Use of Bioluminescent *Escherichia coli* to Determine Retention During the Life Cycle of the Housefly, *Musca domestica* (*Diptera: Muscidae, L*)

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**Abstract**

Researchers have documented that the housefly (*Musca domestica*) can serve as a vector for the spread of foodborne pathogens to livestock, food, and humans. Most studies have investigated *Musca domestica* as a vector only after the fly comes into contact or consumes the pathogen as an adult. The objective of this study was to determine whether the larvae of *Musca domestica* could ingest *Escherichia coli* from bovine manure and whether the *E. coli* could survive the metamorphosis process and be transmitted. Larvae (*n*=960) were incubated in sterilized bovine manure inoculated with 0, 3, 5, and 8 log10 colony-forming units (CFU)/mL of bioluminescent *E. coli* for 24 (larvae stage), 48 (larvae stage), 120 (pupae stage), and 192 h (adult stage). Larvae incubated for 24 h in bovine manure possessed 0.0, 2.7, 2.9, and 3.5 log10 CFU/mL of *E. coli*, from inoculated with 0, 3, 5, and 8 log10 CFU/mL of *E. coli*, respectively. Concentrations of *E. coli* within the pupae were 0.0, 1.7, 1.9, and 2.2 log10 CFU/mL for each inoculation concentration, respectively. Flies that emerged from the pupae stage contained 0.0, 1.3, 2.2, and 1.7 log10 CFU/mL of *E. coli* from larvae incubated in manure inoculated with concentrations of *E. coli*, respectively. These results suggest the housefly can emerge with quantities of *E. coli*. While this was an enteropathogenic *E. coli* (EPEC), these data may suggest that if the fly is capable of retaining similar concentrations of an enterohemorrhagic *E. coli* (EHEC), these concentrations may be capable of initiating illness in humans. Furthermore, the *E. coli* concentration within and on adult flies is related to environmental exposure. It must be noted that larvae were incubated in sterilized bovine manure, and there was no other bacterial competition for the *E. coli*. Thus, the rate of positive flies and concentrations present when flies emerged may vary under more realistic conditions.

**Introduction**

Researchers have reported that pathogens that infect both livestock and humans from livestock feces can be disseminated by house flies (*Musca domestica*, L.). Kobayashi et al. (1999) reported that large numbers of *Escherichia coli* O157:H7 can adhere to the surface of the mouth parts and food particles within the gastrointestinal tract of *M. domestica*. Kobayashi et al. (1999) also reported proliferation of *E. coli* O157:H7 within the minute spaces of the labellum portion of the housefly mouth. In an extension of this research, Sasaki et al. (2000) reported that excrement from experimentally exposed *M. domestica* to *E. coli* O157:H7 contained 4 log10 CFU/mL of *E. coli* O157:H7 1 h after exposure and 5 log10 CFU/mL of *E. coli* O157:H7 3 h after exposure. Ahmad et al. (2007) reported that fecal samples from calves naive to nalidixic acid–resistant *E. coli* O157:H7 were positive for nalidixic acid–resistant *E. coli* after 1 d of exposure to *M. domestica* exposed experimentally to nalidixic acid–resistant *E. coli*. These researchers and a multitude of others have presented strong evidence to indicate that *M. domestica* can serve as a mechanical vector for *E. coli* O157:H7 and most likely all enterohemorrhagic *E. coli*.

