 5±2°C and at 25±2°C were enumerated at days 0, 7, 14, 21, 28, 35 and 42 post inoculation. At each sampling point, 10 gm of inoculated lettuce or soil were removed from the storage containers and added to 90 ml of 0.1% peptone water and homogenized in a stomacher for one minute to make a 10-fold dilution. Then serial dilutions of each sample were made in a 0.1% peptone water and surface plated (0.1 ml) in duplicate onto selective and non-selective media containing tetracycline (25 µg/ml) and nalidixic acid (30 µg/ml). Xylose lysine deoxychlate agar (XLD, Oxoid CM04698) was used as a selective media and Tryptic soy agar (TSA, Merk, Germany) was used as a non-selective media (Vandamm *et al*., 2013). Inoculated plates were incubated at 37°C for 24 hours and the suspected colonies of the pathogen were counted. Three presumptive colonies were cultivated on Triple sugar iron agar (TSI, Oxoid CM0277B) at 37°C for 24 hours and confirmed biochemically (Koneman *et al*., 1992).

Statistical analysis

Statistical analysis was performed using the Statistical Package for Social Sciences version 21.0 (SPSS for Windows 21.0, Inc., Chicago, IL, USA). All *S.* Typhimurium counts were converted to the base – 10 logarithm of the number of colony forming units per gram of both lettuce and soil samples (log CFU g^{-1}).The significance of mean differences in terms of log CFU g^{-1} between uncut & shredded lettuce and between manure soil & sterile groups were tested using independent sample T-test. Results were expressed as Mean ± standard errors (SE). The value of $P < 0.05$ was used to indicate statistical significance.

Results

Survival and growth of S. *Typhimurium on uncut and shredded lettuce*

Uncut and shredded lettuce inoculated with $1.5x10^6$ CFU g^{-1} of *S.* Typhimurium were monitored for changes in population of such pathogen during storage at 4 and 22°C for up to 14 days. Investigation of inoculated lettuce was terminated when lettuce tissues began to liquefy and off odour appears. At 4°C, populations of *S.* Typhimurium on uncut and shredded lettuce declined approximately one log throughout the 14 days storage (Table 1). On uncut lettuce leaves, *S.* Typhimurium populations declined from 6.22 to 5.16 log CFU g^{-1}, whereas, on shredded lettuce, population of such pathogen declined from 6.28 to 5.31 log CFU g^{-1}. On contrast, at 22°C populations of *S.* Typhimurium inoculated onto uncut and shredded lettuce increased approximately 2 and 3 log CFU g^{-1}, respectively within three days, where on uncut lettuce, the count of microorganism raised from 6.25 to 8.24 log CFU g^{-1} and on shredded lettuce raised from 6.33 to 9.27 log CFU g^{-1}. In addition, *S.* Typhimurium counts were found to be significantly higher ($P < 0.05$) on shredded than uncut lettuce at 22°C storage.

Survival and growth of S. *Typhimurium in manure-amended and sterile soil*

Manure-amended and sterile soils inoculated with $5x10^7$ CFU g^{-1} of *S.* Typhimurium were followed up

Table 1: Survival and growth of *Salmonella* Typhimurium in uncut and shredded lettuce

Item	CFU g^{-1a}								
	4°C (days)					22°C (days)			
	0	2	6	10	14	0	2	3	7
Uncut lettuce	6.22±0.17[b]	5.60±0.18[b]	5.43±0.17[b]	5.32±0.15[b]	5.16±0.20[b]	6.25±0.12[b]	7.74±0.24[c]	8.24±0.23[c]	ND[d]
Shredded lettuce	6.28±0.22[b]	5.75±0.15[b]	5.59±0.16[b]	5.43±0.18[b]	5.31±0.16[b]	6.33±0.17[b]	8.69±0.14[b]	9.27±0.14[b]	ND[d]

[a] Mean values of replicate samples (n = 3)
[b, c] Means within the same column carrying these subscripts are significantly different at $P < 0.05$
[d] Not performed because of lettuce deterioration.

for variations in population of this organism during storage at 5 and 25°C for up to 42 days. At 5°C storage, *S.* Typhimurium population in manure-amended soil declined from 7.60 to 2.32 log CFU g^{-1} within 35 days and was not detected after that, whereas in sterile soil, population of microorganism decreased from 7.72 to 2.67 log CFU g^{-1} within 42 days and was detectable up to the end of investigation period (Table 2). However at 25°C, the level of *S.* Typhimurium in manure–amended soil was decreased from 7.74 to 2.75 log CFU g^{-1} within the first 21 days and not detected latter, whereas in sterile soil, such pathogen population declined from 7.85 to 2.43 log CFU g^{-1} within 35 days before becoming undetectable. Significant higher levels and survival ($P < 0.05$) for *S.* Typhimurium were detected in sterile soil than manure –amended soil and at 5°C than 25°C storage.

