ORIGINAL ARTICLE

doi:10.5216/rpt.v43i3.32217

# *Musca domestica* (DIPTERA: MUSCIDAE) BREEDING IN VARIOUS PIG TISSUES

*Laís Vaz da Costa and Júlio Mendes*[1]

## ABSTRACT

*Musca domestica* (L. 1758) is a cosmopolitan species that presents a high degree of association with human environments. This fly has been encountered carrying several pathogens and has been found associated with animal carcasses and human corpses in various countries. In this study, the capacity of larvae of *M. domestica* to breed in various pig tissues was tested. Larvae of this species were transferred to flasks containing liver, abdominal fat, meat, lung and brain tissues. The flasks were maintained in an incubator at 25 ºC ± 1 ºC, 70%± 10% humidity, and with a 12h/12h light/dark photoperiod until the emergence of the imagoes. The rates of development of larvae were assessed. The larvae did not reach adulthood in fat and liver. Comparisons between data from different tissues indicated differences in the size, weight and development of larvae of the same age. Brain and muscle were the substrates that had the lowest frequencies of emergence of imagoes. A longer time for development was observed in the brain, while shorter times were observed in lung and intestine. Lung and intestine were also the substrates from which greater numbers of imagoes emerged. Larvae in muscle presented smaller size and lower weight. Because *M. domestica* is capable of breeding in various tissues in the laboratory, its breeding capacity and abundance in carrion may depend on the biological and environmental conditions encountered outdoors. The influence of the factors studied when used in forensic entomology, should be taken into account, especially as an indicator of postmortem interval.

KEY WORDS: Forensic entomology; forensic indicator; vector of pathogens; postmortem indicator.

## RESUMO

Desenvolvimento de *Musca domestica* em vários tecidos suínos

*Musca domestica* (L. 1758) é uma espécie cosmopolita que apresenta elevado grau de associação com ambientes humanos. Esta mosca tem sido encontrada veiculando um grande número de patógenos e em associação com carcaças de animais e cadáveres humanos em vários países. Neste

1 Setor de Parasitologia, Instituto de Ciências Biomédicas, Universidade Federal de Uberlândia, Uberlândia, MG, Brazil.

Adress for correspondence: E-mail: jmendes@ufu.br

Received for publication: 11/12/2013. Reviewed: 4/8/2014. Accepted: 21/9/2014.

estudo, foi testada a capacidade de larvas de *M. domestica* de se desenvolver em vários tecidos suínos. Larvas foram transferidas para frascos contendo fígado, gordura abdominal, carne, pulmão e tecidos cerebrais. Os frascos foram mantidos em estufa a 25 ± 1 ºC, 70 ± 10 % de umidade e fotoperíodo 12h/12h até a emergência dos imagos. As taxas de desenvolvimento dos imaturos foram acompanhadas. As larvas não se desenvolveram até adultos na gordura e no fígado. As comparações entre os dados originários de diferentes tecidos indicaram diferenças no tamanho, peso e o desenvolvimento das larvas. Cérebro e músculo foram os substratos que apresentaram menores frequências de emergência de imagos. Observou-se um maior tempo de desenvolvimento no cérebro, enquanto que menores tempos foram observados no pulmão e no intestino. Pulmão e intestino também foram os substratos em que a mosca teve maior número de imagos emergidos. Larvas criadas no músculo apresentaram menor tamanho e menor peso. Considerando que *M. domestica* é capaz de criar em vários tecidos/substratos em laboratório, sua capacidade de reprodução e abundância em carcaças pode depender das condições biológicas e ambientais encontradas no campo. Deve-se considerar a influência dos fatores estudados, quando esta espécie for utilizada como indicador na entomologia forense, especialmente como indicador de intervalo post-morte.

DESCRITORES: Entomologia forense; indicador forense; veiculador de patógenos; indicador pós-morte.

## INTRODUCTION

Flies are a highly diverse group of insects and many of them are associated with human environments. Association with anthropic environments and contaminated substrates make many of them important vectors of pathogens to humans and other animals (Greenberg, 1971). Several species also use decaying organic matter as a food source for their immature and/or adult forms and play an important role in the decomposition of carrion (Amendt et al., 2004; Hanski, 1987). As a consequence, these species may be used as forensic indicators in Forensic Entomology (FE) (Marchenko, 2001; Oliveira-Costa et al., 2001; Arnaldos et al., 2005).