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The focus of the aforementioned research has been to investigate the mechanical vector of *M. domestica* related to contamination of the fly occurring after emergence from the pupae. However, *M. domestica* utilize feces as a habitat for offspring and the larvae of *M. domestica* utilize bacteria as a food source, and thus come into contact with pathogens prior to the pupae stage. Rochon et al. (2004) reported that housefly larvae will ingest *E. coli* and utilize this bacterium as a readily available food source. Rochon et al. (2005) reported that 98% of *M. domestica* pupae from larvae fed *E. coli* during the third larval stage retained the bacteria. Rochon et al. (2005) also reported that populations of *E. coli* obtained by the *M. domestica* larvae can persist through the pupae stage and remain with the adult fly upon emergence. Flies emerging from these pupae contained an *E. coli* population of 2 log_{10} CFU/mL. The results of this study clearly indicate that infection of *M. domestica* larvae with nonspecific *E. coli* during the third larval stage can result in the emergence of an adult fly contaminated with *E. coli*. The purpose of this study was to determine whether *E. coli* could be ingested by the larvae of *M. domestica* in a habitat commonly utilized by flies within cattle feedlots (bovine manure) at varying concentrations of *E. coli* (0, 3, 5, and 8 log_{10} CFU/mL) and then persist through metamorphosis to the adult stage, thus emerging from the pupa as a possible mechanical vector for dissemination of *E. coli*.

**Materials and Methods**

**Larvae collection**

Two individual trials were conducted to determine whether *E. coli* ingested during the larval stage of *M. domestica* could persist throughout metamorphosis and the adult fly could emerge as an immediate vector for *E. coli* transmission. *M. domestica* larvae for both trials were collected from the manure run-off basin at the Bearden Dairy Research Center at Mississippi State University. Collected larvae were counted and sorted into categories based upon larval stages; the second larval stage (instar 2) was utilized for this study.

Collected larvae were then counted and processed through a sanitation protocol. For external sterilization, larvae were rinsed with distilled water to remove all organic matter. After rinsing, larvae were individually immersed into a 10% sodium hypochlorite solution for 1 min then immersed in distilled water for 1 min; sanitation was replicated three times. After surface sanitation was completed, larvae were placed on a non-nutrient water agar for 24 h at 20°C (10 larvae/Petri plate; Fisher Scientific, Pittsburgh, PA). After 24 h, larvae were removed from non-nutrient agar and placed into sterile 5-mL microcentrifuge tubes until utilized (<30 min after removal from agar).

**Manure preparation**

For both trials, manure preparation was based upon methods described by Perrotti et al. (2002) with a target of 72% moisture. Fresh manure (9.5 kg for each trial) was rectally removed from Holstein cows at the Bearden Dairy Research Center 7 d prior to start of each trial. Manure was placed in a drying oven for 48 h at 100°C. Dried manure was ground with a commercial food grinder to produce a consistent material and was autoclaved. For each trial, manure was reconstituted to 72% moisture. For reconstitution, 14 g of dried ground manure was placed into 296 mL sterile clear plastic cups and was mixed with 32.5 mL of distilled water and 3.5 mL of Luria-Bertani (LB) broth (Difco, Lawrence, KS) containing varying concentrations of *E. coli*. Escherichia coli-Xen-14 (*E. coli*-Xen-14; Caliper Life Science, Hopkinton, MA) was utilized for these two trials. Escherichia coli-Xen-14 was derived from the parent strain *E. coli* WS2572, which possesses a stable copy of the *Photobacterium luminescens* lux operon. The luxCDABE operon (Rocchetta et al., 2001) consists of the luxAB genes coding for the luciferase enzyme and the luxCDE genes responsible for biosynthesis of the polypeptide substrate for the luminescence reaction. To determine approximate concentration, *E. coli*-Xen-14 was grown for 12 h prior to inoculation of manure in LB broth to achieve an optical density of 0.34. With optical density of 0.34, *E. coli*-Xen-14 concentrations were expected to be 9 log_{10} CFU/mL (previous unpublished data). *Escherichia coli*-Xen-14 was diluted in 3.5 mL of LB broth to achieve the desired inoculation concentration of 0, 3, 5, and 8 log_{10} CFU/mL of LB broth.

**Larvae study**

To determine uptake of *E. coli*-Xen-14 by larvae of *M. domestica*, a 4×2 factorial design was incorporated with two incubation times (24 h and 48 h), and four *E. coli*-Xen-14 inoculation concentrations (0, 3, 5, and 8 log_{10} CFU/mL). Ninety-six cups of reconstituted manure were prepared for inoculation, 48 cups per time period (24 h and 48 h), with 12 cups per treatment (0, 3, 5, and 8 log_{10} CFU/mL). For all treatments, once broth was added, each cup was thoroughly mixed for 3 min with sterile inverted L-shaped cell spreaders (Fisherbrand, Fisher Scientific, Pittsburgh, PA). After all cups were inoculated, 10 larvae were added to each cup. Cups were then covered with sterile cheesecloth and incubated at 23°C for 24 h or 48 h.

