

ROUTE OF CONTAMINATION

EFFECTIVENESS OF CLEANERS AND SANITIZERS IN KILLING *SALMONELLA* NEWPORT IN THE GUT OF A FREE-LIVING NEMATODE, *CAENORHABDITIS ELEGANS* (S. J. Kenney, G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat)

Large microbial populations in the soil matrices, such as those amended with manure, have been reported to attract free-living nematodes. *Caenorhabditis elegans*, a free-living, microbivorous nematode found in the soil of temperate regions, has been reported to ingest *Escherichia coli* O157:H7, *Listeria monocytogenes*, several serotypes of *Salmonella enterica*, *Bacillus cereus*, and *Staphylococcus aureus*. Depending on environmental conditions, bacteria may persist within the gut of *C. elegans* for several days after consumption. Release of pathogens as a result of rupturing of the cuticle or defecation are ways that infected nematodes can contaminate the soil environment. Commercial cleaners and sanitizers used by the produce industry may contain surfactants to aid in the release of microorganisms, and perhaps also nematodes, from the surface of produce. Nematodes may subsequently become resident in or on produce contact areas such as water baths, belts, tables, and sorters in processing facilities. Pathogens from a single worm released onto processing equipment could theoretically release ingested cells of a pathogen and contaminate large amounts of produce. The plausibility of this series of events happening on a commercial level can be more easily assessed if the effectiveness of cleaners and sanitizers in killing pathogens ingested by nematodes is known. A study was undertaken to determine the effectiveness of two commercial cleaners and four sanitizers in killing *E. coli* OP50 and *S. Newport* in the gut of *C. elegans*. The effectiveness of these treatments in killing planktonic cells of *E. coli* OP50 and *Salmonella Newport* was also evaluated.

The efficacy of cleaners and sanitizers in killing *S. Newport* in the gut of *C. elegans* was studied. Adult worms were fed nalidixic acid-adapted cells of *E. coli* OP50 (control) or *S. Newport* for 24 h, washed, placed on paper discs, and incubated at 4 or 20°C and relative humidities of 33 or 98% for 24 h. Two commercial cleaners (Enforce[®] and K Foam Lo[®]) and four sanitizers (2% acetic acid, 2% lactic acid, Sanova[®], and chlorine [50 and 200 µg/ml]) were applied to worms for 0, 2, or 10 min. Populations of *E. coli* and *S. Newport* (cfu/worm) in untreated and treated worms were determined by sonicating worms in 0.1% peptone and surface plating suspensions of released cells on tryptic soy agar containing nalidixic acid. Populations of *S. Newport* in worms exposed to 33 or 98% relative humidity at 4°C or 33% relative humidity at 20°C were significantly ($P \leq 0.05$) lower than the number surviving exposure to 98% relative humidity at 20°C. In general, treatment of desiccated worms with cleaners and sanitizers was effective in significantly ($P \leq 0.05$) reducing the number of ingested *S. Newport*. Results indicate that temperature and relative humidity influence the survival of *S. Newport* in the gut of *C. elegans*, and cleaners and sanitizers may not eliminate the pathogen.

THE ROLE OF AEROSOL IN TRANSMISSION OF MICROORGANISMS (INCLUDING *LISTERIA*) TO READY-TO-EAT MEAT/POULTRY PRODUCTS. (L. Ma, C. M. Lin, Z. Yan, J. Kornacki, O. Oyarzabal, and M.P. Doyle)

Airborne contamination of *Listeria monocytogenes* in food processing facilities may or may not be an important contributing factor in disseminating *L. monocytogenes* in such facilities. However, aerosol studies in food processing plants have been limited by lack of a suitable surrogate microorganism for *L. monocytogenes*. The objectives of this study were to investigate the potential of using *Jonesia denitrificans* as a surrogate for aerosol studies of *L. monocytogenes* and to study the role of aerosols in the transmission of microorganisms (including *L. monocytogenes*) to ready-to-eat meat/poultry products.

