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Effects of the Antibiotics Gentamicin on the Postembryonic Development of *Chrysomya putoria* (Diptera: Calliphoridae)

Adriana C. P. Ferraz,[1,2] Daniele L. Dallavecchia,[1,3] Débora C. Silva,[1,4] Adriana L. Figueiredo,[1] Barbara Proença,[1,5] Renato G. Silva-Filho,[1] and Valéria M. Aguiar[1,2,3,6]

[1]Departamento de Microbiologia e Parasitologia, Laboratório de Estudo de Dípteros, Instituto Biomédico, Universidade Federal do Estado do Rio de Janeiro, UNIRIO, Rua Frei Caneca, 94, Centro, Cep 20211-040, Rio de Janeiro, RJ, Brasil
[2]Universidade Federal Rural do Rio de Janeiro, UFRRJ, Programa de Pós-Graduação em Biologia Animal PPGBA, Rod. BR 465, Km 7, CEP 23890-000 Seropédica, RJ, Brasil
[3]Universidade Federal do Estado do Rio de Janeiro, UNIRIO, Programa de Pós-Graduação em Ciências Biológicas PPGBIO, URCA, RJ, Avenida Pasteur, 296, CEP 22290-240, Rio de Janeiro, RJ, Brasil
[4]Departamento de Estudos Básicos e Instrumentais, Universidade Estadual do Sudoeste da Bahia, UESB, Praça Primavera s/n, Bairro Primavera, CEP 45700-000, Itapetinga, BA, Brasil
[5]Programa de Pós-graduação em Ciências Biológicas (Zoologia), Museu Nacional, Universidade Federal do Rio de Janeiro, Quinta da Boa Vista s/n, RJ, CEP: 20940-040, Rio de Janeiro, RJ, Brasil
[6]Corresponding author, e-mail: valeria@unirio.br

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ABSTRACT. We evaluate the effects the antibiotic Gentamicin on the development of *Chrysomya putoria* (Wiedemann, 1818). Third-generation, first-instar larvae were reared in a climatic chamber on 60 g of homogenate + agar 65% and were treated with three concentrations of Gentamicin: 4.44 mg/ml, 13.33 mg/ml, and 66.66 mg/ml. The control consisted of distilled water. The relationships between mean body mass of mature larvae (measured after diet abandonment, in batches of five individuals), duration of larval and pupal stages, and overall duration of development were analyzed. The actual sex ratio was compared against the expected using the chi square. None of the parameters measured differed significantly among the four treatments, with one exception: when Gentamicin concentration was 13.33 mg/ml, larval viability differed significantly from the control. All larvae from all treatments were considered normal. We conclude that the antibiotic did not significantly alter the development of *C. putoria* (Wiedemann) (Diptera: Calliphoridae).

Key Words: antibiotic, blowfly, forensic study, myiasis

Decomposing animal bodies attract a wide variety of organisms, particularly arthropods, which use this temporary microhabitat for food, breeding, and shelter (Von Zuben 2001). Because insects are usually the first to find a decaying body, they are often used in criminal investigations, particularly to determine the postmortem interval (PMI) (Catts and Goff 1992). To correctly estimate the PMI using insects, however, it is necessary to know their development intervals, succession patterns (Carvalho et al. 2004), and postfeeding larval dispersal rates (Von Zuben 2001).

