A Comparative Survey of Calliphorid Attraction to Microbe-Laden and Antibiotic Treated Beef Liver

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Abstract

The goal of this study was to determine whether bacteria colonizing decomposing material play a role in attracting consumers, such as blow flies, and whether the use of antibiotics affects attraction. Untreated beef liver and liver treated with antibiotics were used as bait. More than 750 blow flies were collected over six days and three genera of Calliphoridae were identified: *Lucilia, Chrysomya,* and *Cochliomya.* Overall, the untreated beef liver attracted more flies than the antibiotic treated beef liver, with *Lucilia* being the most prevalent genus collected. Although obvious trends were observed, there was no statistical significance associated with preference of treated versus untreated beef liver. Descriptions of bait, collecting methods, and locations used to obtain flies are presented as well as descriptions of the family and each genera of fly collected.

Key Words: Dominica, blow fly, oviposition, forensics, antibiotics

Introduction

Members of the family Calliphoridae, known as blow flies, have proven to be of critical importance to the forensic world in the past century. Calliphorids oviposit inside and on the surface of decaying matter, particularly carrion. The eggs then hatch and larvae feed on the decomposing substrate, thereby helping to eliminate it from the environment. These insects are forensically important due to the rapidity in which they colonize dead bodies, and can therefore give insight into the time elapsed since death. Recent studies, such as Burkepile et al. (2006), have aimed more focus on microbes as competitors on carrion. This study found that fresh carrion was more likely to be fed upon by large animals than older, microbe-laden carrion. The microbes in this case can be qualified as competitors by rendering the carrion unattractive to other consumers. Burkepile et al. (2006) also tested whether carrion treated with antibiotics would affect attraction and found that it neither inhibited nor stimulated feeding by consumers. Antibiotics are compounds that hinder or cease the growth of bacteria. The aim of my study was to determine whether or not antibiotics would affect the preference for oviposition location in calliphorids, and also to expand upon the Survey of Necrophilous Diptera of Dominica conducted by Jonathan Cammack (2006).

By treating fresh beef liver with a broad-spectrum, bactericidal antibiotic, such as Enrofloxacin (Baytril®), bacterial cell death occurs within 20-30 minutes after exposure. Calliphorids are attracted to carrion due to the volatiles exuded by bacteria breaking down compounds in the body. Because of this, they should not be attracted to bait that has been treated with any agent that represses bacterial growth.

Materials and Methods

The study was conducted on the island of Dominica, West Indies, from May 24 to May 29, 2009. The study took place at the Archbold Tropical Research and Education Center (ATREC), Springfield (15°20'33.9"N 61°22'41.4" W). Fresh beef liver was used as bait to attract necrophilous flies, primarily members of the family Calliphoridae.

The beef liver was cut into nine pieces, each of which weighed approximately 30g. Three portions were soaked in 1L of untreated water, another three portions were soaked in 1L of water treated with .5 mL of the antibiotic Enrofloxacin, and the remaining three portions were soaked in 1L of water treated with 1mL of Enrofloxacin. Both low and high end doses were given to test whether the concentration of the antibiotic played a role in attraction, or if the mere presence of the antibiotic made a significant difference in preference. All three treatments were covered and left to soak for 36 hours.

Traps were made from nine 2L plastic soda bottles by cutting off the tops and inverting them into the bottom half of the container to form a funnel. After the 36 hour soak, each treatment of meat was taken out of its solution and placed in a Ziplock® bag, which was then labeled. Upon arrival at the designated trap location, each piece of beef liver was placed into an inverted trap along with approximately 100mL of fresh water. All traps were hung with nylon rope from tree branches, were approximately 60 cm away from each other and hung approximately 1.5 m above the ground.

In order to avoid pseudo-replication, the three sets of traps were placed in three different locations around the station on Sunday, May 24, 2009. Set A was placed in a meadow at the top of the Mt. Joy trail (15°21.117'N 61°21.793'W), at an elevation of 530m above sea level , set B was placed on a fig tree on the Massacre Trail (15°20.738'N 61°22.132'W), at an elevation of 448m above sea level, and set C was placed by the Checkhall River (15°20.737'N 61°22.148'W), at an elevation of 273m above sea level.