At the conclusion of each time period, cups were individually emptied on 91-cm² sections of bench paper (Daigger & Co., Veron Hills, IL). Larvae (living and dead) and pupae were removed from the manure using sterile forceps and counted, and all living larvae were placed into a sterile 10-mL conical tube. Two larva from each cup (n = 192) were randomly selected, and exposed to the same procedures used to surface sterilize the larvae prior to use in the study.

After surface sterilization, individual larvae were placed into individual 5-mL microcentrifuge tubes containing 200 μL of LB broth and macerated. Macerated larvae and fluid were transferred to 5-mL microcentrifuge tubes with 800 μL of LB broth for serial dilution (total volume of 1000 μL). Dilutions of macerated larvae were plated on LB agar and incubated overnight (37°C). Dilution plates were counted for total CFU and photonic images of plates were taken utilizing a Berthold/NightOwl camera equipped with the WinLight 32 software, version 2.51.11901 (Berthold Technologies, Oak Ridge, TN); images were analyzed using Image J software (NIH). *Escherichia coli*-Xen-14 log_{10} values were determined based upon total CFU and photonic images of plates were taken utilizing a Berthold/NightOwl camera equipped with the WinLight 32 software, version 2.51.11901 (Berthold Technologies, Oak Ridge, TN); images were analyzed using Image J software (NIH). *Escherichia coli*-Xen-14 log_{10} values were determined based upon total CFU considered as the non-emitting colonies (log_{10} = Total CFU – non-emitting CFU).

**Pupae and adult study**

To determine survival of *E. coli*-Xen-14 ingested by larvae of *M. domestica* through the pupae stage and emergence as an adult, the same protocol as previously described was utilized.
with the following deviations. Incubation time for larvae in inoculated manure was 120 h (48 cups total). After incubation, larvae (live and dead) and pupae were removed from the manure and recorded. From each cup, two pupae (n=96) were randomly selected for imaging, and followed the previously mentioned protocol. An additional four pupae from each cup were randomly selected for incubation until completion of metamorphosis (48 pupae per inoculation concentration). These pupae were exposed to the same surface sterilization protocol and then placed into containment grids. Four containment grids were constructed from acrylic parabolic louvers (cell shape 1.27×1.27 cm²×1.11 cm deep), one grid for each E. coli Xen-14 concentration. The parabolic louver was cut into 14.5×14.5-cm sections containing 10 cells per grid. Fiberglass replacement screens (17×17 cm; Phifer, Tuscaloosa, AL) were affixed to one side of the containment unit. Pupae were placed into individual cells within the containment grid and the grid was covered with 17×17 cm×3-mm-thick clear Plexiglas,* secured by 1.27-cm binder clips. Containment grids were then placed into rigid plastic containers (41.9×29.2×8.9 cm; Newell Rubbermaid, Sandy Springs, GA) and covered with fiberglass replacement screen. Containment grids were incubated at 20°C for 72 h. After incubation for 72 h, flies were euthanized with exposure to carbon monoxide for 5 min. Twelve flies from each containment grid were randomly selected for analysis. The procedure for analysis of pupae and adult flies was identical to those reported for larvae, with the exception of adult flies not being exposed to the surface sterilization prior to analysis.

Statistical Analysis

Sample size was determined based upon a confidence interval of 95% and a power of 80% using the mean and standard deviation of that reported by Rochon et al. (2005) using the Epi-Info sample size calculator for comparing means (EpiInfo, CDC, Atlanta, GA). Data for the larval trial were analyzed as a 4×2 factorial randomized design using PROC GLM procedures in SAS (SAS Institute Inc., Version 9.2; Cary, NC), while data for pupa and adult flies were analyzed as a completely randomized design. Experimental units for both trials were defined as larvae, pupa, or adult fly, and significance was declared at p<0.05. Pairwise differences among least-squares means were evaluated with the PDIFF statement when F-test ≤0.05.