*Musca domestica* L., 1758 (Diptera: Muscidae) is a cosmopolitan species that presents a high degree of association with human environments. Its larvae usually develop in decaying organic substrates such as: human and domestic animal carcasses, food waste, and waste from industrial and agro-pastoral activities (Greenberg, 1971; Mendes & Linhares, 1993; Larraín & Salas, 2008). This fly has been encountered carrying several pathogens such as: viruses, bacteria, cysts of protozoa, and eggs of helminths. Because its adults habitually visit unprotected human food, this species is considered an important vector of several human disease agents (Greenberg, 1971; Graczyk et al. 2001).

This fly has been found associated with animal carcasses and human corpses in various countries (Barreto et al., 2002; Carvalho et al., 2004; Arnaldos et al., 2005; Kimberly et al., 2005; Salazar, 2006; Vitta et al., 2007; Oliveira & Vasconcelos, 2010; Chandna, 2012). In Brazil, there are also records of *M. domestica* associated with rodent and pig carcasses as well as human corpses

(Monteiro-Filho & Penereiro, 1987; Salviano et al., 1996; Oliveira-Costa et al., 2001; Rosa et al., 2009; Oliveira & Vasconcelos, 2010; Faria et al., 2013). These studies have shown that this species is commonly attracted to carcasses and, less frequently than blow flies, its larvae breed in these substrates (Carvalho et al., 2004; Rosa et al., 2009; Beuter et al., 2012; Faria et al., 2013) and can, thus, be used as a forensic indicator (Chandna, 2012).

The success of an insect in the colonization of carrion is associated with its ability to utilize the resources available throughout the decomposition process and to interact with other species in this temporary substrate (Hanski, 1987). The various tissues/organs in corpses exhibit different physical and biochemical characteristics that may influence their availability as a food resource for scavenger insects (Byrd & Castner 2001; Kaneshrajah & Turner 2004; Rabelo et al., 2011). Studies have demonstrated that the characteristics of these tissues may be determinant in their viability for colonization by flies and/or result in different rates of development of larvae in the different tissues of the carcass (Byrd, 2001; Clark et al., 2006; Ujvári et al., 2009; Beuter & Mendes, 2013). In this study, experiments were conducted to identify the capacity of *M. domestica* larvae to develop in different tissues of pigs. Development of the larvae was monitored and compared using parameters of forensic entomology (FE) for calculating post-mortem intervals (PMI) such as: weight, size and time of development (Marchenko 2001; Rabelo et al. 2011).

## MATERIALS AND METHODS

### Establishment and maintenance of the colony of *Musca domestica*

The experiments were carried out in the Laboratory of Veterinary and Medical Entomology (LEMV), department of Parasitology, Institute of Biomedical Sciences (ICBIM) of the Federal University of Uberlândia (UFU), Uberlândia, MG, Brazil. Initially, larvae of *M. domestica* were collected at a farm and maintained in the LEMV. The larvae were kept in plastic flasks of 12 × 15 cm containing ground beef mixed with commercial ration produced for egg laying hens, at a ratio of 1/1. The substrate was offered in flasks with a layer of three cm of sawdust. The sawdust served as the site of pupation for the larvae developed in food substrates. The emerging adults were kept in entomological cages. The substrate used for the maintenance of the larvae was also used for feeding and oviposition of the adult flies. A solution of sugar water was offered *ad libitum* for flies in a second flask. They had access to the solution on wet gauze, kept partially immersed in the liquid in a bottle.

The flies were maintained at room temperature and the temperature and humidity were monitored using a thermo-hygrometer. A heater was used when the temperature fell below 20 °C.

Experimental procedures

Following establishment of the colony, the flies were maintained under the conditions described above for oviposition. The substrate described above was also used during this process. The eggs were transferred to Petri dishes containing filter paper, humidified with distilled water and kept in a B.O.D. incubator at 25 ºC ± 0.5 ºC. Monitoring of the oviposition and hatching of eggs in the incubator was done hourly. Newly hatched larvae were transferred, with the aid of a fine brush, to flasks containing the substrates to be tested: brain, liver, intestine, muscle, lung, fat, ground muscle and the control substrate (50% ground muscle + 37.5% ration for egg laying hens + 12.5% distilled water). The samples of intestine used as breeding substrate were offered with respective residues from alimentary digestion. The fat was from the abdominal region and was offered with the adjacent skin. Substrates/tissues were kept at ambient temperature (23 ºC ± 3 ºC) for 24 hours before being offered to the larvae for breeding. In each test, five flasks, each containing 120 g of the respective tissues and 100 newly-hatched larvae (up to one hour after hatching) were used. Samples of larvae were taken from four of the five flasks containing each of the substrates for analysis, measuring and weighing. The sampled larvae were not returned to their respective breeding media. No larvae were taken from the fifth flask. The test with liver was submitted to three repetitions with the same number of larvae used in the other tissues. The muscle and fat were purchased from a commercial establishment in Uberlândia. Other substrates were donated by a local slaughterhouse.