The traps were checked three times during the study: Monday, May 25, Wednesday, May 27, and Friday, May 29, 2009. The flies were collected by taking down each trap individually and decanting the water and its contents into a handheld stainless steel filter. BioQuip® forceps were also necessary to collect flies adhering to the beef liver , and the specimens were placed into a labeled vial of ethanol (C_2H_6O). The beef liver was then placed back inside the trap along with approximately 100 mL of fresh water and the trap was hung back into place. Traps were checked at approximately the same time each morning of collection, with the exception of set A on May 29, due to the close proximity of four large territorial bovids. Set A, then, was checked mid-afternoon on May 29, as opposed to late morning.

After each collection, the flies were taken back to the lab for identification. All non-calliphorid flies were thrown out. Calliphorids were then pinned on #2 Kostal® black enameled

insect pins and placed inside a Schmidt box. All identifications were made to genus using Triplehorn and Johnson (2005) and Whitworth (2006).

Results

The following genera of the family Calliphoridae were collected during this study:

Lucilia

Chrysomya

Cochliomya

Lucilia can be recognized by its metallic green, blue or bronze abdomen, cluster of setae on the suprasquamal ridge and a lower calypter lacking setae (Whitworth, 2006).

Figure 1: *Lucilia*, dorsal view

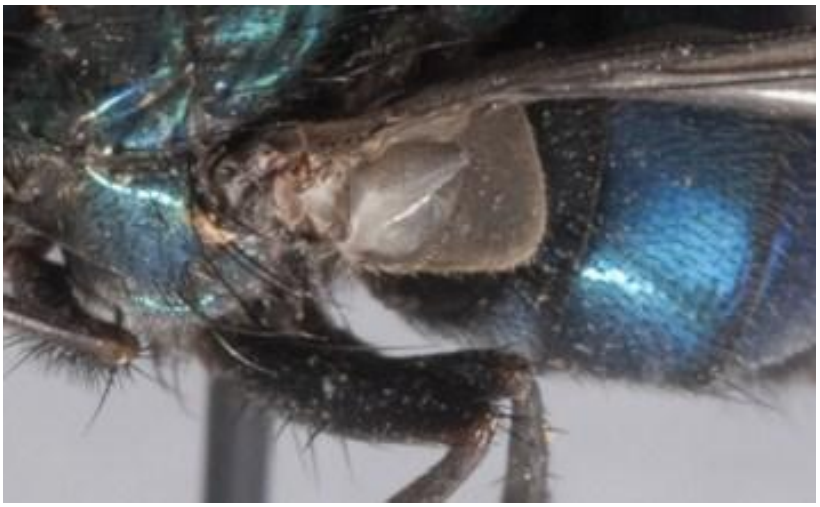

Figure 2: *Lucilia,* lateral view showing bare lower calypter

Chrysomya is distinguished from other calliphorids by a setose stem vein, stiff, erect setae on the greater ampulla, and the dorsum of the 1+2 abdominal tergum and posterior margins of tergites 3 and 4 being black (Whitworth, 2006).

Figure 3: *Chrysomya,* dorsal view

Figure 4: *Chrysomya,* lateral view showing setose lower calypter

Cochliomya can be identified by a setose stem vein, a genal dilation with an orange ground color and yellow setae, dark vittae on the mesonotum, pale setae on the posterior region of the hind coxa, and a filiform palp (Whitworth, 2006).

Figure 5: *Cochliomya,* dorsal view showing dark vittae

Figure 6: *Cochliomya,* anterior view showing orange genal dilation

Results of the trend in attraction of flies in the three genera to each of the treatments are summarized in the graphs and tables below.