The rate of development of scavenger insects can be affected by substances that had been previously introduced into the body (e.g. through the food) (Introna et al. 2001) and may affect the PMI (Estrada et al. 2009). In forensic entomology, calliphorid flies are frequently subjected to toxicological analyses because they are among the first necrophagous insects to colonize a dead body. PMI estimates that do not take the results of these analyses into consideration may be inaccurate, because certain substances ingested before death may alter the development rate of necrophagous species (Introna et al. 2001, Gosselin et al. 2011). For instance, previous studies have found that flies reared on a diet containing scopolamine, buscopam, malathion, diazepam, and methadone take longer to develop (Carvalho et al. 2001, 2012; Grella et al. 2007; Liu et al. 2009; Gosselin et al. 2011). Conversely, when heroin, methamphetamine, codeine, and paracetamol are present in the larval food, individuals develop faster (Goff et al. 1991, 1994; Carvalho et al. 2004; O'Brien and Turner 2004). IPM estimates, particularly when made between 2 hr and 4 wk after death, are based on the assumption that the insect develops according to a predetermined rate, which is calculated taking environmental conditions and the effects of drugs into consideration (Goff and Lord 2001). For this reason, it is important to analyze how certain common substances influence the development of each insect species used in PMI estimates.

Antibiotics interact with microorganisms that cause infections by inhibiting microbial metabolism or reproduction. They either kill microorganisms directly or allow the host's immune system to fight the infection more effectively. One such antibiotic is Gentamicin, an aminoglycoside bactericidal that inhibits bacterial protein synthesis and acts against a wide range of Gram-negative and Gram-positive bacteria. Clinical studies have demonstrated the efficacy of Gentamicin sulphate injections against bacteraemia, septicaemia, serious infections of the central nervous system, kidneys, genitourinary tract, respiratory, and gastrointestinal tracts, as well as infections of the skin, bones, soft tissues (including infected wounds), intra-abdominal tract (including peritonitis), and eyes (Gentamicina 2011).

The objective of this study was to analyze the effects of Gentamicin on the postembryonic development of *Chrysomya putoria* (Wiedemann) (Diptera: Calliphoridae), a blowfly that occurs in many different environments, including forests (Ferraz et al. 2010). Because these flies frequent decaying organic matter, they are important to forensic studies, besides carrying pathogens and causing myiasis in animals and humans (Zumpt 1965, Guimarães et al. 1978, Baumgartner and Greenberg 1984, Ferraz et al. 2011a). We believe that this study has the potential to contribute to forensic entomology by providing information on how Gentamicin alters the following biological parameters of *C. putoria*: rate of body mass gain, rate of development, normality, sex ratio, and viability of the different developmental stages.

This information, in turn, can be used in the calculation of the IPM following toxicological analyses.

Materials and Methods

The blowflies (*C. putoria*) were reared at the Laboratory for the Study of Diptera, Department of Microbiology and Parasitology, Universidade Federal do Estado do Rio de Janeiro.

Adults were obtained from a stock colony originated from insects collected at the Rio de Janeiro Zoo, Quinta da Boa Vista Park, city of São Cristóvão, RJ. Three traps following the model of Mello et al. (2007) and containing sardine as bait were used. They remained exposed for about 5 h in the morning. Adults and larvae of muscoid flies were taken to the Laboratory, where they were sorted and identified following Mello (2003). Experimental flies were reared in plastic cages (40 by 30 by 20 cm) with an opening at the top for aeration and an anterior opening to allow access to the inside of the cage, which was covered with escaline-coated fabric. Each cage received water, honey and water (50%) solution, and chicken gizzard or beef (which are protein sources and serve as substrate for oviposition and ovary maturation). The rearing methodology followed the description of Barbosa et al. (2004) and Ferraz et al. (2011b).

First-instar larvae of the third laboratory generation were transferred with the help of a fine paintbrush to 100-ml beakers containing 60 g gizzard/agar 65% homogenate (Ferraz et al. 2012). This diet was selected because it is practical and sterile (autoclaved), allowing the antibiotic to act fully and only on the larvae. We used 40 mg of the antibiotic Hytamicina (Gentamicin sulfate). Each repetition received 1 ml of the antibiotic, resulting in the following three different diet concentrations: 4.44 m/ml, 13.33 mg/ml, and 66.66 mg/ml. The concentrations were chosen from the serum Gentamicin concentration (established according to the dosage indicated in the prescription advisor), as follows: 2–3 daily intravenous or intramuscular doses; maximum concentration 30 min/1 h after administration: 4–6 μg/ml. Avoid concentrations above 12 μg/ml for extended periods (Gentamicina 2011). The first concentration (T1) is close to the intravenous serum concentration; the second (T2) is close to the maximum intravenous serum concentration; the third (T3) is approximately five times the maximum serum intravenous concentration. Each antibiotic concentration tested and the control were replicated four times using 40 larvae in each repetition. Consequently, a total of 120 larvae were analyzed in each treatment. The control consisted of distilled water.