The sampling of larvae from four of the five test flasks occurred with the removal of one larva from each flask, at intervals of six hours, during the first 12 hours of the experiment and every eight hours during the next 24 hours. In the remaining period, the larvae were sampled every 12 hours until the formation of pupae. Thereafter, the sampled larvae were submerged in water at approximately 100 °C and deposited in flasks containing 80% ethanol. At the end of the experiment 12 larvae were sampled from each replicate flask, totaling 48 larvae sampled from the substrates in which larvae developed to the point of imago emergence. Subsequently the larvae were taken from the flasks and placed for three minutes on filter paper, before being weighed on an analytical scale and measured with a digital caliper. The measurement and verification of larval instars were performed with a stereo-microscope. After the beginning of pupae formation, observation of imago emergence occurred every 24 hours. The experiment was conducted in a B.O.D. incubator at 25 ºC ± 0.5 °C and a 12 hour photoperiod.

Statistical analysis

The results of size and weight measurements were compared in post-fed larvae (pre-pupae) at ages of 84 and 96 hours. The results of these experiments with weight, size and frequency of imago emergence were submitted to an analysis of

variance (ANOVA). The frequency of emergence of imagoes was first transformed in log (x +1), and then submitted to ANOVA. The multiple comparisons that produced significant results were subjected to the Tukey test. The percentages of emergence were compared using the chi-squared ($\chi^2$) test. A significance level of 0.05 was used for all analyses (Zar, 1999).

## RESULTS

The fly larvae hatched 12 hours after oviposition and were able to develop to the point of emergence of imagoes in four of the six substrates/tissues tested. The exceptions were liver and fat. In the liver, larvae of up to 12 hours of age were found. Thereafter, they were no longer found in any of three repetitions of the experiment with this substrate. In the fat, larvae were found until the 60th hour after hatching. These larvae were still in stage II and no more were found in the subsequent samples. In both substrates - liver and fat – emergence of imagoes was not observed, including the fifth vial of each of the two substrates, where no sampling of larvae had been conducted (Table 1). A longer time for development was observed in the brain substrate, while shorter times were observed in lung and intestine (Table 1). Lung and intestine were also the substrates in which the flies had greater numbers of emerged imagoes. However, the respective frequencies did not differ statistically from the control group (Table 2).

*Table 1.* Time of development of immature stages of *Musca domestica* in various pig tissues in the laboratory.

| Tissues | Immature stages (time in hours) | | | | | Imago emergence |
|---|---|---|---|---|---|---|
| | Eggs | Larvae I + larvae II | Larvae III | Post-feeding larvae | Pupae | Time (hours) |
| Meat | 12(±1) | 36(±6) | 60(±12) | 36(±12) | 168(±24) | 324(±48) |
| Brain | 12(±1) | 36(±6) | 60(±12) | 24(±12) | 192(±24) | 348(±24) |
| Liver | 12(±1) | 12* | - | - | - | - |
| Fat | 12(±1) | 60* | - | - | - | - |
| Intestine | 12(±1) | 24(±4) | 60(±12) | 24(±12) | 144(±24) | 288(±24) |
| Lung | 12(±1) | 20(±4) | 72(±12) | 24(±12) | 120(±24) | 264(±24) |
| Control | 12(±1) | 24(±4) | 72(±12) | 36(±12) | 168(±24) | 310(±24) |

*= Larvae were not encountered in the subsequent attempts of sampling at the respective substrates/tissues.

Brain and muscle were the substrates that had the lowest frequencies of emergence of imagoes. No significant differences were observed between the proportions of genders emerging from the different substrates (Table 2). The larvae reared in the intestine and lung showed greater sizes compared with those bred in the other substrates (Table 3). The groups bred in these substrates showed variations in size and weight similar to those observed in the control group. On the other hand, larvae in muscle presented smaller sizes and lower weights (Table 3).

*Table 2.* Development of immature stages of *Musca domestica* in various pig tissues.

| Substrate | Initial number | Larvae sampled | Pupae obtained | Viability of pupae (%) | Total number |
|---|---|---|---|---|---|
| Muscle | 500 | 48 | 166 | 9.6 | 16 C* |
| Brain | 500 | 48 | 136 | 88.2 | 120 B |
| Liver | 500 | 12 | - | | - |
| Lung | 500 | 48 | 353 | 85.9 | 303 A |
| Intestine | 500 | 48 | 326 | 95.1 | 310 A |
| Fat | 500 | 32 | - | | - |
| Control | 500 | 48 | 392 | 95.2 | 373 A |

* = Same letters are not different statistically at 5% of significance.