Results and Discussion

Survivability of larvae

The survivability of the M. domestica larvae incubated for 24 or 48 h in bovine manure inoculated at 0, 3, 5, and 8 log_{10} CFU/mL of E. coli-Xen-14 is reported in Table 1. During 24-h incubation, larvae within the 5 and 8 log_{10} CFU/mL inoculation had a greater (p=0.05) percentage of living larvae compared to 0 and 3 log_{10}. Following the same pattern, the percentage of larvae that died during the incubation period was less (p=0.04) for larvae incubated at 5 and 8 log_{10} compared to 0 and 3 log_{10} inoculation concentrations. After 48-h incubation, E. coli-Xen-14 concentration did not affect (p=0.20) the percentage of larvae living during the 48-h incubation period. A greater (p=0.001) percentage of larvae incubated in manure inoculated at 0 or 8 log_{10} CFU/mL entered the pupa stage, while the greatest (p<0.001) percentage of larvae that died was observed in the 3 log_{10} inoculated manure. The results for the 24-h incubation would be expected, as researchers have reported that larvae of M. domestica utilize bacteria as a readily available food source (Schmidtman and Martin, 1992; Zurek et al., 2000; Rochon et al., 2004). Having a greater percentage of larvae dead after 24 h in the 0 log_{10} CFU/mL of E. coli-Xen-14 is expected based upon previous research as this manure was devoid of all bacteria (autoclaved prior to use). However, interpretation of combined results from 24- and 48-h results provide no clear pattern to the role of bacteria in the development of M. domestica (this was not an objective of this trial) and is most likely a result of the physical and nutritional stress that the larvae experience prior to being placed into the manure (nutritional deprivation for 24 h and sterilization protocol).

Table 1. Percent survival of larvae of the housefly incubated for 24, 48, 120, and 192 h in bovine manure inoculated at 0, 3, 5, and 8 log_{10} Colony-Forming Units (CFU)/mL of Escherichia coli-Xen-14

<table>
<thead>
<tr>
<th>Parameter</th>
<th>0 log_{10} CFU/mL</th>
<th>3 log_{10} CFU/mL</th>
<th>5 log_{10} CFU/mL</th>
<th>8 log_{10} CFU/mL</th>
<th>p-Value</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Survivalability—24 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alive,%</td>
<td>77.5^a</td>
<td>80.8^{ab}</td>
<td>90.0^b</td>
<td>91.7^b</td>
<td>0.05</td>
<td>1.32</td>
</tr>
<tr>
<td>Pupated</td>
<td>1.7^a</td>
<td>0.0^{ab}</td>
<td>0.8^a</td>
<td>0.0^a</td>
<td>0.75</td>
<td>0.24</td>
</tr>
<tr>
<td>Dead</td>
<td>18.3^a</td>
<td>18.3^{ab}</td>
<td>8.3^b</td>
<td>8.3^b</td>
<td>0.04</td>
<td>1.24</td>
</tr>
<tr>
<td>Survivalability—48 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alive</td>
<td>58.3</td>
<td>58.3</td>
<td>66.7</td>
<td>60.8</td>
<td>0.20</td>
<td>1.83</td>
</tr>
<tr>
<td>Pupated</td>
<td>35.0^a</td>
<td>17.5^{b}</td>
<td>23.3^{b}</td>
<td>30.0^{ab}</td>
<td>0.001</td>
<td>1.89</td>
</tr>
<tr>
<td>Dead</td>
<td>6.7^a</td>
<td>23.3^{b}</td>
<td>10.8^{a}</td>
<td>9.2^{a}</td>
<td>&lt;0.001</td>
<td>1.32</td>
</tr>
<tr>
<td>Survivalability—120 h</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pupated</td>
<td>70.0</td>
<td>73.3</td>
<td>75.8</td>
<td>74.2</td>
<td>0.28</td>
<td>0.82</td>
</tr>
<tr>
<td>Living</td>
<td>9.2</td>
<td>7.5</td>
<td>5.8</td>
<td>6.7</td>
<td>0.24</td>
<td>0.97</td>
</tr>
<tr>
<td>Dead</td>
<td>19.2</td>
<td>18.3</td>
<td>16.7</td>
<td>16.7</td>
<td>0.34</td>
<td>1.15</td>
</tr>
<tr>
<td>Survivalability—pupae</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hatched (%)</td>
<td>44.0</td>
<td>47.0</td>
<td>46.0</td>
<td>48.0</td>
<td>0.34</td>
<td>1.26</td>
</tr>
</tbody>
</table>