Trends Observed Over Location and Time

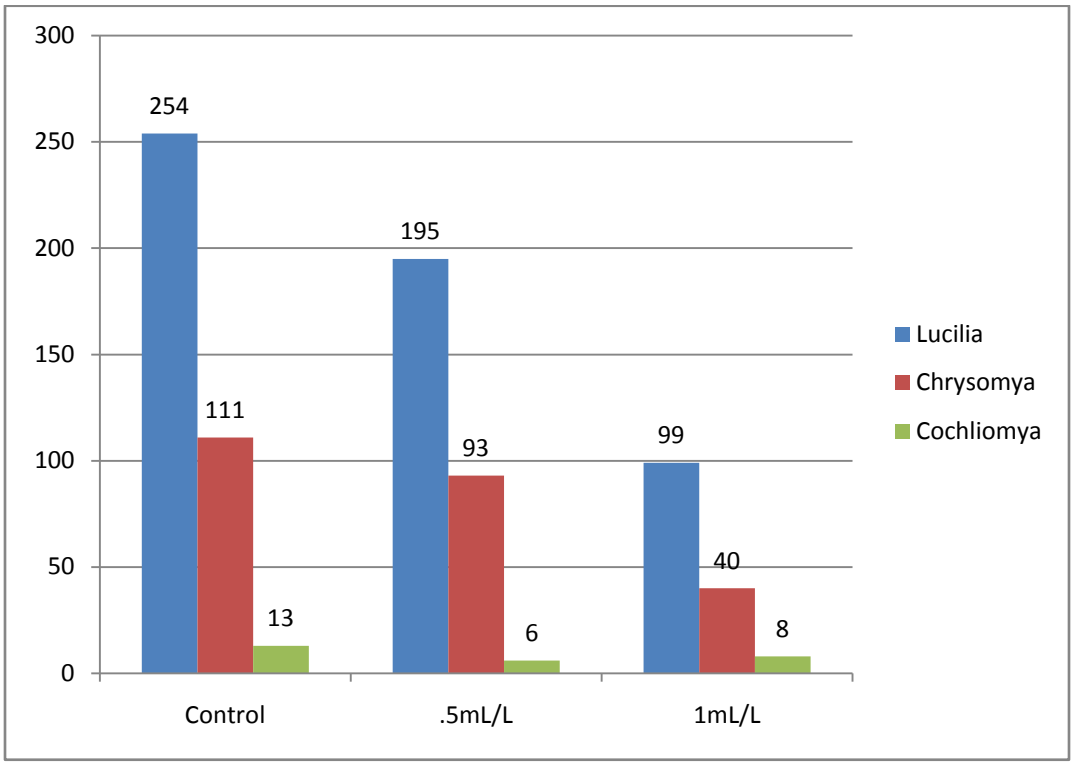

Table 1

On May 25, more flies were attracted to the control at site A, the 1mL/L treatment at site B, and .5mL/L treatment at site C, with at least ten times as many *Lucilia* as either of the other two genera at all three sites. On May 27, more flies were attracted to the .5mL/L treatment at sites A and B, while site C had a greater abundance of flies attracted to the control. Once again, *Lucilia* appeared much more than the other two genera, although *Chrysomya* numbers increased at least ten times more than on May 25.On May 29, more flies were attracted to the .5mL/L treatment at sites A and B, while the control at site C attracted more flies. *Chrysomya* appeared in greater abundance on this day than the other two genera.

Overall, the control attracted 378 flies, the .5mL/L antibiotic treatment attracted 294 flies and the 1mL/L antibiotic treatment attracted 147 flies. *Lucilia* was the most abundant Calliphorid, with 548 specimens collected. *Chrysomya* was the second most collected genus, with 244 specimens. *Cochliomya* appeared to be the least abundant Calliphorid attracted to any of the traps, with only 27 specimens collected.

