SHORT COMMUNICATION

Vector Competence of *Musca domestica* (Diptera: Muscidae) for *Yersinia pseudotuberculosis*

L. ZUREK, S. S. DENNING, C. SCHAL, AND D. W. WATSON

Department of Entomology, North Carolina State University, Raleigh, NC 27695

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ABSTRACT The vector potential of adult house flies, *Musca domestica* L., for *Yersinia pseudotuberculosis* (Pfeiffer), a pathogen of domestic animals and humans, was investigated. Adult flies were allowed to feed on trypticase soy broth (TSB) containing *Y. pseudotuberculosis* for 6 h and then transferred to sterile containers with sterile TSB as a source of water and nutrients. At 6-h intervals, all flies were transferred to sterile containers with sterile TSB and 10 randomly selected flies were examined for the pathogen. *Yersinia pseudotuberculosis* did not establish a permanent population in the house fly colony; however, viable cells were detected from the digestive tract of flies for up to 36 h after the initial exposure, and flies contaminated their environment (sterile TSB) for up to 30 h after the exposure. These results demonstrated that house flies can carry *Y. pseudotuberculosis* for a considerable period and therefore must be considered as a potential mechanical vector of pseudotuberculosis infection.

KEY WORDS house fly, *Yersinia pseudotuberculosis*, vector potential, turkeys

Yersinia (Pasteurella) pseudotuberculosis (Pfeiffer), a member of the family Enterobacteriaceae, is a gram-negative, nonspore-forming, motile, facultative anaerobe. It is primarily an animal pathogen although human infections have also been reported (Aleksic and Bockemmuhl 1999). *Yersinia pseudotuberculosis* has been identified as the etiological agent in epizootics in wild birds and rodents, including grackles, rats, and rabbits (Beaudette 1940, Hacking and Sileo 1974, Hubbert 1972, Kaneko et al. 1979, Wise and Uppal 1972). Outbreaks of *Y. pseudotuberculosis* in stocks of domestic turkeys can cause high bird mortality (Wallner-Pendleton and Cooper 1983). Since the first case report of this disease in turkeys in 1941 (Rosenwald and Dickinson 1944), other outbreaks have been reported from England and the United States (Blaxland 1947, Mathey and Siddle 1954, Kilian et al. 1962, Wise and Uppal 1972, Wallner-Pendleton and Cooper 1983). However, the source of infection of these outbreaks has not been determined although ground squirrels were suggested as potential vectors in one of the outbreaks (Wallner-Pendleton and Cooper 1983).

House flies are known as vectors of many human and animal bacterial pathogens such as *Campylobacter jejuni* (Jones) (Shane et al. 1984), *Helicobacter pylori* (Goddwin) (Grubel et al. 1997), *Salmonella* sp. (Fobert 1971), and *Listeria* sp. (Gershun 1976). Recently, we have isolated *Y. pseudotuberculosis* from the intestinal tract of house fly larvae collected from turkey bedding (Zurek et al. 2000). Because house flies are commonly associated with turkey flocks and house fly larvae develop in turkey bedding, it is possible that adult house flies play a role in transmission and maintenance of the infection.

In this study, we examined the vector potential of adult house flies for *Y. pseudotuberculosis* as well as their capacity to contaminate the environment.

Materials and Methods

Several hundred house fly pupae were selected from our laboratory colony (larvae maintained on wheat bran and calf food [13:1 ratio], adults on sugar and powdered milk [3:1 ratio] and water ad libitum), placed into a sterile plastic container for eclosion, and emerged adults were used in the later experiments. (Our laboratory colony was started 3 yr ago from the stock colony maintained at the Department of Entomology, Cornell University, Ithaca, NY).

One hundred and twenty randomly selected adult flies were transferred in four groups of 30 into sterile plastic containers. Three containers were supplied with a glass dish containing 1.0 ml of trypticase soy broth (TSB, Difco, Detroit, MI) with *Y. pseudotuberculosis* (2.0×10^7 cells per milliliter), one container was provided with a dish containing 1.0 ml of sterile TSB (control group).

At 6-h intervals, all flies were immobilized with carbon dioxide and transferred to new sterile containers containing 1 ml of sterile TSB. The treated colony was monitored for *Y. pseudotuberculosis* for 78 h, the control colony was examined for the pathogen at 6 h and 36 h after the treatment (Table 1). Before each transfer the old TSB was sampled, and 100 μl was spread on a selective medium, Yersinia base agar (YBA) (Difco) undiluted and in two dilutions in PP buffer ($10^{-1}, 10^{-2}$), and incubated aerobically at 27°C to detect *Y. pseudotuberculosis* contamination (TSB

Table 1. Viability of *Y. pseudotuberculosis* in adult house flies

Sampling imte, h	Detection of *Y. pseudotuberculosis*		
	TSB droplet contamination	Gastrointestinal tract	YBA plate contamination
	Test flies		
0[a]	−	−	−
6	+	+	+
12	+	+	+
18	+	+	+
24	+	+	−
30	+	+	−
36	−	+	−
42	−	−	−
78	−	−	−
	Control flies		
6	−	−	−
36	−	−	−

+, positive; −, negative; TSB, trypticase soy broth; YBA, Yersinia base agar.