^a,bLeast square means within a column, lacking a common subscript are different if p≤0.05.
For larvae incubated for 120 h in *E. coli*-Xen-14 (Table 1) inoculated bovine manure, there was no difference (p ≤ 0.28) in the percentage of larvae entering the pupae stage, alive, or dead regardless of inoculation rate. During the 120-h incubation period, 70, 73, 76, and 73% of the larvae in the 0, 3, 5, and 8 log_{10} CFU/mL treatment groups entered the pupae stage (not reported in tabular form). Furthermore, the percentage of pupae that hatched were not affected (p = 0.34) by concentration of *E. coli*-Xen-14; 44, 47, 46, and 48% of the larvae that entered the pupae stage hatched for 0, 3, 5, and 8 log_{10} CFU/mL treatment groups, respectively.

Uptake of *E. coli*-Xen-14

For 24-h incubation, 0, 75, 88, and 96% of larva evaluated from 0, 3, 5, and 8 log_{10} CFU/mL of *E. coli*-Xen-14, respectively, were determined to be positive for *E. coli*-Xen-14 (not reported in tabular form). Following 48 h incubation, 0, 92, 88, and 96% of larvae from manure inoculated with 0, 3, 5, and 8 log_{10}, respectively were positive for *E. coli* (not reported in tabular form). These data are very similar to those reported by Rochon *et al.* (2004, 2005), who reported that 90 and 98% of the larvae reared in the presence of *E. coli* ingested the bacterium.

The *E. coli*-Xen-14 uptake of *M. domestica* larvae (24- and 48-h incubation), pupae (120-h incubation), and adult flies (192-h incubation) after incubation in bovine manure inoculated at 0, 3, 5, and 8 log_{10} of *E. coli*-Xen-14 is reported in Table 2. By design (a control group), all larvae, pupae, and flies evaluated from the 0 log_{10} CFU/mL treatment were negative for *E. coli*-Xen-14. For determination of *E. coli*-Xen-14 uptake, larvae (24), pupae (24), or flies (48) were randomly selected from each *E. coli*-Xen-14 concentration at each incubation period (24, 48, 120, and 192 h). Determination of *E. coli*-Xen-14 was based upon total CFU counts on LB agar minus nonemitting CFU as determined by photonic imaging. Larvae positive for *E. coli*-Xen-14 after 24-h incubation had 2.7, 2.9, and 3.5 log_{10} CFU of *E. coli*-Xen-14, respectively, for inoculation rates of 3, 5, and 8 log_{10} CFU/mL (Table 2). The concentration of *E. coli*-Xen-14 was greater (p = 0.05) in larvae incubated in manure inoculated at 8 log_{10} CFU/mL; however, this difference was less than 1 log_{10} CFU/mL. Larvae positive for *E. coli*-Xen-14 after 48-h incubation had 3.0, 3.3, and 3.9 log_{10} CFU/mL of *E. coli*-Xen-14, respectively, for inoculation rates of 3, 5, and 8 log_{10} CFU/mL. These results are similar to that of 24-h incubation. The difference observed at 48 h was different (p = 0.03), but was again less than 1 log_{10} CFU/mL. The results for 24-h exposure at 8 log_{10} CFU/mL from the present study were slightly less than those reported by Rochon *et al.* (2004) for 24-h exposure at 8 log_{10} CFU/mL (3.5 log_{10} CFU/mL compared to 4.4 log_{10} CFU/mL). On the other hand, larvae exposed to 3 or 5 log_{10} CFU/mL in the present study were approximately 1.5 log_{10} less than those reported by Rochon *et al.* (2004; 2.7 and 2.9 log_{10} CFU/mL, respectively). As for 48-h exposure, results from Rochon *et al.* (2004) and the current study are similar, regardless of the concentration of *E. coli*-Xen-14. Rochon *et al.* (2004) reported that larvae exposed to direct contact with *E. coli* at a concentration of 8 log_{10} resulted in larvae possessing 3.8 log_{10} CFU/mL of *E. coli*. Based upon these results, it appears that regardless of exposure through direct contact at 8 log_{10} or inoculation of sterile manure at varying concentrations (3, 5, and 8 log_{10} CFU/mL), after 48 h larvae of *M. domestica* are capable of containing 3 to 4 log_{10} CFU/mL of *E. coli*.