The settling rates of aerosol-borne *J. denitrificans* released into a bioaerosol chamber were determined. Studies revealed that settling rates depend on particle size and relative humidity of the environment. Larger particles settled from the air more rapidly than smaller particles, with 5- μm particles completely settled out of the air within a few minutes of releasing and 0.3- μm particles remaining airborne ($<1 \log_{10}$ reduction) for 4 hours. In most instances, relative humidity (RH) at 40 or 75% had minimal effect on settling rates, although settling rates of *J. denitrificans* were slightly greater at 75% RH than at 40% RH. Overall, *J. denitrificans* had similar settling rates as *L. monocytogenes* (previous studies).

The contamination level of *J. denitrificans* on turkey meat following its aerosolization in the bioaerosol chamber was similar to that of *L. monocytogenes* which was dependent on initial inoculum cell numbers, exposure time, and relative humidity. The greater the number of cells in the aerosol, the greater the number of contaminated turkey samples and the less exposure time for contamination to occur. No turkey samples were *J. denitrificans* or *L. monocytogenes* positive within 4 hours exposure time when the initial cell number was $\leq 2.5 \times 10^2$ or $\leq 1.5 \times 10^2$ cfu/L air, respectively, and all samples were positive within 5 to 30 minutes of exposure when cell inoculum populations were $\geq 3.5 \times 10^5$ cfu/L air. More samples were positive in the 75% RH environment when the inoculum was 10^3 cfu/L air but relative humidity had little influence on the number of contaminated samples for higher or lower levels of inoculum. Both the detectable cell numbers of *J. denitrificans* and *L. monocytogenes* on positive samples of non-cured turkey meat were generally low, ranging from 1 to 12 cfu per three slices. These results suggest that even when relatively large cell numbers are in the aerosols in a room, relatively small numbers contaminate the surface of products during a short exposure.

Releasing *J. denitrificans* at 10^3 cfu/L as an aerosol into a deboning room of a poultry processing pilot facility revealed that the distance from the air conditioning units from which the bacteria were aerosolized influenced the level of *J. denitrificans* contamination that occurred. The greatest degree of contamination occurred at 100 to 150 cm from the air conditioners, and least at 50 and 250 cm from the units. For samples obtained at 100 cm, the greatest average number of *J. denitrificans* on agar media was 2.4×10^2 cfu/plate, and greatest percentage of meat samples positive at a sampling distance was 40%. Results indicate that releasing as an aerosol at a high population (10^3 cfu/L), *J. denitrificans* can contaminate agar plate and meat surfaces at a range of 250 cm from air conditioning units with the greatest degree of contamination occurring within 100 to 150 cm of an air conditioning unit. Interestingly, swab sampling of environmental surfaces of the deboning room immediately after aerosolizing *J. denitrificans* yielded negative results; indicating *J. denitrificans* is not a good environmental survivor.

MIGRATION OF *CAENORHABDITIS ELEGANS* TO MANURE AND MANURE COMPOST AND POTENTIAL VECTORING OF *SALMONELLA* NEWPORT TO FRUITS AND VEGETABLES (S. J. Kenney, G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat)

It is not uncommon for animal manure and manure compost to be applied to cropland soil as fertilizers. The application of manure and manure compost to soil may attract nematodes that feed on bacteria. Free-living, microbivorous nematode populations have been reported to increase in soils to which cattle manure slurry has been applied. The extent to which various types of manure and manure composts are incorporated into the soil can influence populations of nematodes. Sand homogeneously amended with a humus-litter mixture has been reported to support higher populations of *Caenorhabditis elegans* compared to sand containing isolated patches of the humus-litter mixture. It is hypothesized that free-living nematodes such as *C. elegans* and possibly other genera may ingest human pathogens occasionally found in the soil and transport them through the soil matrix. As a worm migrates through soil it may come in contact with external tissues of plants, either by attraction mechanisms or by random chance. A study was undertaken to determine if *C. elegans* is attracted to bovine manure, turkey manure, composted bovine manure, composted turkey manure, and manure-amended soil inoculated with *Salmonella* Newport. Survival and reproduction of *C. elegans* in the same matrices not inoculated with *S.*

Newport were investigated. Movement of *C. elegans* to lettuce, strawberries, and carrots on an agar medium and the ability of the nematode to transport *S. Newport* in soil to the surface of produce were also studied.