[a] Before *Y. pseudotuberculosis* exposure.

droplet contamination, Table 1). In addition, at each 6-h interval, 10 flies from each treated colony (flies in the control colony were examined at 6- and 36-h intervals only) were randomly selected and divided into two equal groups. Flies in one group were surface sterilized by submersion in ethanol (70%) for 1 min, rinsed with sterile water, submerged in sodium hypochlorite (0.05%) for 1 min, and then rinsed twice in sterile water and homogenized in 1.0 ml of sterile PP buffer. The homogenate (100 μl) was spread undiluted and in two dilutions in PP buffer (10^{-1}, 10^{-2}) on YBA and incubated aerobically at 27°C and regarded as a representation of the gastrointestinal microbial population (Table 1). The second group of flies was transferred to a petri plate with YBA medium containing a 100-μl droplet of PP (3.0 mM potassium phosphate) buffer on the surface. This group was maintained for 6 h to detect their potential to contaminate the plate (YBA plate contamination, Table 1).

After 48 and 72 h of incubation, each YBA plate was examined for the pathogen. Morphologically different colonies were isolated on YBA. The presence of *Y. pseudotuberculosis* was confirmed by colony and cell morphology, oxidase (BBL Oxidase, Becton Dickinson, Cockeysville, MD) and catalase test (3% H_2O_2), motility test (stabbing into soft agar medium: tryptose 10.0 g/liter, NaCl 5.0 g/liter, agar 5.0 g/liter), and inoculation on Simmon's citrate agar (Difco) (Holt et al. 1994). Other bacterial colonies growing on YBA were isolated and characterized by cell morphology, gram staining (BBL, Becton Dickinson), oxidase and catalase test, motility test, growth on MacConkey agar (Difco) and Simmon's citrate agar and tested for carbohydrate (glucose, lactose, sucrose) fermentation on triple sugar iron agar (TSI) (Difco) (Garcia et al. 1998).

Results and Discussion

Our results showed that adult house flies can transmit *Y. pseudotuberculosis.* The pathogen was carried in the digestive tract of flies for 36 h after the initial exposure (Table 1), although the number of colony forming units declined over time, suggesting that the pathogen did not replicate in the gut lumen. Two other gram-negative bacterial isolates from the digestive tract growing on YBA plates successfully outcompeted *Y. pseudotuberculosis* after 36 h. Phenotypic tests revealed that these bacteria were also members of the family Enterobacteriaceae and were a part of the natural gut microbiota of house flies. Both isolates were gram-negative, motile rods, catalase positive, oxidase negative, grew on MacConkey agar and Simmon's citrate agar and fermented glucose. They were detected in the digestive tract of control flies as well as house flies from our regular laboratory colony.

Our results also show that house flies can contaminate the environment (droplet of TSB) with *Y. pseudotuberculosis* for up to 30 h after the initial exposure (Table 1). It is likely that the TSB droplet became contaminated by regurgitation of the crop content during feeding, although contamination from the feces is also possible. House flies kept on YBA plates contaminated the medium for up to 18 h; heavy growth of two other enteric bacteria made detection of the pathogen impossible after this period (Table 1).

These results indicate that *Y. pseudotuberculosis* was not a part of the natural bacterial community in the digestive tract of the house fly, and it was probably eliminated by competitive exclusion over a relatively short period. Nevertheless, from the vector potential perspective, this period is considerable because flies could carry viable cells of *Y. pseudotuberculosis* for up to 36 h after exposure. Clinical symptoms of the pseudotuberculosis infection in turkeys include watery diarrhea (Wallner-Pendleton and Cooper 1983) that likely becomes a source of contamination for flies. In addition, because *Y. pseudotuberculosis* causes high mortality in turkey flocks, carcasses may also become a source of inoculum. Consequently, it is feasible that house flies transmit the pathogen throughout a turkey farm and the surrounding environment. Because house flies can fly distances of >32 km (Schoof and Siverly 1954), the transmission of this pathogen over a relatively wide area is also possible.

Many other studies have shown that house flies can serve as vectors for other pathogenic microorganisms of humans and animals. For example, Grubel et al. (1997) investigated viability of *H. pylori* in adult house flies. Their results of reisolation trials of this pathogen were similar to the results in our study. House flies carried *H. pylori* in the digestive tract for up to 30 h (Grubel et al. 1997). This suggests that the conditions in the digestive tract of the house fly are favorable for only a certain microbial community and new microorganisms are probably eliminated by competitive exclusion as well as by physical and chemical factors in the gut lumen.

In conclusion, adult house flies can carry viable cells of *Y. pseudotuberculosis* for up to 36 h, and therefore must be considered a potential mechanical vector of pseudotuberculosis infection. Special efforts should be taken to reduce house fly populations on turkey farms during pseudotuberculosis outbreaks.

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