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

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**Abstract**

A study was undertaken to determine the persistence of *Escherichia coli* O157:H7 and salmonellae in the gut of a free-living nematode, *Caenorhabditis elegans*, as affected by temperature and relative humidity and to determine if infected worms transmit *Salmonella enterica* serotype Newport to progeny and uninfected worms. Worms were fed cells of a non-pathogenic strain of *E. coli* (OP50), *E. coli* O157:H7, *S. enterica* serotype Newport, and *S. enterica* serotype 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 log\textsubscript{10} 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 log\textsubscript{10} 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

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contaminating preharvest fruits and vegetables, thus potentially increasing the risk of enteric infections associated with consumption of raw produce.

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1. Introduction

Consumption of raw and minimally processed fruits and vegetables has increased substantially in the United States during the past two decades (Putnam et al., 2000). Concurrent with this increase, outbreaks of human microbial infections associated with the consumption of raw produce have increased (Mead et al., 1999; NACMCF, 1999; IFT/FDA, 2001; Beuchat, 2002). These outbreaks have raised interest in identifying preharvest and postharvest sources from which raw and minimally processed fruits and vegetables can become contaminated with microorganisms capable of causing human diseases.

Cropland soil may become contaminated with human pathogenic bacteria in a variety of ways (Nicholson et al., 2000). Application of raw or improperly composted manure (Food and Drug Administration, 1998), contaminated irrigation water, runoff from pastureland, or excreta from wild animals that inhabit cropland have been suggested as vehicles through which soil and preharvest produce may become contaminated with microorganisms capable of causing human diseases (Williams and Dusenbery, 1990; Centers for Disease Control and Prevention, 1997; Cody et al., 1999). Depending upon environmental conditions, the population density of microorganisms within the soil matrix changes and is not homogeneous (Williams and Dusenbery, 1988). Higher populations of microfauna are known to reside in the rhizosphere of plants compared to areas in the soil distant from plant roots (Khanna et al., 1997).

Free-living, bacterivorous nematodes are attracted to areas in soil in which large populations of bacteria are present (Opperman et al., 1993), 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 enterica serotype Poona (Caldwell et al., 2003b), S. enterica serotype Typhimurium (Aballay et al., 2000), Bacillus cereus (Anderson et al., 2003), and Staphylococcus aureus (Sifri et al., 2003), as well as on Pseudomonas aeruginosa (Mahajan-Miklos et al., 1999; Tan et al., 1999) and Enterococcus faecalis (Ewbank, 2002). Ingestion of S. Typhimurium (Aballay et al., 2000), S. aureus (Sifri et al., 2003), and P. aeruginosa (Mahajan-Miklos et al., 1999) shortens the life span of C. elegans.

Bacterial cells ingested by C. elegans have been shown to remain viable after treatment of worms with acidic sanitizers at concentrations lethal to planktonic cells (Caldwell et al., 2003a). Solutions containing high concentrations of NaOH and NaOCl are used in the laboratory to kill juvenile and adult nematodes and to surface sterilize their eggs without loss of viability, indicating that active components in these solutions can penetrate or compromise the cuticle of the adult worm. C. elegans is relatively sensitive to drying. Worms that may be present on the surface of postharvest fruits and vegetables may become desiccated and would be expected to die but bacteria in their gut may remain viable. The influence of temperature and relative humidity on the survival of ingested pathogens, however, has not been determined. The cuticle of the dead worm may serve as a barrier to prevent or inhibit produce sanitizers from coming in contact with ingested bacteria that survive in desiccated worms, thus enabling them to survive treatment with sanitizers.

We hypothesize 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 *S. enterica* serotype Newport to adult progeny of *C. elegans* and to uninfected worms. The efficacy of an alkaline NaOCl solution for killing cells of pathogens ingested by worms and on the surface of eggs was also examined.