Each beaker was put into a larger beaker containing sterilized sawdust, which was sealed with elastic escaline. The sawdust serves as a pupation substrate for the larvae after they abandon the diet. Larvae were kept in a climatic chamber at 30°C per day and 28°C per night, 70 + 10% relative humidity (RH), and 14-h photoperiod. Observations were made daily, always at the same time (12 h).

As a precaution, all treatments in this study ensured that each individual larva had >1 g of food available. We ensured this quantity because Aguiar-Coelho and Milward-de-Azevedo (1996) had previously suggested that this is the optimal amount of meat for rearing Calliphoridae. Ensuring that larvae have the optimal amount of food prevents exploitative competition and the stress associated with it, and with the chemical changes in the food substrate resulting from larval metabolism (Khazaeli et al. 1993, Bubli et al. 1998).

The body mass after diet abandonment was recorded in batches of five larvae, with the help of an analytical scale. Larvae were then stored in test tubes sealed with nylon and elastic fabric for further observation. The dates of pupation and emergence, as well as the sex ratio and morphological abnormalities in the adults, when present, were recorded.

The program Microsoft Excel was used to analyze the raw data, and further analyses were conducted in the program PAST. Variations in the mean body mass of larvae and the duration of larval and pupal stages and total development (from neolarvae to adult) were analyzed using the Student's *t*-test ($\alpha = 5\%$). Viability and normal rates were compared by analysis of variance. The observed sex ratio was tested against the expected frequency, using chi square.

Results

The four treatments did not differ significantly in the following parameters: body mass of *C. putoria* larvae (Table 1); mean duration of larval inoculation to abandonment; and larval, pupal, and total development times (d) (Table 2).

The rates of diet abandonment, pupation, and emergence (%) obtained for *C. putoria* in the four different treatments (Control = gizzard and agar homogenate; T1 = gizzard and agar homogenate + Gentamicin 3.33 mg/ml; T2 = gizzard and agar homogenate + Gentamicin + 13.33 mg/ml; T3 = gizzard and agar homogenate + Gentamicin 66.66 mg/ml) is shown in Fig. 1. Diet abandonment peaked in the control group between 5 and 6 d, and predominantly on the fifth day in the three

Table 1. Body mass of larvae (g) of the blowfly *C. putoria* (Wiedemann, 1830) (Diptera, Calliphoridae) after four treatments with different concentrations of Gentamicin, reared in a climatic chamber (30°C day and 28°C night, 70 + 10% RH, and 14-h photoperiod)

Treatment	Mean individual mass (g) ± standard deviation	Variation interval	*P* = significance value (*t*-test)			
			Control	T1	T2	T3
Control	0.049a ± 0.007	0.035–0.059	—	0.569	0.239	0.483
T1	0.053a ± 0.012	0.021–0.068	0.569	—	0.909	0.974
T2	0.054a ± 0.003	0.041–0.062	0.239	0.909	—	0.826
T3	0.053a ± 0.008	0.037–0.064	0.483	0.974	0.826	—

Means followed by same letter in same column do not differ significantly by the *t*-test, 5% confidence. Control = gizzard homogenate agar; T1 = gizzard homogenate agar + Gentamicin 3.33 μg/ml; T2 = gizzard homogenate agar + Gentamicin 13.33 μg/ml, T3 = gizzard homogenate agar + Gentamicin 66.66 μg/ml.

