

## First use report flies (Diptera) to Estimate time of death in an indoor case in the Brazilian Midwest

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### Resumo

As moscas constituem uma classe importante de insetos para a entomologia forense. Eles são os primeiros a colonizar o corpo e são os mais representativos durante a decomposição. Esses insetos são usados principalmente para a estimativa do intervalo post-mortem (PMI), bem como um indicador de morte violenta ou natural. Assim, este estudo relata, pela primeira vez, o uso de dados entomológicos como ferramenta complementar para auxiliar na elucidação de uma investigação criminal realizada no Estado de Mato Grosso do Sul, na região centro-oeste do Brasil. Neste caso de homicídio, cinco espécies de Diptera pertencentes a duas famílias, Muscidae e Calliphoridae, foram coletadas e identificadas. O cálculo de Grau-Hora Acumulado (ADH) foi realizado utilizando a taxa de desenvolvimento de *Chrysomya megacephala* (Fabricius, 1974), obtida de pesquisas realizadas em condições de laboratório, na mesma temperatura em que as espécies e o corpo foram submetidos. Com base na idade das larvas e seu padrão alimentar, os resultados permitiram estimar o IPM<sub>min</sub> e, também, inferir sobre a natureza do crime. Nossos resultados demonstram que as moscas podem ser usadas como evidência em investigações criminais, indicando o potencial da entomologia forense na região.

*Palavras-Chave:* Intervalo Pós-Morte; Diptera; Estágio de Desenvolvimento; Grau-Hora Acumulado; Natureza da morte.

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### Abstract

Flies comprise an important class of insect for forensic entomology. They are the first to colonize the body, and they are the most representative during decomposition. These insects are mainly used for Post-Mortem Interval (PMI) estimation, as well as an indicator of violent or natural death. Thus, this study reports, for the first time, the use of entomological data as a complementary tool to assist in the elucidation of a criminal investigation that took place in the State of Mato Grosso do Sul, in the central-western region of Brazil. In this homicide case, five species of Diptera belonging to two families, Muscidae and Calliphoridae, were collected and identified. The Accumulated Degree Hour (ADH) calculation was made using the rate of development for *Chrysomya megacephala* (Fabricius, 1974) obtained from research carried out under laboratory conditions at the same temperature in which both species and the body were submitted. Based on the age of the larvae and their feeding pattern, results allowed the estimation of PMI<sub>min</sub> and, inference about the nature of the crime. Our results show that flies can be used as evidence in criminal investigations, demonstrating the potential of forensic entomology for the region.

*Keywords:* Post-Mortem Interval; Diptera; Developmental stage; Accumulated Degree Hour; Nature of death.

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## 1. INTRODUCTION

The Calliphoridae, commonly known as blow flies, are considered the most important group for forensic entomology because they locate in the body soon after death and then perform oviposition [1]. This allows more accurate PMI estimate because usually Calliphorids are the first to arrive at the crime scene [2]. Flies of the family Muscidae are another group used as entomological evidence [3]. This group has a wide geographic distribution, is associated with humans in many different environments, and is considered the third family of Diptera in importance for forensic entomology [3].

For a more accurate PMI estimation, the duration of the development cycle of species found on the body is taken into account; therefore, correct identification of fly species is important because each species has a different rate of development [4-6]. It is also necessary to consider abiotic factors at the crime scene, such as temperature and humidity [4]. These factors can significantly affect the development time of fly species since high temperatures accelerate their life cycle, while lower temperatures delay it [7]. Moreover, in order to perform PMI estimation, it is necessary to collect the most advanced stages at the crime scene, i.e., those specimens that were first laid and therefore can provide the exposure time of the corpse [6].

Forensic entomology has also been used to infer the nature of a crime since flies exhibit preferences for certain oviposition sites [8]. Thus, it is common to find oviposition and larval masses in natural orifices, such as eyes, mouth, nose, anus and genitals [9]. On the other hand, when a corpse features wounds, these sites will also be favorable for oviposition and larval feeding mostly because of the blood and due to soft tissue exposure [10]. Thus, oviposition by dipterans will occur primarily in orifices or wounds on the corpse [4].

Access to the body by insects also plays an important role in decomposition and, consequently, in criminal investigations [11]. Fauna composition of carcasses exhibit differences between indoor and outdoor environments, whether by fly species richness/abundance and/or eggs laid [10, 12, 13]. Not only will fauna be different between these two sites, but also the microclimate, accessibility, and lifecycle, among other factors that should be considered when using entomological data in cases of bodies found indoors [11].

In Brazil, forensic entomology is not often used by criminal experts, even in cases where the information provided by insects could be important to resolving forensic questions [14]. Few real cases are published in Brazil using insects as a tool for investigations, especially due to the lack of trained professionals, making the field of forensic entomology unusual in criminal investigations [15]. The use of clues provided by insects can be very

useful in criminal investigations, however, especially in countries like Brazil, more studies on fauna of forensic importance in different regions are needed due to its large territory with different climatic and geographical conditions. [15]. A fact that makes the diversity of fauna associated with carcasses a rich source of forensic data.

Successful criminal investigations may well encourage the use of insect fauna [16]. In this sense, we herein demonstrate the importance of using forensic entomology as a complementary investigative tool in forensics by presenting the first case report performed in the State of Mato Grosso do Sul, Brazil, using data provided by flies of forensic interest to resolve important issues in a criminal investigation.

## 2. METHODOLOGY

Collections were performed by the Forensic Entomology Research Group of the Laboratório de Ecologia Comportamental (LABECO/UEMS). Temperature at the death scene was measured with a Multifunction Meter ITMP-600. We also obtained the mean temperature on September 3rd to 6th, using the data provided by Weather Station #86858, Instituto Nacional de Meteorologia (INMET), Dourados, MS, located at latitude 22° 11'38.11" S and longitude 54° 54'40.88" W. Larval mass temperature was measured by Infrared Thermometer MT-350PG.

