Effects of Antibiotic Use on Larval House Fly Development

H. Griese and C. Olds

Summary

House flies (*Musca domestica* L.) are persistent pests in dairy operations, thriving in manure and other waste products that also may contain antibiotic residues. To evaluate the effect of antibiotic residues on house fly development, laboratory-reared flies with no prior antibiotic exposure and wild flies collected from a dairy were raised in tetracycline- and enrofloxacin-infused growth media. Laboratory colony flies showed reduced larval weight, while wild-caught flies were less affected, suggesting prior environmental exposure may increase tolerance to chemical stressors. These findings suggest that antibiotics in manure may act as chronic chemical stressors, driving future tolerance to insecticides in adult house flies.

Introduction

House flies are synanthropic insects that thrive in livestock production systems, particularly confined animal feeding operations such as dairies, where manure and organic waste provide abundant breeding sites (Geden et al., 2021). Eggs are laid directly onto moist decomposing organic matter, and first instar larvae hatch within 24 hours after the eggs are laid. Larvae feed on soluble matter and microbes and proceed through three instars, gaining size with each molt. Once sufficient weight has been gained in the third instar, larvae move to drier areas to pupate. Once fully developed within the puparium, an adult fly emerges and reaches sexual maturity within three days (Geden et al., 2021).

While adult house flies are well recognized for their ability to develop resistance to insecticides (Kaufman et al., 2010), much less is known about how chemical exposure during the larval stage influences survival, development, and fitness. Accordingly, the purpose of this study was to evaluate how house fly larvae respond to chemical stressors that may be present in typical dairy environments. Two antibiotics were evaluated in this study, enrofloxacin and tetracycline. The antibiotics were selected for their different antibacterial modes of action. Tetracycline is used to treat bacterial infections and targets the bacterial ribosomes to inhibit protein synthesis, leading to cell death. While not permitted for use in cattle older than 20 months, enrofloxacin is used to treat bovine respiratory disease by targeting enzymes associated with DNA replication, which halts any replication or cellular repair.

This study aims to enhance the understanding of how environmental exposures influence house fly ecology and resilience, with implications for pest management strategies and the broader issue of antimicrobial resistance in livestock systems.

Experimental Procedures

House fly colony history and rearing

Carolina 2024 (CR) house flies were purchased from Carolina Biological Supply, Burlington, NC, in 2024 and maintained at Kansas State University, Department of Entomology. This colony is maintained without exposure to insecticides or antibiotics. Egg-laying media is placed in the cages each week to raise new generations of adults. Larvae are raised on a mixture containing wheat bran (500 g), Nutrena SafeChoice pelleted horse food (150 g), and water (350 ml). Adult flies are maintained in cages with about 200 flies per cage with access to water and a mixture of sugar and milk powder *ad libitum*. Eggs for the assay were collected by providing flies with a moistened paper towel containing yeast and milk powder, which is used to lay eggs.

Three times over the summer of 2025, adult house flies were collected from feed bunks of the Kansas State University Dairy Teaching and Research Center, Manhattan, KS. Approximately 200 adult flies were collected directly from the feed bunks by sweep-netting and immediately placing the flies into cages with food and water *ad libitum*. The adults were provided with a moistened paper towel with yeast and milk powder three days post-collection to lay eggs. These flies were referred to as the Dairy strain (DR).

Larval antibiotic exposure

To assess the effects of antibiotics on larval development, three biological replicates were conducted for each treatment of antibiotics on larvae from each fly colony. Concentrations of enrofloxacin and tetracycline were selected based on published reports of antibiotics found in the environment. For tetracycline, environmental residues of approximately 43.5 mg/kg have been reported (Daghrir and Drogui, 2013). Environmental concentrations of enrofloxacin have been observed at approximately 10.5 mg/kg (Zhao et al., 2010). In addition, dosages of 10 times (436 and 105 mg/kg for tetracycline and enrofloxacin) and 100 times (4,360 and 1,050 mg/kg) the environmental concentration were tested.

Antibiotics were applied to house fly larval growth media (30 g per cup) and mixed thoroughly to ensure homogeneity. Each antibiotic treatment was performed in triplicate. Approximately 30 eggs were placed into each cup, which was then covered with vented lids and wrapped in aluminum foil to prevent degradation of the antibiotics by light. All treatments were incubated at 28 °C at a maintained humidity of 50 ± 5% and monitored throughout the development period. At the second and third instar larval stages (days 2 and 5, respectively), 10 larvae were removed from the cup, and weight measurements were taken. Larvae were returned to their experimental units after their weights were recorded. One day after pupation began, 10 pupae were removed from their cups, weighed, and placed into new cups to measure adult emergence. The entire assay was repeated three times for each strain.

Bacterial community analysis

While second- and third-instar flies were removed for weight measurement, 0.1 g of media was removed from each cup and added to sterile phosphate-buffered saline (PBS). Ten-fold serial dilutions with PBS were performed until a dilution of 10^{-10} was achieved, after which 100 mL was spread plated onto Luria-Bertani (LB) agar plates. The plates were incubated at 37 °C overnight, and the number of colonies was counted. Colonies with distinct morphotypes were further grown on blood agar plates and submitted to the Kansas State University Veterinary Diagnostic Laboratory

(Manhattan, KS, USA) for bacterial identification via Matrix-Assisted Laser Desorption/Ionization-Time of Flight Mass spectrometry (MALDI-TOF MS).