Discussion

Monitoring growth and survival of *S.* Typhimurium on uncut and shredded lettuce stored at 4 and 22°C for up to 14 days revealed that *S.* Typhimurium population on both uncut and shredded lettuce decreased approximately 1 log CFU g^{-1} when stored at 4°C for 14 days, meanwhile 2 and 3 log CFU g^{-1} increase in population of such microorganism were recorded on uncut and shredded lettuce respectively within 3 days of storage at 22°C. Moreover, significantly higher log counts of *S.* Typhimurium were detected during investigation on shredded than uncut lettuce stored at 22°C. Similar findings were previously recorded in Florida, USA (Vandamm *et al*., 2013). The authors found that *Salmonella* species populations inoculated into fresh-cut and uncut celery declined by 0.5–1 log CFU g^{-1} over 7 days when stored at 4°C, while at 22°C, population of such pathogen increased by 2 logs with majority of growth during first 17 hours, even more, populations on cut surfaces were significantly higher than those on uncut surfaces. In Taiwan, population of *S.* Typhimurium inoculated into shredded iceberg lettuce declined 1 log CFU g^{-1} at 4°C over 14 days storage, however at 22°C the population increased 3 logs within 3 days (Chang and Fang, 2007). Survival and persistence of *S.* Typhimurium were also detected in tomato leaves, where populations of this pathogen decreased during the first two weeks after inoculation but remained unchanged at about 10^4 CFU g^{-1} in the third week (Gu *et al.*, 2011). The persistence and viability of *S.* Typhimurium on uncut and shredded lettuce at 4 and 22°C in this study imposed a potential health risk to consumers and ensure the previous reports of lettuce incrimination in human outbreaks of salmonellosis (Stafford *et al.*, 2002; Horby *et al*., 2003). On the other hand the higher log counts of *S.* Typhimurium recorded in the present study on shredded than uncut lettuce may postulated to easy internalization and more available nutrients on shredded lettuce. In a previous study (Brandl and Amundson, 2008), exudates and nitrogen content of leaf surface contribute to shaping of bacterial communities of lettuce. Furthermore, in India *S.* Typhimurium count was found to be higher on phytopathogen damaged produce than healthy produce (Ansingkar and Kulkarni, 2013).

In many previous studies, the influence of factors like temperature, manure addition or protozoan predation on the survival of *Salmonella* species in environmental samples was shown to be most important (Arrus *et al*., 2006; Garcia *et al*., 2010). In our study, significant differences in *S.* Typhimurium survival in manure–amended and sterile soil at 5 and 25°C were detected by plate counting technique. The greatest and fastest decline in *S.* Typhimurium levels was recorded in manure–amended soil within 21 days of storage at 25°C and the pathogen was not detected latter, meanwhile, *S.* Typhimurium inoculated into sterile soil and stored at 5°C showed the lowest decrease and remained within detectable limit up to the end of investigation (42 days). These results are in agreement with those of Garcia *et al*. (2010) who determined better *S.* Typhimurium survival in soil at 5 than at 25°C and in non amended than in manure-

Table 2: Survival and persistence of *Salmonella* Typhimurium in manure-amended and sterile soil

Item	CFU g^{-1a}													
	5°C (days)							25°C (days)						
	0	7	14	21	28	35	42	0	7	14	21	28	35	42
Manure-amended soil	7.60± 0.11 [b]	6.59± 0.18 [c]	5.51± 0.20[c]	3.58± 0.16[c]	2.65± 0.11 [c]	2.32± 0.15 [c]	ND [d]	7.74± 0.23 [b]	4.70± 0.19 [c]	3.80± 0.18 [c]	2.75± 0.26 [c]	ND [d]		
Sterile soil	7.72± 0.13 [b]	6.97± 0.12 [b]	6.87± 0.17 [b]	5.91± 0.11 [b]	3.91± 0.16 [b]	2.83± 0.10 [b]	2.67± 0.13	7.85± 0.15 [b]	5.84± 0.24 [b]	4.82± 0.14 [b]	3.87± 0.22 [b]	2.81± 0. 16	2.43± 0.11	ND [d]