For 120-h incubation, 25, 75, and 63% of pupae were positive for *E. coli*-Xen-14 from 3, 5, and 8 log_{10} CFU/mL inoculation (not reported in tabular form). These data differ from those reported by Rochon *et al.* (2004), who reported that 98, 96, 98, and 100% of the pupae evaluated 1, 3, 5, and 6 d after pupation, respectively, were positive for *E. coli*. Pupae positive for *E. coli*-Xen-14 after 120-h incubation possessed 1.7, 1.9, and 2.2 log_{10} CFU/mL of *E. coli*-Xen-14, respectively, for inoculation rates of 3, 5, and 8 log_{10} CFU/mL of *E. coli*-Xen-14 (Table 2). These data also differ from those reported by Rochon *et al.* (2004), who reported an average of 4.7 log_{10} CFU/mL infection of *E. coli* in *M. domestica* pupae. The difference in concentrations observed in this study compared to Rochon (2004) could be a result of the stage of the larvae utilized. Rochon *et al.* (2004) utilized larvae in the third stage, which are physiologically closer to pupation; thus, there might have been less time for the digestion of the *E. coli* consumed by the larvae.

From each inoculation concentration, 48 pupae were randomly selected to continue incubation for an additional 72 h. At the conclusion of 192 h incubation (120 h for pupae

<table>
<thead>
<tr>
<th>Parameter</th>
<th>3 log_{10} CFU/mL</th>
<th>5 log_{10} CFU/mL</th>
<th>8 log_{10} CFU/mL</th>
<th>p-Value</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Larvae <em>E. coli</em> uptake—24 h Emitting log_{10} CFU/mL</td>
<td>2.69&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.89&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.52&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.05</td>
<td>1.53</td>
</tr>
<tr>
<td>Larvae <em>E. coli</em> uptake—48 h Emitting log_{10} CFU/mL</td>
<td>3.01&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.29&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.92&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.03</td>
<td>1.36</td>
</tr>
<tr>
<td>Pupae <em>E. coli</em> uptake—120 h Emitting log_{10} CFU/mL</td>
<td>1.66</td>
<td>1.92</td>
<td>2.16</td>
<td>0.19</td>
<td>0.93</td>
</tr>
<tr>
<td>Fly <em>E. coli</em> uptake—192 h Emitting log_{10} CFU/mL</td>
<td>1.33</td>
<td>2.15</td>
<td>1.73</td>
<td>0.65</td>
<td>0.67</td>
</tr>
</tbody>
</table>

<sup>a</sup>No values are reported for the 0 log_{10} CFU/mL as there were no *E. coli*-Xen-14 present.