2. Materials and methods

2.1. Maintenance of *C. elegans*

A wild type strain (N2) and a green fluorescent protein (gfp) labeled strain (PD4792) of *C. elegans* were used. Worms were maintained on K agar (pH 6.5), which contains (per liter of deionized water): potassium chloride (2.36 g), sodium chloride (3.0 g), Bacto peptone (2.5 g; BBL/Difco, Sparks, MD, USA), and agar (17.0 g) (*Williams and Dusenbery*, 1988). *E. coli* OP50, an avirulent strain routinely used as a feed source for *C. elegans*, was cultured at 37°C for 24 h in OP50 broth (*Brenner*, 1974), which contains (per liter of deionized water): sodium chloride (5.0 g) and Bacto peptone (10.0 g). The K agar was surface inoculated with 0.1 ml of a 24-h culture of *E. coli* OP50 and incubated at 37°C for 24 h to establish confluent growth. Approximately 50 adult worms were deposited on the surface of K agar and incubated at 20°C for up to 3 days prior to transferring worms to a fresh K agar plate with a 24-h lawn of *E. coli* OP50, *E. coli* O157:H7, or *S. Newport* and *S. enterica* serotype Poona.

2.2. Preparation of *C. elegans* for enteric pathogen assay

The surface of ten K agar plates, each containing 500–1000 eggs and 30–50 adult worms, was washed by depositing 5 ml of sterile K medium (*Williams and Dusenbery*, 1990) and gently rubbing with a sterile bent glass rod. The suspended eggs and worms were aseptically transferred to a sterile 15-ml centrifuge tube. The wash and transfer procedure was repeated to enable efficient harvesting of eggs. Eggs and worms were collected by centrifugation (500×g, 2 min), supernatants were removed, and a pellet from pooled suspensions was resuspended in 10 ml of 0.013 M NaOH solution containing 1% NaOCl (pH 13.0) then incubated at 20°C for 15 min to kill all life cycle forms of the worm except the eggs. The suspension was centrifuged (500×g, 2 min) and the supernatant was removed. Worms and eggs in the pellet were resuspended in 10 ml of K medium and centrifuged again. The supernatant was removed and the eggs and dead worms were resuspended in K medium. The suspension (0.1 ml containing 400–600 eggs) was deposited on the surface of a K agar plate on which a lawn of *E. coli* OP50 had formed, followed by incubation at 20°C for 3 days. This procedure ensured that all worms used in assays were the same age. Adult worms were used in assays to confirm that they will ingest pathogenic bacteria, and to determine the effects of temperature and relative humidity on persistence in the gut of ingested pathogen.

2.3. Bacteria used and preparation for ingestion and persistence assay

Two strains of *E. coli* O157:H7 (SEA13B88 and E0018), two serotypes of *S. enterica* (Poona and a multidrug-resistant strain of Newport), and *E. coli* OP50 (control) were evaluated. All strains were adapted to grow in tryptic soy broth (TSB, pH 7.3; BBL/Difco) supplemented with 50 μg/ml nalidixic acid (Sigma, St. Louis, Mo., USA) (TSBN). Nalidixic acid-adapted cells were grown in 10 ml of TSBN at 37°C for 24 h. Cultures were transferred twice to 10 ml of TSBN at successive 24-h intervals. Each inoculum (0.1 ml of a 24-h culture) was spread on K agar supplemented with 50 μg/ml nalidixic acid (KN agar) and incubated at 37°C for 24 h. Cultures were transferred twice to 10 ml of TSBN at successive 24-h intervals. Each inoculum (0.1 ml of a 24-h culture) was spread on K agar supplemented with 50 μg/ml nalidixic acid (KN agar) and incubated at 37°C for 24 h to produce a lawn of cells. Three-day-old adult worms from a synchronized culture grown on K agar were placed on the surfaces of KN agar plates which carried a lawn of *E. coli* OP50 or a test pathogen. The worms were allowed to feed on cells for 3 h at 20°C. Then, K medium (10 ml) was deposited on the surface of each plate and worms and bacteria were suspended using a sterile glass rod. The suspension was placed in a sterile 15-ml centrifuge tubes, centrifuged (500×g, 2 min), resuspended in K medium, and centrifuged again. The
supernatant was decanted and 100–200 worms were placed on K agar containing a lawn of nalidixic acid-sensitive *E. coli* OP50 cells and incubated at 20 °C for up to 120 h. Nalidixic acid-sensitive *E. coli* OP50 was used to facilitate detection of nalidixic acid-adapted pathogens on tryptic soy agar (BBL/Difco) supplemented with 50 µg/ml nalidixic acid (TSAN) on which worms that had ingested nalidixic acid-adapted pathogens would eventually be placed. Sensitive *E. coli* OP50 cells were not expected to grow on TSAN, but resistant cells of pathogens voided by the worm were expected to form colonies.