Table 2. Average duration of the postembryonic development stages (d) of larvae of the blowfly *C. putoria* (Wiedemann, 1830) subjected to four treatments with different concentrations of Gentamicin and reared in a climatic chamber (30°C day and 28°C night, 70 + 10% RH, and 14-h photoperiod)

		Days	Variation interval	Standard deviation	*P* = significance value (*t*-test)			
					Control	T1	T2	T3
Duration until abandonment	Control	4.478a	4–5	0.517	—	0.214	0.488	0.418
	T1	4.114a	4–5	0.089	0.214	—	0.645	0.150
	T2	4.225a	4–5	0.450	0.488	0.645	—	0.231
	T3	4.989a	4–6	1.056	0.418	0.150	0.231	—
Larval stage	Control	5.477a	5–6	0.516	—	0.528	0.573	0.494
	T1	5.270a	5–6	0.340	0.528	—	0.986	0.264
	T2	5.275a	5–6	0.441	0.573	0.986	—	0.287
	T3	5.845a	5–7	0.871	0.494	0.264	0.287	—
Pupal stage	Control	3.874a	3–5	0.214	—	0.406	0.181	0.652
	T1	3.610a	3–6	0.551	0.406	—	0.163	0.741
	T2	4.057a	4–5	0.114	0.181	0.163	—	0.278
	T3	3.741a	2–5	0.518	0.652	0.741	0.278	—
Total stage	Control	9.339a	9–10	0.322	—	0.124	0.954	0.532
	T1	8.785a	8–11	0.530	0.124	—	0.168	0.139
	T2	9.323a	9–10	0.435	0.954	0.168	—	0.531
	T3	9.642a	9–11	0.854	0.532	0.139	0.531	—

Means followed by same letter in same column do not differ significantly by the *t*-test, 5% confidence. Control = gizzard homogenate agar; T1 = gizzard homogenate agar + Gentamicin 3.33 μg/ml; T2 = gizzard homogenate agar + Gentamicin 13.33 μg/ml, T3 = gizzard homogenate agar + Gentamicin 66.66 μg/ml.