<sup>b,c</sup>Least square means within a column, lacking a common subscript are different if p ≤ 0.05.
evaluation and an additional 72 h to allow hatching of pupae), 44, 47, 46, and 48% of the pupae hatched for 0, 3, 5, and 8 log_{10} CFU/mL, respectively (Table 1). After incubation, 13, 17, and 17% of flies emerging from the pupae were positive for E. coli-Xen-14 from 3, 5, and 8 log_{10} CFU/mL inoculation (not reported in tabular form). The percentages of flies emerging from the pupae contaminated with E. coli in the current study are less than those reported by Rochon et al. (2005), who reported that 66% of the flies emerging from the pupae were infected with E. coli. Flies emerging from the pupae positive for E. coli-Xen-14 after 120-h incubation possessed 1.3, 2.2, and 1.7 log_{10} CFU/mL for inoculation rates of 3, 5, and 8 log_{10} CFU/mL of E. coli-Xen-14, respectively (Table 2). In terms of concentration of E. coli associated with emerging flies, there is a relative agreement between the current study and Rochon et al. (2005). Rochon et al. (2005) reported 2.3 log_{10} contamination of E. coli on the emerging flies; however, it must be taken into account that the fly concentrations reported by Rochon et al. (2004) are from flies that were rinsed upon emergence, and the reported log_{10} CFU/mL value is only representative of the E. coli located within the fly, whereas in the current study flies were not rinsed prior to enumeration, and this log_{10} CFU/mL reported is simply a total log10 CFU/mL with no indication of where the E. coli was located (includes both internal and external E. coli present at the time of enumeration).

The objective of this study was to further investigate the findings of Rochon et al. (2005), which indicated that M. domestica flies could emerge from the pupae stage contaminated with E. coli consumed by the larvae. The design of the study differs from that of Rochon et al. (2005) in three areas. First, the larvae were placed into a more natural environment (bovine manure) inoculated with E. coli, while Rochon et al. (2004 and 2005) utilized a Petri plate contaminated with E. coli. Secondly, for the current study we utilized 0, 3, 5, and 8 log_{10} CFU/mL concentrations of E. coli compared to only 8 log_{10} concentration of E. coli. Finally, for the current study we utilized larvae collected from the wild and utilized larvae from the second larval stage, whereas Rochon et al. (2004 and 2005) utilized lab-reared larvae in the third larval stage. As a result, there were differences in the percentage of larvae, pupae, and flies contaminated with E. coli and the concentration of E. coli within the pupae, pupae, and flies compared to that of Rochon et al. (2004 and 2005). However, the overall conclusions are the same. Larvae of M. domestica can consume E. coli during the larval stage, survive the metamorphosis process, and emerge containing E. coli, thus allowing the adult fly to emerge as an immediate mechanical transmission vector of E. coli. However, it must be noted that for the current study an enteropathogenic E. coli strain was utilized, and there may be some difference between the enteropathogenic strain and that of an enterohemorrhagic strain of E. coli. While the conclusion of this study and Rochon et al. (2005) are in agreement, there is still further research that needs to be conducted. Manure utilized in the current study was devoid of bacterial competition that could obfuscate some of the current findings related to the percentage of larvae and flies contaminated with an E. coli and the concentrations of an E. coli isolated from within the larva and the flies. Thus, there is a need to determine whether the current results would remain similar when there is bacterial competition within the inoculated manure. Secondly, larvae utilized for the current study were collected from a manure run-off basin; thus, there was no exact known age for the larvae utilized. Utilization of larvae hatched from sterile eggs and allowed to be in contact with E. coli throughout the entire life cycle of the housefly is needed to validate these findings. Finally, for this trial an enteropathogenic strain of E. coli strain was utilized. There is a need to determine whether these results will hold consistent when an enterohemorrhagic strain of E. coli is utilized.

Conclusions

These results indicate that an adult M. domestica can emerge from the pupa stage contaminated with an E. coli and possibly serve immediately as a transmission vector. While in specifics these results vary from those of Rochon et al. (2004 and 2005), the conclusions of both trials are similar. Furthermore, results of this trial continue to demonstrate the beneficial use of photonically transformed bacteria as a research model for investigating migration and/or transmission of bacteria.

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Disclosure Statement

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References


