



ELSEVIER

Available online at www.sciencedirect.com

SCIENCE @ DIRECT®

International Journal of Food Microbiology 93 (2004) 259–262

INTERNATIONAL JOURNAL OF
Food Microbiology

www.elsevier.com/locate/ijfoodmicro

Short communication

Quantitative contamination and transfer of *Escherichia coli* from foods by houseflies, *Musca domestica* L. (Diptera: Muscidae)

Antonio J. De Jesús*, Alan R. Olsen, John R. Bryce, Richard C. Whiting

U.S. Food and Drug Administration/Center for Safety and Applied Nutrition, 5100 Paint Branch Parkway, College Park, MD 20740, USA

Received 12 February 2003; received in revised form 30 October 2003; accepted 1 December 2003

Abstract

The housefly, *Musca domestica* L. (Diptera: Muscidae), is recognized as an important factor in the dissemination of various infectious diseases such as cholera, shigellosis, and salmonellosis. They can also serve as a cross-contamination vector for other foodborne pathogens. However, the potential for bacterial transfer by houseflies has been demonstrated in a qualitative rather than quantitative manner. In this study, the numbers of bacteria a housefly can carry on its body and transfer to a clean surface after exposure to a sugar–milk aqueous solution, steak, and potato salad contaminated with a fluorescent gene *Escherichia coli* ($8 \log_{10}$ CFU/ml) were determined. In the first series of experiments to quantify bacterial numbers on the flies, about 40–60 flies were transferred into a sterile cage, exposed to the food for 30 min, the flies immobilized and the attached *E. coli* on each fly enumerated. Detectable *E. coli* ($>1.7 \log_{10}$ CFU/fly) were found on 43% (29/67), 53% (23/43), and 62% (32/52) of the flies in the cages with sugar/milk, steak, and potato salad, respectively. For the positive flies, the geometric mean carriage (\log_{10} CFU/fly) was 2.93 ± 1.24 for sugar–milk, 3.77 ± 1.28 for steak, and 2.25 ± 0.64 for the potato salad. In the second series of experiments, the transfer of bacteria by individual flies from contaminated food to the inner surface of a sterile jar per each landing was determined. *E. coli* transferred from the sugar–milk was $3.5 \pm 0.7 \log_{10}$ CFU/fly-landing, 3.9 ± 0.7 for steak and 2.61 ± 1.16 for the potato salad. From the initial contamination levels of bacteria and the number of transferred bacteria, it can be calculated that flies contaminate clean surfaces with approximately 0.1 mg of food per landing.

© 2004 Elsevier B.V. All rights reserved.

Keywords: Houseflies; Bacterial transfer; Food

1. Introduction

The U.S. Food and Drug Administration (FDA) has classified flies as filth adulterants and requires the exclusion of flies and other pests from food and from establishments that manufacture, pack or store food

products. Flies are also considered to be mechanical vectors of various pathogens such as bacteria, protozoa, and viruses. The housefly (*Musca domestica*) is categorized by FDA as an important contributing factor in the dissemination of various infectious foodborne diseases such as cholera, shigellosis and salmonellosis (Olsen et al., 2001). Adult houseflies have been demonstrated to transmit pathogens from the sponging mouthparts, through vomitus, on body and leg hairs, on the sticky parts of the feet, and through the intestinal tract. Microorganisms on the fly's body

* Corresponding author. Tel.: +1-301-436-1663; fax: +1-301-436-2632.

E-mail address: adejesus@cfsan.fda.gov (A.J. De Jesús).

are disseminated by direct contact, in fly feces and through the air for short distances from insect-electrocuting traps (Olsen, 1998).

The sources of approximately 25% of epidemics of foodborne illnesses reported each year are unknown. Foods contaminated by flies may contribute to some of these epidemics. In an epidemic of enterohemorrhagic colitis in a nursery school in Japan, *Escherichia coli* O157:H7 was isolated from both patients and houseflies collected in the area and all isolates were indistinguishable by molecular typing methods. Flies were traced back to a cattle farm located near the nursery school (Moriya et al., 1999). Other studies have shown that pathogen-carrying flies travel between pathogen reservoirs and exposed foods. In Mexico it was demonstrated that houseflies transported *Salmonella* from slaughterhouses to nearby markets and residential areas (Greenberg et al., 1963). An earlier study found houseflies transporting *Salmonella enteritidis* from a sewage pool to a kitchen 3 miles away (Pepler, 1944).

The potential of the housefly to carry different pathogenic bacteria has been studied extensively. Fukushima et al. (1979) isolated *Yersinia enterocolitica* from flies collected from a piggery. Rosef and Kapperud (1983) isolated *Campylobacter jejuni* from houseflies captured from a chicken farm and a piggery. Recently, Olsen and Hammack (2000) isolated *Salmonella* spp., among them *S. enteritidis*, from different types of flies collected at caged-layer chicken facilities that had produced eggs implicated in two recent outbreaks of *S. enteritidis*.