Descriptive Statistics

Dependent Variable: Count

Treatment	Day	Mean	Std. Deviation	N
1	1	4.22	8.303	9
	2	35.67	67.836	9
	3	2.11	2.934	9
	Total	14.00	41.040	27
2	1	8.56	16.195	9
	2	19.33	17.088	9
	3	4.78	11.443	9
	Total	10.89	15.822	27
3	1	7.89	14.658	9
	2	6.78	7.726	9
	3	1.67	1.936	9
	Total	5.44	9.657	27
Total	1	6.89	13.107	27
	2	20.59	40.858	27
	3	2.85	6.786	27
	Total	10.11	25.918	81

Table 2

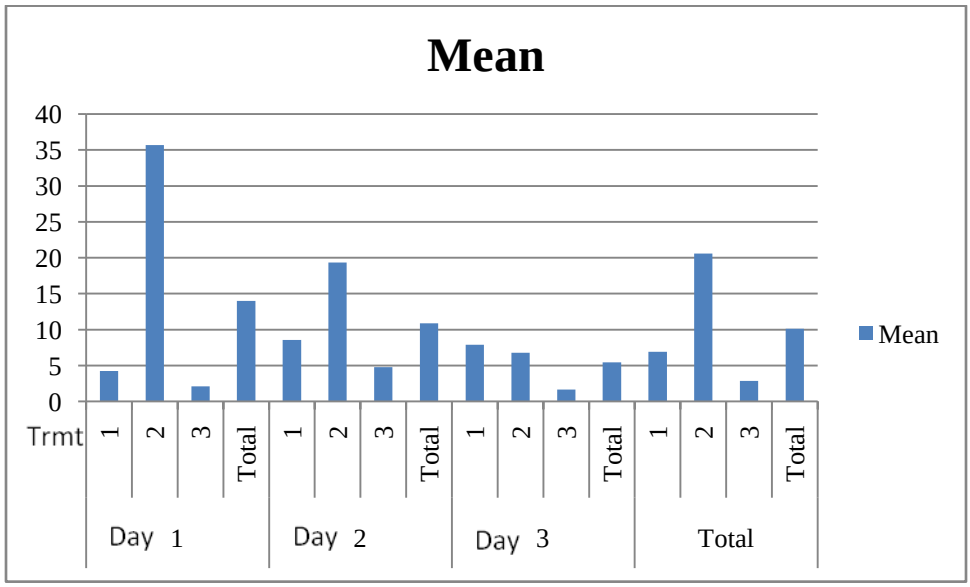

Table 3

Tests of Between-Subjects Effects

Dependent Variable: Count

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
Corrected Model	8595.333(a)	8	1074.417	1.714	.110
Intercept	8281.000	1	8281.000	13.208	.001
Treatment	1012.667	2	506.333	.808	.450
Day	4669.407	2	2334.704	3.724	.029
Treatment * Day	2913.259	4	728.315	1.162	.335
Error	45142.667	72	626.981		
Total	62019.000	81			
Corrected Total	53738.000	80			

R Squared = .160 (Adjusted R Squared = .067)

Table 4

Discussion

The trend observed across the location and time period of data collected in this study showed that all three genera of flies were more attracted to the untreated beef liver rather than beef liver that had been treated with a bactericidal agent (Table 1). The beef liver treated with the lowest dose of antibiotic, however, attracted almost as many flies as the control, possibly because of a negligible reduction in bacteria that would have made preference between the control and the .5mL/L treatment difficult (Table 1). Two genera, *Lucilia* and *Chrysomya*, were much less attracted to the 1mL/L antibiotic treatment, whereas *Cochliomya* preferred it only slightly more than the .5mL/L treatment (Table 1). The lack in attraction to the high-end treatment of the antibiotic could be due to the reduction in bacteria. This would lead to a reduction in the volatiles released by bacteria that attract consumers, such as blow flies. Therefore, since the attractive agent for flies had been reduced in the 1mL/L antibiotic treatment, less blow flies would prefer this beef liver as a prime oviposition location.

Although the trends in the data observed across the location and time period showed obvious preference for the control, there was no statistical significance which would support a preference in any one treatment by the flies. The standard deviation is double the mean in almost every treatment for the three sample days. High variability in the data due to differences in the number of flies at each treatment over three sample periods in three different locations makes it difficult to observe any statistical trend in preference without a larger number of replicates or observation periods.

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