At 24-h intervals, 10 worms were removed from each plate, washed in 10 ml of K medium, and centrifuged (500×g, 2 min) to remove most of the nalidixic acid-adapted *E. coli* OP50 cells from the outer cuticle. Washed worms were suspended in 10 ml of sterile 0.1% (w/v) peptone (BBL/Difco) water and sonicated (Sonicate 450, Branson Ultrasonics, Danbury, CT, USA) using a duty cycle of 25% for 25 s at 21 °C to rupture the cuticle of *C. elegans* and release ingested bacteria. Sonicate was serially diluted in 0.1% peptone water and surface plated (0.1 ml in duplicate) on TSAN. Plates were incubated at 37 °C for 24 h before colonies of test pathogens were counted.

### 2.4. Persistence of pathogens in *C. elegans* as affected by temperature

Worms were fed on lawns of nalidixic acid-resistant *E. coli* OP50, *E. coli* O157:H7, *S. Poona*, or *S. Newport* on K agar for 3 h at 20 °C. Worms (100–200) were removed from K agar containing pathogens or *E. coli* OP50 using the procedure described above, washed, placed on the surface of uninoculated Bacto agar (2.0%, wt/vol; BBL/Difco), and incubated at 4, 20, or 37 °C for up to 72 h. Populations of ingested pathogens and *E. coli* OP50 were determined at 24-h intervals as described above.

### 2.5. Persistence of *E. coli* OP50 and *S. Newport* in *C. elegans* as affected by relative humidity

*C. elegans* (100–200 worms) was fed on a lawn of nalidixic acid-adapted *E. coli* OP50 or *S. Newport* on TSAN for 24 h at 20 °C. Worms were harvested from the TSAN by flooding plates twice with 5 ml of K medium, collecting by centrifugation, and washing twice with K medium as described above. Washed worms (100–200 worms) in 0.5 ml of K medium were placed on the surface of uninoculated K agar and the K medium was allowed to absorb into the agar at 20 °C for 1 h. Relative humidities of 33%, 75%, or 98% inside 1.7-l tubs were achieved by depositing 300 ml of saturated magnesium chloride, sodium chloride, or disodium phosphate, respectively, beneath an elevated surface of a platform on which paper discs (6 mm diameter) inoculated with *C. elegans* that had ingested test bacteria would be eventually placed. A suspension (5 µl) containing 10 worms in sterile 0.1% peptone water was placed on discs, which were then placed in tubs with known relative humidities and sealed by applying a lid. Worms were incubated in tubs at 4 or 20 °C for 24 h before analyzing for populations of bacterial cells. Each disk was aseptically transferred to a sterile 15-ml centrifuge tube containing 10 ml of 0.1% peptone water, and sonicated as described above. The suspension was spread plated on TSAN as described above. Plates were incubated at 37 °C for 24 h before colonies were counted.

### 2.6. Transmission of ingested *S. Newport* to progeny of *C. elegans*

*C. elegans* was fed on a lawn of nalidixic acid-adapted *S. Newport* on KN agar for 3 h at 20 °C. Worms were harvested by flooding plates with K medium, collected by centrifugation, and washed twice with K medium before placing, one per plate, on uninoculated Bacto agar and incubating at 20 °C for 96 h. Ten adult worms were removed from the Bacto agar using a sterile platinum wire, suspended in 10 ml of sterile 0.1% peptone water, and sonicated as described above. Sonicate (0.25 ml in quadruplicate) was surface plated on bismuth sulfite agar (BSA; BBL/Difco) supplemented with 50 µg/ml nalidixic acid (BSAN) and TSAN. Plates were inverted and incubated at 37 °C 24 h and colonies were counted and randomly selected for confirmation using latex agglutination (FT 0203A; Oxoid, Basingstoke, Hampshire, UK). Concurrently, individual adult progeny were aseptically transferred to uninoculated plates of Bacto agar and incubated at 20 °C for 96 h. Ten
second-generation worms were analyzed for the presence of ingested *S. Newport* as described above.