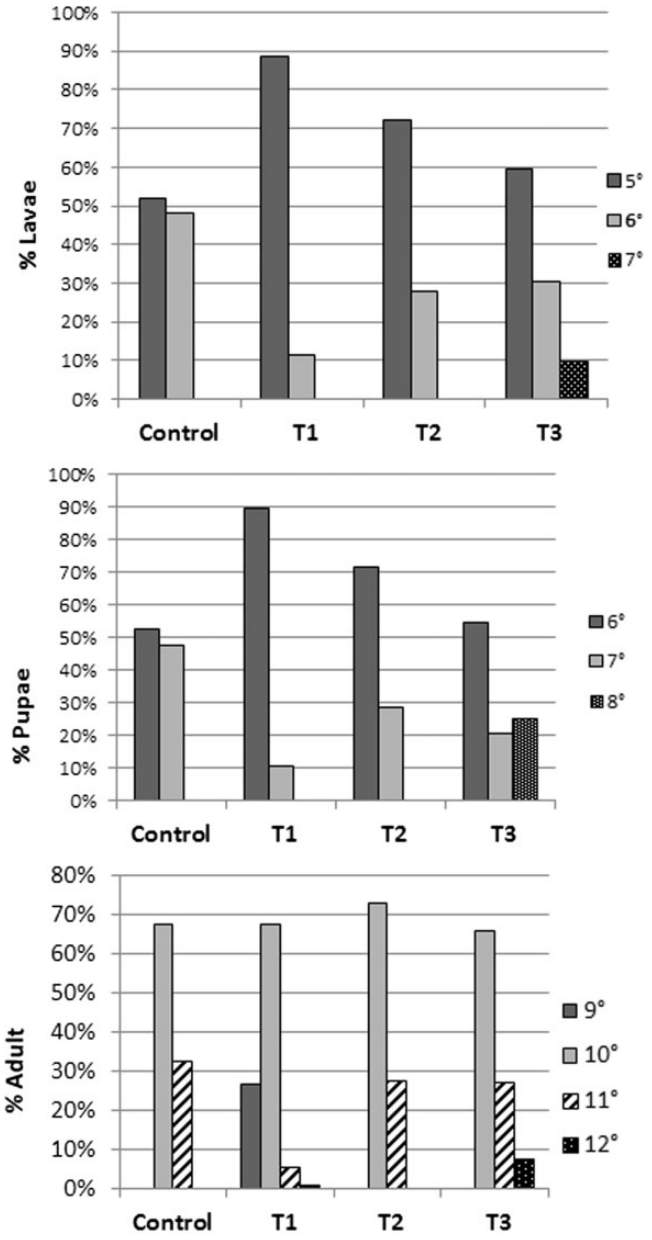

Fig. 1. Rate of abandonment of the diet, pupation, and emergence (%) of *C. putoria* (Wiedemann, 1830) subjected to four treatments: control = gizzard homogenate agar; T1 = gizzard homogenate agar + Gentamicin 3.33 mg/ml, T2 = gizzard homogenate agar + Gentamicin 13.33 m/ml, T3 = gizzard homogenate agar + Gentamicin 66.66 mg/ml.

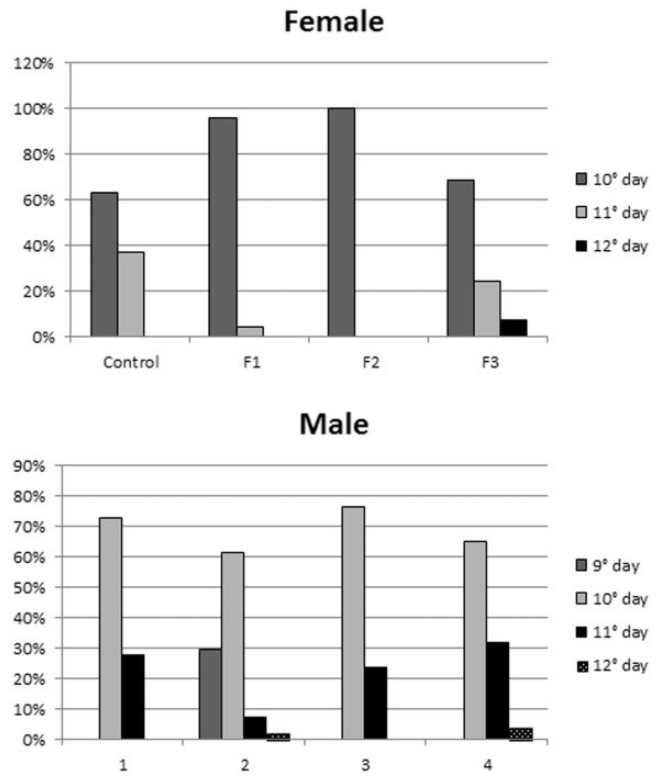

Fig. 2. Rate of emergence, in days, of males and females *C. putoria* (Wiedemann, 1830) subjected to four treatments: control = gizzard homogenate agar; T1 = gizzard homogenate agar + Gentamicin 3.33 mg/ml, T2 = gizzard homogenate agar + Gentamicin 13.33 m/ml, T3 = gizzard homogenate agar + Gentamicin 66.66 mg/ml.

treatments. In T3, and only in this treatment, larval abandonment lasted until the seventh day. The pupation peak of control flies happened between the sixth and seventh days, whereas in the other treatments, it happened on day 6, extending up to day 8 in T3. In all treatments, the emergence peak occurred on day 10, lasting to day 12 only in T1 and T3.