Few studies have reported quantitative data on the numbers of bacteria a housefly can potentially transfer. In 1999, Kobayashi et al., reported that 10^6 – 10^7 *E. coli* O157:H7 per fly were present in the alimentary canal of all the flies tested immediately after feeding. In the same study, the authors reported that flies were able to disseminate several hundreds of colonies of *E. coli* O157:H7 onto the surface of an agar plate. In a separate study, Sasaki et al. (2000) reported that 5×10^6 CFU/crop of *E. coli* O157:H7 were detected in houseflies immediately after feeding and that the numbers of the same bacteria in one drop of excrement 1 h after feeding were approximately 10^4 CFU. Although these studies are of great significance, they do not provide an estimate of the numbers of bacteria transferred by the houseflies

onto a clean surface. In the present study, the numbers of bacteria a housefly can carry on its body and transfer to a clean surface after exposure to a contaminated food were determined to provide an estimate of the bacterial concentrations a fly could potentially transfer to a food.

2. Material and methods

2.1. Bacterial strain and food

E. coli ATCC 11775 containing the green fluorescent protein plasmid (GFP) was obtained from the Food and Drug Administration's Center for Food Safety and Applied Nutrition culture collection. *E. coli*-GFP colonies fluoresce upon UV illumination and are ampicillin resistant, which allows them to be enumerated in the presence of background microflora. The working culture was grown in brain heart infusion broth (BHI, Difco) supplemented with ampicillin (100 mg/ml, Sigma) for 24 h at 37 °C and stored at 5 °C for up to 2 weeks. Inoculum was prepared by transferring 0.1 ml of the working culture to 10 ml of BHI broth and incubating it for 24 h at 37 °C. Three different foods were used as the contamination source: a sugar–milk solution, uncooked steak and commercial potato salad. Sugar–milk solution and potato salad were inoculated with the 24-h culture to obtain a concentration of $8 \log_{10}$ CFU/g of food. Sterile cotton was used as a reservoir in the sugar–milk solution experiments. The steak was inoculated with 250 μ l of the 24-h culture spread over approximately 3 cm² of meat.

2.2. Flies

Adult houseflies, *M. domestica*, were reared in sanitary facilities from pupae obtained from Carolina Biological Supply, Burlington, NC. Fly cultures were maintained in the FDA insectary at the Center for Food Safety and Applied Nutrition (CFSAN) at 25 °C under a 12-h day/night light cycle. Adult flies were provided with water and fed an approximate 1:1 (v/v) mixture of granulated sugar and powdered milk. Voucher specimens of the adult flies were deposited in the FDA repository collection located at CFSAN, College Park, MD.

2.3. Contamination of houseflies with *E. coli*

About 15–20 flies were transferred into a sterile stainless steel cage and exposed to the inoculated food for 30 min. The flies were quickly immobilized by refrigerating the cage for about 5 min and each fly was transferred into 5 ml 0.1% peptone water. After vortexing for 45 s, appropriate dilutions were made and plated onto BHIA plates supplemented with ampicillin. Plates were incubated at 37 °C for 24 h and the colonies counted. *E. coli*-GFP colonies were confirmed by fluorescence under UV light.

2.4. *E. coli* transfer from inoculated food to a clean surface by houseflies

Experiments were performed in 250-ml sterile polymethylpentene jars (Nalgene) containing a small dish of food. Contaminated food was placed into the sterile dish, which was then carefully placed inside the sterile jar avoiding any contact of the food with the jar's interior surface. Individual flies were transferred to the jars and exposed to the contaminated food for different time periods and the numbers of landings on the food were recorded. After 5–10 landings–take offs, the flies were immobilized by refrigerating the jar for about 5 min and the fly and food dish were removed. The jar interior surface was rinsed by vigorous shaking with 10 ml 0.1% peptone buffer and appropriate dilutions were plated in BHIA plates supplemented with ampicillin. Plates were incubated at 37 °C for 24 h. *E. coli*-GFP colonies were confirmed by fluorescence under UV light. The total number of *E. coli* rinsed off the inside surface of the jar was divided by the appropriate number of landings on the food and reported as CFU/fly-landing.

3. Results and discussion

As mechanical vectors, flies can transfer pathogens by contact with contaminated legs or mouthparts, and by the excreta or regurgitated fluid within a short time after exposure to the contaminated source. In the first set of experiments, only the numbers of *E. coli* adhering to the fly's body after multiple landings were measured. Detectable *E. coli* ($>1.3 \log_{10}$ CFU/fly) were found on 43% (29/67) of the flies exposed to

the sugar–milk solution. *E. coli* counts ranged from 1.7 to 5.5 \log_{10} CFU/fly with a mean carriage of $2.93 \pm 1.24 \log_{10}$ CFU/fly. Of the flies exposed to the inoculated steak 53% (23/43) were found positive for *E. coli*. Counts ranged from 1.7 to 5.5 \log_{10} CFU/fly with a mean carriage of $3.77 \pm 1.28 \log_{10}$ CFU/fly. Sixty-two percent (32/52) of the houseflies exposed to contaminated potato salad were found positive for *E. coli*. Counts ranged from 1.4 to 3.8 \log_{10} CFU/fly with an averaged carriage of $2.25 \pm 0.64 \log_{10}$ CFU/fly.