### 2.7. Transmission of ingested *S. Newport* to uninfected *C. elegans*

Approximately 50 *C. elegans* (strain CB5584) with their pharynx labeled with green fluorescent protein (gfp) were supplied by the Caenorhabditis Genetic Center, Minneapolis, MN. This strain contains a gfp reporter gene that is continuously expressed and is fully integrated in the genome. Descendants of these worms were fed on a lawn of gfp-transformed *S. Newport* (SN78gfp) on TSA supplemented with 100 μg/ml ampicillin (TSAA) for 72 h at 20 °C. Worms were removed from the plate, washed, and centrifuged as described above before ca. 50 were deposited on TSAA. Approximately 50 wild type *C. elegans* were removed from K agar plates on which they had fed on *E. coli* OP50 72 h at 20 °C. The wild type worms were washed and centrifuged using the same procedure described for gfp-labeled nematodes. Wild type worms (ca. 50) were deposited on the surface of the same TSAA plate containing the gfp-labeled worms. Plates were incubated at 20 °C for 24 h.

Sterile, molten Bacto agar (0.5 ml) was placed on a sterile glass microscope slide, and allowed to solidify and cool to 20 °C for 1 h. Ten microliters of 37% formalin (Sigma, St. Louis, MO) was deposited on the agar pad. A sterile platinum wire was used to remove 10 worms from the TSAA. Worms were placed in the formalin on the agar pad, allowed to fix for 5 min, covered with a coverslip, and sealed with melted valap (equal parts petroleum jelly, lanolin, and paraffin). Wild type worms were examined microscopically for the presence of gfp-transformed *S. Newport* in their gut.

### 2.8. Survival of ingested *E. coli* OP50 and *S. Newport* as affected by treatment of worms with alkaline NaOCl

This experiment was done to determine the effectiveness of an alkaline NaOCl solution for killing *E. coli* OP50 and *S. Newport* ingested by *C. elegans* or on the surface of worms or eggs. Approximately 50 wild type *C. elegans* were fed on lawns of nalidixic acid-adapted *E. coli* OP50 or *S. Newport* on TSAN for 24 h at 20 °C. K medium was deposited on the surface of TSAN and suspensions of worms were removed and collected by centrifugation as described above. All but 0.5 ml of the K medium was removed from the centrifuge tube. Worms in the pellet were resuspended in 10 ml of 0.013 M NaOH solution containing 1% NaOCl or 0.1% peptone water (control) and held at 20 °C for 15 min. The suspension was centrifuged (500 × g, 2 min) and the supernatant was removed using a sterile pasteur pipette. Worms and eggs were resuspended in 10 ml of K medium and centrifuged again. A suspension (0.1 ml containing 400–600 eggs) was deposited on the surface of a TSAN plate and incubated at 37 °C for 24 h before examining for the presence of *E. coli* OP50 and *S. Newport* colonies.

### 2.9. Statistical analysis

All experiments were replicated three times. Data were analyzed using the general linear models procedure of the Statistical Analysis Software (SAS).
Institute, Cary, NC). Significant differences \((P \leq 0.05)\) between mean values were determined using Duncan’s multiple range test.

### 3. Results

The initial populations of ingested pathogens \((2.52–3.20 \text{ log}_{10} \text{ cfu/worm})\) significantly \((P \leq 0.05)\) increased by up to 2.93 \text{ log}_{10} \text{ within 1 day after removal from respective feed sources and remained constant for an additional 4 days on K agar incubated at 20 °C (Table 1). Populations of ingested nalidixic acid-adapted \textit{E. coli} OP50 (control) recovered from worms incubated on K agar remained constant for 5 days. There were no significant \((P > 0.05)\) differences between populations of the two strains of \textit{E. coli} O157:H7 or between populations of \textit{S. Newport} and \textit{S. Poona} on each day of analysis.