[a] Mean values of replicate samples (n = 3)
[b, c] Means within the same column carrying these subscripts are significantly different at $P < 0.05$
[d] Not detected

amended soil. Semenov *et al*. (2007) showed that *S*. Typhimurium cells inoculated into cow manure decreased faster at 23°C than at 7°C and similar results were reported by Holley *et al.* (2006). Islam *et al*. (2004) found that survival of *S*. Typhimurium was least in soil containing dairy cattle manure compost. Furthermore, the recorded lower *S*. Typhimurium survival in manure–amended than in sterile soil, both at 5 and 25°C in this study might be attributed to the high nutrient availability due to manure addition and subsequent increase in the activity of native soil microbial community (Jiang *et al.*, 2002), increasing the general competition between bacteria and leading to decrease in the survival of introduced *S.* Typhimurium pathogen (Franz *et al*., 2005). In addition, evolution in protozoan level in manure-amended soil might contribute to the faster decrease in *S*. Typhimurium colony forming unit, since predation by protozoa is another factor affecting bacterial survival in soil (Recobet *et al*., 1992). In Denmark, after inoculation of *S*. Typhimurium into manure-amended soil at 5 and 15°C, blooms in number of protozoa occurred within 24 days, while in soil without manure, the highest level of protozoa was not observed until end of the assay at 42 days and a significant negative correlation was found between abundance of protozoa and *S*. Typhimurium plate counting results (Garcia *et al.*, 2010).

The prolonged survival and viability of *S.* Typhimurium cells in soil recorded in this study and other previous studies (Jones, 1986; Islam *et al*., 2004) is of great concern from public and zoonotic point of view and pose an infection risk. Several studies have revealed that pathogens applied directly to plants survive for shorter periods of time than those applied to soil (Jones, 1986), even more when contaminated manures are applied to soil, there is higher risk for contamination of roots of vegetables with *Salmonella* species for several months (Islam *et al*., 2004). In addition, the pathogen could move through the soil matrix, both vertically and horizontally and reaching surface or underground water. If this water is subsequently used for irrigation of produce for livestock consumption, there will be implications on food safety. Factors that influence the horizontal movement of pathogens across soil comprise soil type, rainfall, temperature, soil water content, soil pH, density of microorganism and transportation through plant roots (Mawdsley *et al.*, 1995). Factors influencing the vertical movement of pathogens through soil include the proximity of pollutant source, agricultural practice, weather, amount and intensity of rainfall and season of application (Pike and Carrington, 1986).

Conclusion

Overall, results of this study clarified that populations of *S*. Typhimurium on lettuce were slightly reduced but remained survive at 4°C, however, at 22°C, the microorganism grew and its count was elevated. In addition, counts of *S*. Typhimurium were found to be significantly higher on shredded than uncut lettuce at 22°C. The results suggested the need for effective mitigation strategies for the microorganism on lettuce. Strict control of temperature during transportation, storage and consumption appeared to be essential to reduce human cases of salmonellosis from lettuce consumption. On the other aspect, monitoring the survival of the microorganism in soil revealed better *S*. Typhimurium survival in soil at 5 than 25°C and in sterile than manure-amended soil. Thus temperature and manure addition are among the important factors influencing the survival of *S*. Typhimurium in soil.

Acknowledgment

The authors are thankful to Mohamed Afifi for his valuable assistance in performing the statistical analysis.

References

Ansingkar, V. M. and Kulkarni, N. S. 2013. Studies on survival of *Salmonella* Typhimurium in phytopathogen damaged and healthy fresh produce. *http://www.webmedcentral.com/article_view/3988.*

Arrus, K. M., Holley, R. A., Ominski, K. H., Tenuta, M. and Blank, G. 2006. Influence of temperature on *Salmonella* survival in hog manure slurry and seasonal temperature profiles in farm manure storage reservoirs. Livestock Science 102: 226-236.

Bailey, J. S. 1998. Detection of *Salmonella* cells within 24-26 hours in poultry samples with the polymerase chain reaction BAX system. Journal of Food Protection 61: 792-795.

Brandl, M.T. and Amundson, R. 2008. Leaf age as a risk factor in contamination of lettuce with *Escherichia coli* O157.H7 and *Salmonella enterica*. Applied and Environmental Microbiology 74 (8): 2298-23006.