In the second set of experiments, counts recovered from the jar interior surface could also reflect bacteria transferred in the regurgitated fluid of the flies. *E. coli* numbers transferred from food to jar ranged from 2.4 to 4.7 \log_{10} CFU/fly-landing for the sugar–milk solution, from 2.5 to 4.7 \log_{10} CFU/fly-landing for the steak and from 1.1 to 4.5 \log_{10} CFU/fly-landing for the potato salad. The numbers of *E. coli* transferred averaged 3.5 ± 0.7 , 3.9 ± 0.7 , and $2.61 \pm 1.16 \log_{10}$ CFU/fly-landing for sugar–milk, steak, and potato salad, respectively.

Our results provided quantitative data on bacterial transfer by the houseflies and showed that they can cross contaminate other surfaces with approximately 0.001% of the original numbers in the contaminated source. The food contained approximately 10^8 CFU/g and an average of 10^3 CFU per landing was observed. Based on these data, it was estimated that the flies could transfer approximately 0.1 mg of food per landing (Fig. 1). Greater numbers of *E. coli* were transferred per landing from the steak, which could be attributed to the higher concentration of inocula on the

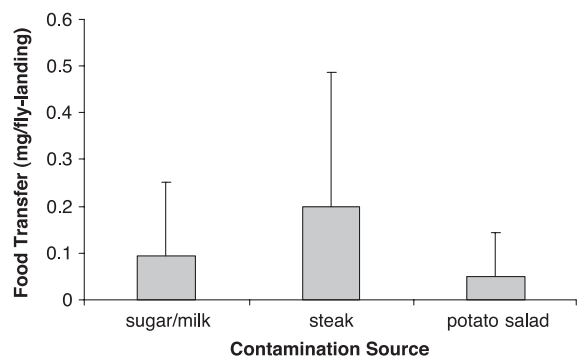


Fig. 1. Averages and standard deviations of food transferred by flies per landing from sugar–milk, steak and potato salad.

surface of the meat. It is possible some of the transfer is via regurgitation but most is the transfer of food or liquid that contains the bacteria. These type of quantitative data are essential when estimating the potential risk that a single housefly or a population of houseflies might represent in the transmission of pathogens into the food supply and can be used for risk assessments purposes.

Acknowledgements

The authors would like to thank Brian. S. Eblen and Karen Carson (FDA/CFSAN, College Park, MD) for critically reading this manuscript.

References

- Fukushima, H., Ito, Y., Saito, K., Tsubokura, M., Otsuki, K., 1979. Role of the fly in the transport of *Yersinia enterocolitica*. Appl. Environ. Microbiol. 38, 1009–1010.
- Greenberg, B., Varela, G., Bornstein, A., Hernandez, H., 1963. Salmonellae from flies in a Mexican slaughterhouse. Am. J. Hyg. 77, 177–183.
- Kobayashi, M., Sasaki, T., Saito, N., Tamura, K., Suzuki, K., Watanabe, H., Agui, N., 1999. Houseflies: not simple mechanical vectors of enterohemorrhagic *Escherichia coli* O157:H7. Am. J. Trop. Med. Hyg. 61, 625–629.
- Moriya, K., Fujibayashi, T., Yoshihara, T., Matsuda, A., Sumi, N., Umezaki, N., Kurahashi, H., Agui, N., Wada, A., Watanabe, H., 1999. Verotoxin-producing *Escherichia coli* O157:H7 carried by the housefly in Japan. Med. Vet. Entomol. 13, 214–216.
- Olsen, A.R., 1998. Regulatory action criteria for filth and other extraneous materials: III. Review of flies and foodborne enteric disease. Regul. Toxicol. Pharmacol. 28, 199–211.
- Olsen, A.R., Hammack, T., 2000. Isolation of *Salmonella* spp. from the housefly, *Musca domestica* L., and the dump fly, *Hydrotaea aenescens* (Wiedemann) (Diptera: Muscidae), at caged-layer houses. J. Food Prot. 63, 958–960.
- Olsen, A.R., Gecan, J.S., Ziobro, G.C., Bryce, J.R., 2001. Regulatory action criteria for filth and other extraneous materials: V. Strategy for evaluating hazardous and nonhazardous filth. Regul. Toxicol. Pharmacol. 33, 363–392.
- Peppler, H.J., 1944. Usefulness of microorganisms in studying dispersion of flies. Bull. U.S. Army Med. Dep. 75, 121–122.
- Rosef, O., Kapperud, G., 1983. Houseflies (*Musca domestica*) as possible vectors of *Campylobacter fetus* subsp. *jejuni*. Appl. Environ. Microbiol. 45, 381–383.
- Sasaki, T., Kobayashi, M., Agui, N., 2000. Epidemiological potential of excretion and regurgitation by *Musca domestica* (Diptera: Muscidae) in the dissemination of *Escherichia coli* O157:H7. J. Med. Entomol. 37, 945–949.