Compared to day 0, populations of ingested test pathogens did not differ by more than 0.47 \text{ log}_{10} \text{ cfu/worm} when worms that had fed on pathogens were subsequently incubated on Bacto agar at 4 °C for up to 3 days (Table 2). At 20 °C, populations of ingested pathogens decreased significantly within 1 or 3 days. Populations recovered from worms incubated at 37 °C

### Table 2

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Microorganism</th>
<th>Population (log_{10} cfu/worm)</th>
<th>Day 0</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<td>2.97 a</td>
<td>2.78 a</td>
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<td>1.89 b</td>
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* Mean values in the same row that are not followed by the same letter are significantly different \((P \leq 0.05)\).
significantly increased \((P \leq 0.05)\) within 1 day and remained constant for two additional days.

Placement of *C. elegans* on K agar for 1 h at 20 °C did not significantly \((P > 0.05)\) reduce the number of ingested *E. coli* OP50 and *S. Newport* (Table 3). Initial populations \((0 \text{ h})\) of *E. coli* OP50 and *S. Newport* ingested by *C. elegans* decreased by up to 1.65 and 3.44 log\(_{10}\) cfu/worm, respectively, when inoculated discs were incubated at 20 °C and 33% relative humidity for 24 h. The number of *S. Newport* that survived in worms exposed to 33% or 75% relative humidity at 4 °C or 33% relative humidity at 20 °C was significantly \((P \leq 0.05)\) lower than the number surviving exposure to higher relative humidities at respective temperatures.

Cells of *S. Newport* labeled with gfp were detected in the gut of wild type *C. elegans* progeny after contact for 24 h with gfp-labeled *C. elegans* (Fig. 1). Cells of nalidixic acid-resistant *S. Newport* were isolated from all adult *C. elegans* analyzed from two successive generations removed from exposure to the pathogen.

An interesting phenomenon in this series of studies was the observed presence of gfp-labeled juvenile
worms inside infected wild type worms (Fig. 2). Also, worms were observed feeding on the gut contents of other worms with ruptured cuticles.

No *E. coli* OP50 or *S. Newport* cells were recovered from worms or eggs treated with the alkaline NaOCl solution.

4. Discussion

Results indicate that once *C. elegans* has ingested enteric pathogens, progeny may remain infected until they are voided of the pathogens or environmental conditions become unsuitable for the bacteria to survive. This is the first demonstration that pathogens from infected *C. elegans* can be transmitted to progeny as a result of excretion of viable cells into the environment, either by discharge of cells as a result of rupture of the cuticle, or by juvenile worms eating their way out of an infected adult worm and thereby ingesting bacteria in the process. If the environmental conditions are favorable, excreted or discharged bacteria may grow. Growth of bacteria may, in turn, attract uninfected worms that may ingest pathogens, thus repeating the cycle of infection. The practical implications of these processes are substantial in terms of prolonging exposure times of produce to foodborne pathogens in the soil.

Worms that have ingested *Salmonella* have been reported to have a decreased life span (Aballay et al., 2000). The time to death for 50% (TD50) of the worms ranged from 3.7 to 9.0 days after ingesting various serotypes of *S. enterica* compared to 9.9 days after ingesting *E. coli* OP50. The reduced life span of worms that carry foodborne pathogens, however, may have little impact on their ability to contaminate preharvest fruits and vegetables. Caldwell et al. (2003b) showed that *C. elegans* vectors *S. Poona* to cantaloupe rind in contact with soil within 1 day of ingesting the pathogen. Our observation that *S. Newport* is present in *C. elegans* two generations removed from exposure to the pathogen raises further concern that the nematode can serve to extend the presence of enteric pathogens that are occasionally present in soils in which fruits and vegetables are grown.

Populations of ingested pathogens increased when worms were incubated on TSAN at 20 °C; however, reduced numbers of cells were recovered from worms incubated on Bacto agar at 20 °C. TSAN is rich in nutrients. Bacto agar, on the other hand, is devoid of nutrients required for bacterial growth, and was used only as a physical support for the worms and to prevent them from desiccating. Bacteria from the cuticle surface or excreted from the worms would be expected to grow on the TSAN but not on Bacto agar. Bacterial cells may grow on TSAN at a rate faster than they are consumed by *C. elegans*. Aballay et al. (2000) reported that *S. Typhimurium* populations in the intestinal lumen of *C. elegans* increased when the worm was placed on nutrient agar.