Burnett, S. L. and Beuchat, L. R. 2000. Human pathogens associated with raw produce and unpasteurized juices and difficulties in decontamination. Journal of Industrial Microbiology and Technology 25: 281-287.

Centers for Disease Control and Prevention, 1991. Multistate outbreak of *Salmonella* Poona infections–United States and Canada, 1991. Journal of American Medical Association 266: 1189-1190.

Centers for Disease Control and Prevention, 1999. Outbreak of *Salmonella* serotype Muenchen infections associated with unpasteurized orange juice-United States and Canada, June, 1999. Morbidity and Mortality Weekly Report 48: 582-585.

Centers for Disease Control and Prevention, 2000. Foodborne outbreak response and surveillance unit. *http.//www.ede.gov/foodborneoutbreaks/us_outb/fbo2000/bacteria100.htm.in.*

Chang, J. M. and Fang, T. J. 2007. Survival of *Escherichia coli* O157:H7 and *Salmonella enterica* serovars Typhimurium in iceberg lettuce and the antimicrobial effect of rice vinegar against *E. coli* O157:H7. Food Microbiology 24: 745-751.

Costerton, J. W., Stewart, P. S. and Greenberg, E. P. 1999. Bacterial biofilms: a common cause of persistent infections. Science. 284: 1318-1322.

Ercolani, G. L. 1976. Bacteriological quality assessment of fresh marketed lettuce and fennel. Applied and Environmental Microbiology 31: 847-852.

Franz, E., van Diepeningen, A. D., de Vos, O. J. and van Bruggen, A. H. C. 2005. Effects of cattle feeding regimen and soil management type on the fate of *Escherichai coli* O157.H7 and *Salmonella enterica* serovar Typhimurium in manure–amended soil and lettuce. Applied and Environmental Microbiology 71: 6165-6174.

Garcia, R., Baelum, J., Fredslund, L., Santorum, P. and Jacobsen, C. S. 2010. Influence of temperature and predation on survival of *Salmonella enterica* serovar Typhimurium and expression of *inv*A in soil and manure-amended soil. Applied and Environmental Microbiology 76 (15): 5025-5031.

Gu, G., Hu, J., Cevallos-Cevallos, J. M., Richardson, S. M. and Bartz, J. A. 2011. Internal colonization of *Salmonella enterica* serovar Typhimurium in Tomato Plants. PLoS one 6 (11): e27340, pp1-11.

Guan, T. Y. and Holley, R. A. 2003. Pathogen survival in swine manure environment and transmission of human enteric illness. Journal of Environmental Quality 32: 383-392.

Holley, R. A., Arrus, K. M., Ominiski, K. H., Tenuta, M. and Blank, G. 2006. *Salmonella* survival in manure-treated soil during simulated seasonal temperature exposure. Journal of Environmental Quality 35: 1170-1180.

Horby, P. W., O'Brien, S. J., Adak, G. K., Graham, C., Hawker, J. I., Hunter, P., Lane, C., Lawson, A. J., Mitchell, R. T., Reacher, M. H., Threlfall, E. J. and Ward, L. R. 2003. A national outbreak of multi-resistant *Salmonella enterica* serovar Typhimurium definitive phage type (DT) 104 associated with consumption of lettuce. Epidemiology and Infection 130 (2): 169-178.

Islam, M., Morgan, J., Doyle, M. P., Phatak, S. C., Millner, P. and Jiang, X. 2004. Fate of *Salmonella enterica* serovar Typhimurium on carrots and radishes grown in fields treated with contaminated manure composts or irrigation water. Applied and Environmental Microbiology 70 (4): 2497-2502.

Jensen, A. N., Dalsgaard, A., Stockmarr, A., Nielsen, E. M. and Baggesen, D. L. 2006. Survival and transmission of *Salmonella enterica* serovar Typhimurium in an outdoor organic pig farming environment. Applied and Environmental Microbiology 72: 1833-1842.

Jiang, X. P., Morgan, J. and Doyle, M. P. 2002. Fate of *Escherichia coli* O157: H7 in manure–amended soil. Applied and Environmental Microbiology 68: 2605-2609.

Jones, P. W. 1986. Sewage sludge as a vector of Salmonellosis, p. 21-33. in Block, J. C. Haielaar, A.H. and L'Hermite, P. (ed), Epidemiological studies of risks associated with the agricultural use of sewage sludge, Elsevier, London, England.

Koneman, E. W., Allen, S. D., Janda, W. M., Schrecken berger, C. and Winn, W. C. 1992. Diagnostic Microbiology, J.B. Lippincott Company, USA.