*Table 3.* Size and weight of larvae of *Musca domestica* breeding in various pig tissues.

| Parameters | Immature | Tissues | | | | |
|---|---|---|---|---|---|---|
| | | Control | Meat | Brain | Intestine | Lung |
| Size (mm) | Larva III | 13.1(±0.54) a* | 11.7(±0.8) c | 12.3(±0.6) b | 13.0(±0.5) a* | 12.5(±0.9) a b |
| Weight (mg) | Larva III | 28.6(±0.4) A B | 17.9(±3.5) D | 25.9(±3.5) C | 32.7(±4.9) A | 27.3(±5.0) B |

* Same letters are not different statistically at 5% of significance

## DISCUSSION

Liver and fat were not found to be suitable for the development of larvae of *M. domestica*. One explanation for this is that these tissues have very different physical and biochemical characteristics from the others in which the flies bred. While liver presents various nutrients and ample liquid; fat has high lipid content (USDA, 2014) but low concentrations of other nutrients necessary for the development of fly larvae (Vanderzant, 1974; Ujvári et al., 2009). Large amounts of liquid in liver and low concentration of nutrients for larvae in the fat would prevent larval development in these tissues. Studies with species of blow flies have shown disparate results with liver as a breeding substrate. While *Calliphora vomitoria* (Linnaeus) grow relatively well in this substrate (Ireland & Turner, 2006), the development of *Lucilia sericata* (Meigen) is slower compared with other tissues (Clark et al., 2006). In a study by Beuter and Mendes (2013), *Chrysomya albiceps* (Wiedemann) did not breed in pig liver offered in similar conditions to those offered in the present experiments for *M. domestica*. Nevertheless, other authors have observed the development of *C. albiceps* in other mammalian liver (Al-Misned et al., 2002; Carvalho et al., 2007). Since time/stage of decomposition influences the physical and biochemical characteristics of the rearing substrate (Nespoli et al., 1998); Beuter and Mendes (2013) have argued that the discrepancy between their results and those of other authors may be associated with the supply of liver in different stages of decomposition from the other studies. It is possible that the failure of *M. domestica* to grow in this substrate is also associated

with this factor. The finding that this species has been recorded breeding in sheep liver may corroborates this hypothesis (Arong et al., 2011). However, the number of breeding records of this species in carcasses/cadavers is less than the records of visitation by its adults (Kimberly et al., 2005; Rosa et al., 2009; Oliveira & Vasconcelos, 2010; Rosa et al., 2011). For these reasons it is possible that this fly is unable or barely able to grow on this substrate. This same argument could also be used to justify its failure to develop in fat.

Muscle was the least efficient substrate, compared with the other substrates in which *M. domestica* bred. Although rich in protein, this substrate does not seem to provide the necessary nutrients in sufficient concentrations for full development of the larvae. An indication that reinforces this finding was that the flies presented the greatest rates of development in the control group, which was composed of muscle supplemented with ration for egg laying chickens. On the other hand, lung and intestine presented parameters close to those observed in the control group, also being effective for breeding of the flies.

This species visits corpses more often than it breeds in them and is usually surpassed in numbers and frequency by several species of blow flies (Carvalho et al., 2000; Kimberly et al., 2005; Rosa et al., 2009; Faria et al., 2013). *M. domestica* is, however, capable of breeding in various tissues. Its breeding capacity and abundance in carcasses may, therefore, depend on other factors including: local exposure of the carcasses and the occurrence of intra and/or inter specific interaction in this temporary substrate (Hanski, 1987; Lomonaco & Germanos, 2001). Competition with blow fly larvae may be one of the factors influencing its capacity to breed in the animal carcasses. There is also the possibility that some of these same factors interfere in the measurements of parameters such as weight, size and time of development of the fly in the carcasses.

It is thus recommended that when this species is used by FE, particularly as an indicator of the post-mortem interval (PMI), these assumptions should be taken into account, especially since the degree of accuracy of inference of PMI may depend on knowledge of the magnitude of the influences of the factors studied and appointed in this study. Another point to be considered is that, depending on the available breeding media, larvae may migrate and feed in more than one tissue during their development. In such situations, inferences on PMI would consider that the larvae have developed in the tissues localized in the area of the remains where the larvae were collected. The results presented here are also important for public health, since carrion may be a source of pathogens carried by the house fly.

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