*C. elegans* survived at 4 °C but did not move on the Bacto agar surface to forage for bacteria. All test bacteria could be recovered from worms incubated at 4 °C for at least 3 days. Metabolic processes associated with digestion in *C. elegans* would be much slower at 4 °C than at 20 °C. This would suggest that cells ingested by worms prior to exposure to refrigeration temperatures would be less likely to be affected by digestive processes. Pathogens ingested by *C. elegans* that may contaminate the surface of postharvest produce would be protected at refrigeration temperatures. Produce in the retail market is not uncommonly displayed at ambient temperature (ca. 20 °C), which could also protect ingested pathogens, even after death of the worms.

Worms incubated at 20 °C are in their most active state and will rather quickly consume most bacteria. Since Bacto agar is nutrient deficient and would not support the growth of bacteria, *C. elegans* probably used ingested bacterial cells as a nutrient source, resulting in a decrease in populations during the 3-day incubation period. *C. elegans* does not survive at 37 °C. The increase in bacterial populations in worms incubated on Bacto agar at 37 °C is attributed to an increase in the availability of nutrients for growth as the worms undergo autolysis.

It is unclear how labeled worms became internalized in the gut of wild type worms. A possibility would be that the juvenile worm entered the adult wild type worm through a rupture in the cuticle. Juvenile worms in the carcass of a dead adult worm may be a normal occurrence; however, lack of phenotypic differences in the wildtype worms makes it difficult to determine if juveniles were progeny of a dead worm or from another worm. Worms that were observed feeding on the gut contents of dead worms
were probably attracted to viable bacterial cells that had been ingested before rupture of the cuticle. In this way, one infected worm could infect many previously uninfected worms. It is also possible that the wild type worm ingested an egg from a transgenic worm that then developed into a juvenile. Studies have shown C. elegans is capable of ingesting rigid objects smaller than 5 μm in diameter (Boyd et al., 2003). C. elegans eggs are ca. 30 μm in diameter but the eggs may be pliable enough to allow ingestion by adult worms.

At both 4 and 20 °C, incubation of C. elegans at 33% relative humidity resulted in a significant decrease (P≤0.05) in the number of S. Newport recovered. Worms would be more desiccated when exposed to 33% relative humidity compared to 75% or 98% relative humidity. Largest reductions in exposed to 33% relative humidity compared to 75% recovered. Worms would be more desiccated when eggs are ca. 30 μm in diameter but the eggs may be pliable enough to allow ingestion by adult worms.

In the laboratory, an alkaline NaOCl solution is used to surface sterilize eggs and to separate eggs from other life stages of C. elegans. Other disinfectants have been reported to unsuccessfully eliminate bacteria that may be present in the gut of the worm (Chang et al., 1960; Smerda et al., 1970; Caldwell et al., 2003a). In our study, bacterial colonies were not formed on TSAN plates on which worms treated with a 1% alkaline NaOCl solution were incubated at 37 °C for 24 h. This suggests that the NaOCl solution is effective in penetrating the cuticle of the worm and inactivating the bacteria in the gut. The pH of the solution was 13.0, a value at which C. elegans cannot survive (Khanna et al., 1997). When sanitizing produce, a NaOCl solution is most effective at a pH of 6.0–7.5 (Beuchat, 1998).

Results indicate that E. coli O157:H7 and salmonellae can survive for an extended time after ingestion by C. elegans. It is plausible that worms infected with bacteria capable of causing disease in humans may come into contact with preharvest fruits and vegetables and contaminate their surfaces by excreting pathogens. Attachment of infected worms to the surface of produce may be followed by death of the worms but ingested pathogens may survive and be physically protected against treatment with sanitizers, rendering sanitization more difficult. These and other observations provide new insights to the role C. elegans and perhaps other free-living nematodes with similar feeding habits may play in enhancing survival and persistence of foodborne pathogens, with potentially consequent increases in risk of contamination of produce at the point of harvest and during subsequent handling and processing. Field trials to verify associations of C. elegans, foodborne pathogens, and produce are warranted.

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**References**