Lampel, K. A., Orlandi, P.A. and Kornegay, L. 2000. Improved template preparation for PCR-based assays for detection of foodborne bacterial pathogens. Applied and Environmental Microbiology 60: 4539-4542.

Lapidot, A., Romling, V. and Yaron, S. 2006. Biofilm formation and the survival of *Salmonella* Typhimurium on parsley. International Journal of Food Microbiology 109: 229-233.

Lee, W. H., Lee, M. J., Kim, J. S. and Park, S. Y. 2001. Foodborne illness outbreaks in Korea and Japan studies retrospectively. Journal of Food Protection 64: 899-902.

Lim, Y. H., Hirose, K., Izumiya, H., Arakawa, E., Takahashi, H., Terajima, J., Itoh, K., Tamura, K., Kim, S. and Watanabe, H. 2003. Multiplex polymerase chain

reaction assay for selective detection of *Salmonella enterica* serovar Typhimurium. Japanese Journal of Infectious Diseases 56: 151-155.

Mawdsley, J. L., Bardgett, R. D. and Merry, R. J. 1995. Pathogens in livestock waste, their potential for movement through soil and environmental pollution. Applied Soil Ecology. 2: 1-15.

Pell, A. N. 1997. Manure and microbes: Public and animal health problem? Journal of Dairy Science 80: 2673-2681.

Pike, E. B. and Carrington, E. G. 1986. Stabilization of sludge by conventional and novel processes–a healthy future, P.1-43. In proceeding of symposium. The agricultural use of sewage sludge- is there a future? The institute of water pollution control, Maidstone, United Kingdom.

Recobet, G., Steinberg, C. and Faurie, G. 1992. Survival in soil of genetically engineered *Escherichia coli* as related to inoculum density, predation and competition. FEMS Microbiology Ecology 101: 251-260.

Sanchez, G., Elizaquivel, P. and Aznar, R. 2012. A single method for recovery and concentration of enteric viruses and bacteria from fresh-cut vegetables. International Journal of Food Microbiology 152: 9-13.

Sant'Ana, A. S., Barbosa, M. S. and Destro, M. T. 2012. Growth potential of *Salmonella* spp. and *Listeria monocytogenes* in nine types of ready-to-eat vegetables stored at variable temperature condition during shelf-life. International Journal of Food Microbiology 157: 52-58.

Sant'Ana, A. S., Franco, B. D. G. M. and Schaffner, D. W. 2014. Risk of infection with *Salmonella* and *Listeria monocytogenes* due to consumption of ready-to-eat leafy vegetables in Brazil. Food Control 42: 1-8.

Semenov, A. V., van Bruggen, A. H. C., van Overbeek, L., Termor¬shuizen, A. J. and Semenov, A. M. 2007. Influence of temperature fluctuations on *Escherichia coli* O157:H7 and *Salmonella enterica* serovar Typhimurium in cow manure. FEMS Microbiology Ecology 60: 419-428.

Sivapalasingam, S., Friedman, C. R., Cohen, L. and Tauxe, R. V. 2004. Fresh produce, a growing cause of outbreaks of foodborne illness in the United States, 1973 through 1997. Journal of Food Protection 67: 2342-2353.

Stafford, R. J. I., McCall, B.J., Neill, A. S., Leon, D. S., Darricott, G. J., Towner, C. D. and Micalizzi, G. R. 2002. A state wide outbreak of *Salmonella Bovismorbificans* phage type 32 infection in Queensland. Communicable Disease Intelligence Quarterly Report 26 (4): 568-573.

Tsen, H. Y. 2002. Molecular typing of *Salmonella enterica* serovars Typhimurium, Typhi and Enteritidis isolated in Taiwan. Journal of Food Drug Analysis 10: 242-251.

Van Beneden, C. A., Keene, W. E., Strang, R. A., Werker, D. H., King, A. S., Mabon, B., Hedberg, K., Bell, A., Kelly, M. T., Balan, V. K., Mackenzie, W. R. and Fleming, D. 1999. Multinational outbreak of *Salmonella enterica* serotype Newport infections due to contaminated alfalfa sprouts. Journal of American Medical Association 281: 158-162.

Vandamm, J. P., Dili, Harris, L. J., Schaffner, D. W. and Danyluk, M. D. 2013. Fate of *Escherichia coli* O157:H7, *Listeria monocytogenes,* and *Salmonella* on fresh-cut celery. Food Microbiology 34: 151-157.