C. elegans moved most rapidly to turkey manure and strawberries, with 35% and 60% of worms, respectively, associating with samples within 30 min. Survival and reproduction of *C. elegans* in test materials was not affected by the presence of *S. Newport*. Bovine manure and bovine manure compost inoculated with *S. enterica* serotype Newport (8.6 log₁₀ cfu/g) were separately placed in the bottom of a glass jar and covered with a layer of soil (5 cm) inoculated (50 worms/g) or not inoculated with *C. elegans*. A piece of lettuce, strawberry, or carrot was placed on top of the soil before jars were sealed and held at 20°C for up to 10 days. In the system using soil inoculated with *C. elegans*, *S. Newport* initially in bovine manure was detected on the surface of lettuce, strawberry, and carrot samples within 3, 1, and 1 days, respectively. The pathogen was detected on lettuce, strawberry, and carrot within 1, 7, and 1 days, respectively, when initially present in bovine manure compost. With one exception, the pathogen was not detected on the produce over the 10-day incubation period when *C. elegans* was not present in the soil. Results indicate that *C. elegans* has the potential for transporting pathogens in soil to the surface of preharvest fruits and vegetables in contact with soil.

INFECTION OF CANTALOUPE RIND WITH *CLADOSPORIUM CLADOSPORIOIDES* AND *PENICILLIUM EXPANSUM*, AND ASSOCIATED MIGRATION OF *SALMONELLA* POONA INTO EDIBLE TISSUES (G. M. Richards and L. R. Beuchat)

Cantaloupe fruits are often in contact with the ground during their development, enhancing the potential for contamination by microorganisms capable of causing human diseases. They are susceptible to postharvest fungal rots, especially under warm, wet conditions. Complete loss of the commodity occurs when one or a few fungal pathogens invade and begin to breakdown the tissues. We undertook a study to determine if the growth of two molds known to cause decay of cantaloupes, *Cladosporium cladosporioides* and *Penicillium expansum*, in wounds on rinds facilitate migration of *Salmonella* Poona into sub-surface mesocarp tissues.

Two phytopathogens, *Cladosporium cladosporioides* and *Penicillium expansum*, in wounds on cantaloupe rinds, were studied to assess their potential to facilitate migration of *S. Poona* into sub-surface mesocarp tissues. Wounded sites in cantaloupe rind were inoculated with *S. Poona* only, *S. Poona* and mold simultaneously, or mold followed by *S. Poona* 3 days later. A cylindrical plug (ca. 3 cm diameter and 4 cm deep) of inoculated tissue extending from the rind surface into edible tissues was removed and cut transversely into four segments (0 – 1, 1 – 2, 2 – 3, and 3 – 4 cm) representing distances from the rind surface. Regardless of the type of inoculum or the time of storage subsequent to inoculation, the pH of the tissues was significantly higher ($P \leq 0.05$) as the distance from the rind surface increased. Test microorganisms and naturally-occurring microorganisms on the rind surface which were introduced into internal tissues during wounding, as well as physiological changes in cantaloupe tissue, contributed to these changes. *C. cladosporioides* and *P. expansum* were recovered from the inoculated rind and underlying tissues throughout storage at 20°C for 10 days. *S. Poona* persisted and grew in wounds on rinds on inoculated cantaloupe incubated at 20°C. Recovery of *S. Poona* from tissues 3 – 4 cm below the inoculated wound supports the hypothesis that it can migrate from the site of inoculation into adjacent mesocarp tissues. Survival and migration of *S. Poona* into the internal tissues of cantaloupes were enhanced by co-inoculation with *C. cladosporioides* and, to a lesser extent, *P. expansum*. Consumption of cantaloupes from which diseased tissue has been removed is not advisable because *S. Poona* and perhaps other enteric pathogens may still be present in remaining tissues.