Data analysis

A two-way ANOVA was performed to examine the effects of antibiotic treatment of houseflies. All data analyses were conducted using GraphPad Prism version 10.6.1 for Windows, GraphPad Software, Boston, Massachusetts USA, *www.graphpad.com*. Significance levels were annotated as * = $p < 0.05$, ** = $p < 0.01$, ***= $p < 0.001$, ****= $p < 0.0001$ (two-way ANOVA) in Table 2.

Results and Discussion

Bacterial community analysis

To ensure microbial food source abundance was not negatively impacted by antibiotic exposure, samples were taken from the media, and microbial communities were identified. Four morphotypes were selected and identified through MALDI-TOF MS as *Providencia stuartii*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Staphylococcus gallinarum*. These taxa were consistently detected in all groups regardless of antibiotic exposure. Neither enrofloxacin nor tetracycline exposure significantly altered the number of colony-forming units (CFU) (Table 1), indicating that house flies across all treatments would have had equal nutritional resources.

Effect of antibiotic exposure on house fly larval development

Second instar larvae of the CR strain exhibited significant weight reductions when exposed to the highest concentrations of tetracycline and enrofloxacin (Table 2). Untreated larvae averaged 10.1 mg, while those treated with 2,180 mg/kg tetracycline or 1,050 mg/kg enrofloxacin weighed 6.1 mg and 5.6 mg, respectively. In contrast, DR larvae showed minimal response, with only the highest enrofloxacin concentration causing a significant weight reduction.

In the third-instar CR larvae, antibiotic exposure continued to suppress growth across treatments (Table 2). Tetracycline at 436 and 4360 mg/kg significantly reduced the average weight to 13.8 mg and 12.5 mg, respectively, compared to 23.4 mg in the control groups. Larvae could not survive exposure to enrofloxacin at 1,050 mg/kg, which resulted in complete mortality by the third instar, and hence no weights could be recorded. Similar to the second instar, DR larvae remained largely unaffected by antibiotic exposure, showing no significant weight loss across treatments.

In CR pupae, antibiotic exposure continued to suppress growth across treatments (Table 2). Tetracycline at 436 and 4,360 mg/kg significantly reduced average weights to 12.8 mg and 6.1 mg, respectively, compared to 21.3 mg in the control groups. Enrofloxacin exposure at 105 and 1050 mg/kg lowered pupal weights to 14.6 mg and resulted in complete mortality at the highest dose. In contrast, DR pupae showed no significant differences across treatments.

Adult emergence from the puparium showed no significant differences between antibiotic treatment and control.

Conclusions

This study shows that antibiotic residues commonly found in dairy cow manure directly affect larval house-fly development and growth. Due to the adequate levels of nutritional resources across groups, it is most likely that this is not due to a lack of food,

but rather the energy cost associated with detoxification of these chemical stressors. Reductions in larval weights were dose dependent, further supporting this supposition. The ability of wild-caught flies to withstand antibiotic exposure without affecting larval weight may be due to already heightened levels of detoxification due to generational exposure to chemicals. Exposure to antibiotic residues as larvae and the associated changes may result in adult populations that are more tolerant to insecticides, influencing house fly control efforts.

Acknowledgments

We would like to thank the Kansas State University Dairy Teaching and Research Center for allowing us to collect adult house flies used in this study. Funding for this study was provided by USDA Cooperative Agreement 58-3020-3-004 and Hatch Multistate funds.

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Table 1. CFU counts and CFU/ml

	Control CFU/mL	Env CFU/mL	10 × CFU/mL	100 × CFU/mL
Tetracycline	8.36E + 13	6.96E + 13	6.01E + 13	6.72E + 13
Enrofloxacin	3.15E + 13	4.89E + 13	3.28E + 13	4.38E + 13

Table 2. Average weight for 10 house fly larvae and pupae exposed to antibiotics. Values represented as the Mean (Standard Deviation). Significance reduction in weight compared to control represented by * = p < 0.05, **= p < 0.01, ***= p < 0.001, ****= p < 0.0001.

	Tetracycline			
	Control	**43.6 mg/kg (Environmental)**	**436 mg/kg (10×)**	**4,360 mg/kg (100×)**
CR Second Instar	10.1 (0.9)	9.0 (2.7)	7.0 (1.8)	6.1 (1.1) **
CR Third Instar	24.1 (4.3)	23.4 (3.0)	13.8 (1.0) ***	12.5 (1.8) ****
CR Pupae	21.3 (2.4)	20.8 (1.8)	12.8 (1.1) **	6.1 (5.3) ****
DR Second Instar	10.3 (0.5)	10.3 (0.5)	10.2 (0.6)	10.0 (0.5)
DR Third Instar	24.1 (4.3)	23.2 (3.0)	20.6 (2.0)	22.7 (2.9)
DR Pupae	22.3 (3.6)	21.9 (2.9)	19.2 (1.9)	21.8 (3.2)

	Enrofloxacin			
	Control	**10.5 mg/kg (Environmental)**	**105 mg/kg (10×)**	**1050 mg/kg (100×)**
CR Second Instar	10.6 (0.5)	10.4 (1.4)	8.4 (2.1)	5.6 (0.5) ***
CR Third Instar	21.6 (2.2)	21.6 (2.3)	16.4 (3.9)	0.0 (0.0) ****
CR Pupae	20.6 (1.7)	19.3(1.0)	14.6 (3.8) *	0.0 (0.0) ****
DR Second Instar	10.3 (0.3)	10.0 (0.6)	9.4 (0.5)	8.9 (0.7) *
DR Third Instar	22.4 (2.7)	20.7 (2.1)	19.1 (2.0)	17.5 (3.0)
DR Pupae	21.0 (2.6)	19.3 (1.7)	18.2 (2.2)	16.3 (2.6)