The emergence rate of *C. putoria* males and females was similar in the control and T3 (Fig. 2). In T1, males emerged first (beginning on day 9) and continued to do so up to day 12. Females, in contrast, began to emerge a day later (day 10) and continued up to day 11. In T2, all female flies emerged on day 10, whereas males emerged from days 10 to 11. The sex ratio for each treatment was as follows: control, males = 47%, females = 53%; T1, males = 48%, females = 52%; T2 males = 50%, females = 50%; and T3, males = 51%, females = 49%. The chi-square tests revealed that the sex ratios obtained for the four treatments did not differ from the expected (control: $\chi^2 = 0.398$, T1: $\chi^2 = 0.221$, T2: $\chi^2 = 0.006$, T3: $\chi^2 = 0.081$; tabulated $\chi^2 = 3.84$, df = 1, $\alpha = 5\%$).

All adults in the control and in the other treatments were normal. As for larval viability, only T2 differed significantly from the control (Table 3).

Table 3. ANOVA comparison of the viability of larvae, pupae, and total (neolarvae to adult) of *C. putoria* (Wiedemann, 1830) subjected to four treatments: control = gizzard homogenate agar; T1 = gizzard homogenate agar + Gentamicin 3.33 mg/ml, T2 = gizzard homogenate agar + Gentamicin 13.33 m/ml, T3 = gizzard homogenate agar + Gentamicin 66.66 mg/ml

		Viability (%)	*P* = significance value (ANOVA)			
			Control	T1	T2	T3
Larval	Control	81.88a	—	0.196	0.006	0.874
	T1	90.63ab	0.196	—	0.326	0.419
	T2	96.88b	0.006	0.326	—	0.185
	T3	80.00ab	0.874	0.419	0.185	—
Pupal	Control	93.60a	—	0.263	0.739	0.228
	T1	75.40a	0.263	—	0.287	0.468
	T2	92.26a	0.739	0.287	—	0.234
	T3	86.85a	0.228	0.468	0.234	—
Total	Control	76.88a	—	0.717	0.073	0.517
	T1	70.63a	0.717	—	0.284	0.948
	T2	89.38a	0.073	0.284	—	0.093
	T3	69.38a	0.517	0.948	0.093	—

ANOVA, analysis of variance. Means followed by the same letter do not differ at 5% significance.

Discussion

Previous studies have detected toxins and controlled substances in samples collected from carrion in advanced stage of decomposition and

the insects that visit them (Goff and Lord 2001). In some studies, decreased larval mass gain, and larval growth, as well as reduced larval activity, were correlated with the presence of certain drugs (Soto 2008). This was not observed in this study. Consistent with the results of Ferraz et al. (2012), who reared experimental larvae on meat, gizzard, and gizzard and agar homogenate, the mean larval mass obtained in our study was similar in all treatments.

In the treatments, the total duration of development increased with increased antibiotic concentrations (T1 > T2 > T3). However, this difference was not statistically significant. After all treatments in our study, the larval stage lasted longer than in the study of Ferraz et al. (2011b), who reared *C. putoria* on meat and dog food. This was expected, because meat is the natural diet of this species (Leal et al. 1982). Furthermore, Ahmad et al. (2006) found that another blowfly, *Cochliomyia macellaria* (F.) (Diptera: Calliphoridae), when reared under sterile conditions, developed faster and survived longer than flies reared in the presence of bacteria. In this study, no significant differences in the rate of diet abandonment, pupation, and adult emergence were found between the control and each of the treatments. According to our results, the antibiotic Gentamicin did not alter the duration of the postembryonic stages. A similar result was documented by George et al. (2009), who tested the effect of morphine on *Calliphora stygia* (Diptera: Calliphoridae). However, in a study on the effects of cocaine on the development of *C. putoria* (reared on liver containing the substance), treatment larvae developed significantly faster, pupated, and emerged sooner than control larvae (Carvalho 2004). Other substances such as Diazepam and Anfepromona also accelerated the development of *C. putoria* larvae (Carvalho et al. 2001, Carvalho 2004). In contrast, the painkiller Scopolamine slowed down larval development in treatments containing higher concentrations of the substance (Grella et al. 2007), and Buscopan accelerated the rate of growth of *Chrysomya megacephala* (Diptera: Calliphoridae) (Oliveira et al. 2009). Additionally, a study on the effect of Malathion on *C. megacephala* revealed that this insecticide can alter IPM estimates in up to 36 h (Liu et al. 2009).