METABIOTIC ASSOCIATIONS OF MOLDS AND *SALMONELLA* POONA ON INTACT AND WOUNDED CANTALOUPE RIND (G. M. Richards and L. R. Beuchat)

Several national and international outbreaks of salmonellosis have been epidemiologically linked to consumption of fresh cantaloupes. Cantaloupe fruits may be in direct contact with the ground during their development on long, running, non-climbing vines that are prostrate on the soil. The growth habit of cantaloupes enhances the potential for fruits to be contaminated by pathogens that may be present in the soil. Postharvest handling may also bring cantaloupes in direct contact with various sources of foodborne pathogens. Mesocarp tissues of fruits are particularly subject to contamination when rind surface integrity is compromised by disease, bruising, cutting, or peeling. Infection of cantaloupes by plant pathogenic fungi and contamination with foodborne pathogenic bacteria may occur before harvesting, at the time harvest, during handling, storage, transport, and marketing, or after purchase by the consumer. The behavior of foodborne pathogens such as *Salmonella* on or in cantaloupes as affected by metabiotic activities of plant pathogens has not been investigated. The objective of this study was to examine the association between selected molds pathogenic to cantaloupes and *Salmonella* Poona on the surface of intact rind and in wounds in the rind. Changes in pH caused by growth of molds were monitored, as were survival and growth of *S. Poona* in co-infected tissue as affected by temperature.

We tested proteolytic activity and measured changes in the pH of cantaloupe rind caused by growth of *Alternaria alternata*, *Cladosporium cladosporioides*, *Epicoccum nigrum*, *Geotrichum candidum*, and *Penicillium expansum*. Survival and growth characteristics of *S. Poona* co-infected with each mold on the surface rind and in wounded rind tissue as affected by temperature were determined. *C. cladosporioides*, *G. candidum*, and *P. expansum*, but not *A. alternata* and *E. nigrum*, showed proteolytic activity on agar media containing gelatin and/or casein, with concurrent increases in pH, thus favoring survival and growth of salmonellae. Intact and mechanically wounded tissues of cantaloupe rinds were inoculated with a five-strain mixture of *S. Poona* and/or test mold. Five inoculation schemes were used: mold only, *S. Poona* only, mold and *S. Poona* simultaneously, mold then *S. Poona* 3 days later, and *S. Poona* then mold 3 days later. The pH of cantaloupe rinds inoculated with molds and stored at 20°C for 14 days was significantly higher ($P \leq 0.05$) than on day 0. Only the pH of rinds inoculated with *C. cladosporioides* or *G. candidum* was significantly higher ($P \leq 0.05$) on day 21 than on day 0, when cantaloupes were stored at 4°C. An initial population of *S. Poona* increased from 3.3 log₁₀ cfu/sample (ca. 7 cm²) of cantaloupe rind to populations as high as 9.5 log₁₀ cfu/sample during storage at 20°C for up to 14 days, regardless of co-inoculation with molds. Populations of *S. Poona* decreased or remained constant at 4°C for up to 21 days. Results demonstrate that persistence and growth of *S. Poona* on intact, wounded, and decaying cantaloupe rind is not affected by the presence of molds.

SHEDDING OF FOODBORNE PATHOGENS BY *CAENORHABDITIS ELEGANS* IN COMPOST-AMENDED AND UNAMENDED SOIL (G. L. Anderson, S. J. Kenney, L. R. Beuchat, and P. L. Williams)

Nematodes are the most abundant soil metazoa and play an important role in soil ecology. Free-living forms that feed on bacteria respond rapidly to new nutrient sources and have a major impact on soil microfauna. Free-living nematodes harbor ingested bacteria, including human pathogens, and grow in manure-amended soils. Thus, the potential for nematodes to act as vectors of human pathogenic bacteria may be increased if manure or improperly treated compost is used as soil amendments. We conducted a study to determine the time course over which *Caenorhabditis elegans* sheds ingested *Escherichia coli* O157:H7, *Salmonella* Poona, *Listeria monocytogenes*, and a non-virulent strain (OP50) of *E. coli*. The ability of *C. elegans* to survive and reproduce on these bacteria was determined. The effect of adding turkey manure compost to soil on populations of *C. elegans* and *E. coli* O157:H7 ingested by worms before inoculation of soil was studied.

Synchronous populations of *C. elegans* were fed for 24 h on confluent lawns of nalidixic acid-adapted bacteria. *C. elegans* shed viable cells of ingested bacteria on tryptic soy agar supplemented with nalidixic acid (50 µg/ml) (TSAN) throughout a 5-h post-feeding period. *C. elegans* persisted for up to 10 days by feeding on bacteria that had been shed and grew on TSAN. Eggs harvested from *C. elegans* cultured on

shed foodborne pathogens had the same level of viability as those collected from *C. elegans* grown on shed *E. coli* OP50. After 6 - 7 days, 78, 64, 64, and 76% of eggs laid by *C. elegans* that had fed on *E. coli* O157:H7, *S. Poona*, *L. monocytogenes*, and *E. coli* OP50, respectively, were viable. Worms fed *E. coli* O157:H7 were inoculated into soil and soil amended with turkey manure compost. Populations of *C. elegans* persisted in compost-amended soil for at least 7 days but declined in unamended soil. *E. coli* O157:H7 was detected at 4 and 6 days post inoculation in compost-amended and unamended soil, and in unamended soil inoculated with *E. coli* OP50. Populations of *E. coli* O157:H7 in soil amended with turkey manure compost were significantly ($\alpha = 0.05$) higher than those in unamended soil. Results indicate that *C. elegans* can act as a vector to disperse foodborne pathogens in soil, potentially resulting in increased risk of contaminating the surface of pre-harvest fruits and vegetables.

PERSISTENCE OF *ESCHERICHIA COLI* O157:H7, *SALMONELLA* NEWPORT, AND *SALMONELLA* POONA IN THE GUT OF A FREE-LIVING NEMATODE, *CAENORHABDITIS ELEGANS*, AND TRANSMISSION TO PROGENY AND UNINFECTED NEMATODES (S. J. Kenney, G. L. Anderson, P. L. Williams, P. D. Millner, and L. R. Beuchat)

Free-living, bacterivorous nematodes are attracted to areas in soil in which large populations of bacteria are present, so their presence on produce grown in these soils would be likely. *Caenorhabditis elegans* has been reported to feed on human pathogenic bacteria such as *Escherichia coli* O157:H7, *Listeria monocytogenes*, *Salmonella* Poona, *Salmonella* Typhimurium, *Bacillus cereus*, and *Staphylococcus aureus*, as well as on *Pseudomonas aeruginosa* and *Enterococcus faecalis*. Ingestion of *S. Typhimurium*, *S. aureus*, and *P. aeruginosa* shortens the life span of *C. elegans*. We hypothesized that free-living nematodes may ingest human enteric pathogens present in soil matrices and harbor them in their gut. Ingested pathogens may then remain in the gut and be protected against environmental stresses imposed by desiccation or sanitizers used to decontaminate raw fruits and vegetables, even after the worm has died. A preliminary objective of this study was to confirm that *C. elegans* ingests *E. coli* O157:H7 and salmonellae. Major objectives were to determine persistence characteristics of the pathogens in the gut after ingestion, the effects of temperature and relative humidity on survival and growth of ingested cells, and transmission of ingested *Salmonella enterica* serotype Newport to adult progeny of *C. elegans* and to uninfected worms.

Worms were fed cells of a non-pathogenic strain of *E. coli* (OP50), *E. coli* O157:H7, *S. Newport*, and *Salmonella* Poona, followed by incubating at 4, 20, or 37°C for up to 5 days. Initial populations of ingested pathogens significantly increased by up to 2.93 log₁₀ cfu/worm within 1 day at 20°C on K agar and remained constant for an additional 4 days. When worms were placed on Bacto agar, populations of ingested pathogens remained constant at 4°C, decreased significantly at 20°C, and increased significantly at 37°C within 3 days. Worms fed *E. coli* OP50 or *S. Newport* were incubated at 4 or 20°C at relative humidities of 33, 75, or 98% to determine survival characteristics of ingested bacteria. Fewer cells of the pathogens survived incubation at 33% relative humidity compared to higher relative humidities. Populations of ingested *E. coli* OP50 and *S. Newport* decreased by up to 1.65 and 3.44 log₁₀ cfu/worm, respectively, in worms incubated at 20°C and 33% relative humidity. Placement together on K agar of adult worms, labeled with green fluorescent protein (gfp) in the pharynx area, that had ingested gfp-labeled *S. Newport* and uninfected wild type worms resulted in transfer of the pathogen to gut of wild type worms. *S. Newport* was isolated from *C. elegans* two generations removed from exposure to the pathogen. Results of these studies show that *C. elegans* may serve as a temporary reservoir of foodborne pathogens, and could perhaps be a vector for contaminating preharvest fruits and vegetables, thus potentially increasing the risk of enteric infections associated with consumption of raw produce.