In this study, the only statistically significant effect of Gentamicin was on the viability of T2 larvae. Larvae were more viable after this treatment than the others, although viability after all treatments was high. In Diptera, viabilities above 60% are considered promising (Loureiro et al. 2005). In a study using artificial diet plus scopolamine to rear *C. putoria* flies, mortality was higher with increased drug concentrations (Grella et al. 2007), contrasting with the results of our study. It is also possible that, in our study, the drug was not metabolized by the larvae. This is consistent with the results of Grella and Thyssen (2008), who reared *Chrysomya albiceps, C. megacephala,* and *C. putoria* on artificial diet plus oxycodone hydrochloride and failed to find significant differences in development of the control and the experimental groups. Larvae are able to efficiently eliminate various toxic substances during development (Nuorteva and Nuorteva 1982). Also, at least in two other studies, treatments containing low drug concentrations paradoxically resulted in higher survival rates than the control (Goff et al. 1989, Soto 2008).

In all treatments of this study, pupal viability was above 75%. This percentage is considered appropriate and is higher than the values obtained by Soto (2008) for *C. putoria* larvae reared on artificial diet plus chicken heart, both for the control and the treatments with barbiturates.

Total survival was above 69% in all treatments, being higher than in the control and other treatments in the results of Soto (2008). The emergence rates found by Aguiar-Coelho et al. (1995) for several different larval densities of *Chrysomya* ranged between 60 and 98%, also consistent with the findings of this study.

The sex ratio found in our study, after chi-square analysis, was 1:1, indicating that the population is stable (Fisher 1930). Additionally, 100% of the control and treatment flies were normal, indicating that the flies' metabolic adjustments buffered or neutralized the effects of the antibiotics (Lomônaco and Germanos 2001). This result is consistent with the effects of method on the sex ratio of *Lucilia sericata* (Diptera: Calliphoridae). However, this substance delayed the development of those flies and altered their mortality rates (Gosselin et al. 2011).

Entomotoxicology is the analysis of substances in necrophagous arthropods to identify drugs and toxins present in dead tissues, their effects on the development of arthropods, and how they interfere with IPM estimates (Goff and Lord 2001). Such analyses are particularly relevant when blood, urine, and the internal organs of a body are no longer available. According to Liu et al. (2009), understanding the effects of drugs and toxins on the development of flies is very important to make accurate IPM estimates. Though laboratory studies to ascertain how certain drugs affect the development of flies can be made using rodents, rats, and rabbits (George et al. 2009; Liu et al. 2009; Carvalho et al. 2012), artificial diets containing these substances are a viable alternative.

In the study of Gosselin et al. (2011), 100 g of bovine heart plus 8% gelatin was given to every 50 larvae. In this study, we used the diet proposed by Ferraz et al. (2012) for *C. putoria c*onsisting of gizzard/agar homogenate, this diet is low cost and is very rich in nutrients. Because the final product is liquid, it can be easily mixed with different concentrations of Gentamicin. Two grams of diet in four repetitions were made available to the larvae, because this quantity is ideal for the development of this species and results in normal body mass, growth rate, viability, and sex ratio (Ferraz et al. 2012). Even though forensic entomologists have been conducting IPM calculations in different countries, a standard practice has not been adopted yet. The adoption of such practice would be highly desirable (Magni et al. 2013).

The excessive use of drugs can be more harmful than beneficial. United States statistics show that overdosing or drug incompatibility may lead to death, especially in older individuals. Therefore, studies such as ours may foster the precision of PMI estimates using C. *putoria*, especially when antibiotics have been previously used and/or have been the cause of death.

Gentamicin did not alter the parameters tested for *C. putoria* in this work: body mass, larval, pupal, and total development. Therefore, IPM calculations based on the biological parameters of this fly will not be affected when this antibiotic is present.

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