Due to the abundance of larval mass, it was not possible to quantify larvae *in situ*; therefore, all body areas were photographed to be later categorized in order of abundance of larval mass, according to the methodology of [17] who proposed the following categories: up to 1000 larvae, 1000 to 3000, and > 3000. Inferior and superior members, as well as thorax and abdomen, were not categorized.

All developmental stages present in the body were collected with the aid of tweezers and entomological net, including egg masses, larvae from different instars and adults from different species of Diptera. Immature specimens were collected, separated by collection area on the body, and subsequently packed in 250 ml plastic containers, along with fresh ground beef, and transported to the Laboratório de Ecologia Comportamental (LABECO/UEMS). Adults collected at the site were placed in 30cm<sup>3</sup> cages. Around 50 larvae of each instar were fixed in 70% alcohol as vouchers, and the remainder was raised in an incubator under controlled temperature of 32°C with photoperiod of 12:12 (L:D). The specimens collected were identified using the identification key of [18].

To estimate the PMI<sub>min</sub>, the expected ADH values were calculated according to the formula described by [19]:

$$\text{ADH expected} = (\text{Rearing temperature} - \text{temperature threshold}) \times \text{Development time}$$

For the calculation of the ADH, the following parameters are considered: the temperature under which the flies were reared; development time that the species took to reach the stage to be used; and the minimum temperature threshold, which is the minimum temperature required for the species development. According to [19], the minimum threshold for flies of forensic importance is 6°C for temperate regions and 10°C for tropical regions.

Next, we used the equation presented by [20] and performed the calculation of the ADH obtained by the time of daily development in hours and the mean temperature of this exposure period, as already performed by [20] and [21]. After that, the daily ADH is summed until the value equals the expected ADH.

PMI<sub>min</sub> was estimated using the larval instar at the most advanced stage, in this case, third instar larvae. To avoid miscalculations, the temperature used was based on larval developmental stage, as proposed by [19]. Thus, the temperatures used were those of the larval mass for the final instars (2nd and 3rd instar) and room temperature for the early stages (eggs and 1st larval instar).

We used larva of the species *Chrysomya megacephala* (Fabricius, 1974) for calculation, which was at the 3rd instar, based on the suggestion of [22] that post-feeding larvae and pupae should not be used to estimate the Accumulated Degree Day or Hour by linear model because the development time of these stages is highly temperature-dependent with more sensitivity to temperature variation than the early stages.

The expected Accumulated Degree Hour was calculated as proposed by [6], using the species development data under 25°C presented by [23], obtaining the value of 1503 (ADH). This value represents the time (hours) required for the species *C. megacephala* to develop under controlled temperature of 25°C. A retrospective calculation from the developmental stage of the species found in the body until the period of oviposition was conducted, as proposed by [19].

### 3. CASE REPORT

On September 6, 2017, around 2:00 pm, in Dourados, Mato Grosso do Sul, Brazil (22°13'16''S; 54°48'20''W), the body of a 53-year-old man was found inside a residence in an advanced stage of decomposition. The corpse was found inside a brick residence with 4 rooms and approximately 45 m<sup>2</sup> of building area. The body was on the bedroom floor between the bed and the wardrobe, wearing a t-shirt, shorts, and flip-flop slippers.

In the living room, an open tilt and turn window was probably the only access to the body by insects. The corpse

was in supine position, with open arms. A partially consumed cigarette was observed on the right hand between the forefinger and middle finger. Due to the stage of decomposition in which the body was found, it exhaled a strong odor, displayed body parts that had loss of rigor mortis, epidermal detachment, facial skin with darkened aspect caused after the death and gigantism.

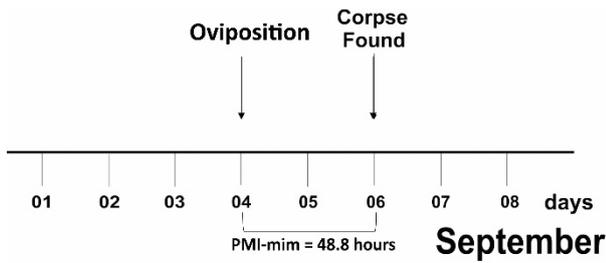
A large amount of dipteran larvae was observed on the body and its surroundings. Larval masses were found in natural orifices of the body, such as eyes, mouth, nose, genital area and anus, as well as a significant larval mass in the neck. Larvae of different sizes and/or instars were observed, including some on the floor already moving away from the corpse, most likely in post-feeding phase looking for a place for pupation.

The temperature inside the residence at the time of arrival on site was 30 °C ± 1, and larval mass temperature was 32 °C ± 1. Mean, maximum and minimum temperatures obtained from the meteorological station for August 28th to September 6th was 25.8°C (±1.1), 26.6°C (±5.0) and 25.0°C (±4.9). And the mean temperatures for September days 3rd to 6th were 26.3°C (±4.5), 26.6°C (±4.9), 24.8°C (±4.5) and 24.4°C (±6.3), respectively.

During body examination at the crime scene and by photographic comparisons, the body parts with greater abundance of larval masses were categorized in the following order according to the methodology of [17]: neck (>3000), head (eyes, nose, mouth) (1000 to 3000), genital area (up to 1000). No larvae in large numbers were feeding on other body parts, such as thorax, abdomen and limbs.

Five species were identified belonging to two families: *Chrysomya albiceps* (Wiedemann, 1819), *Chrysomya putoria* (Wiedemann, 1818), *Chrysomya megacephala* (Fabricius, 1974), *Lucilia eximia* (Wiedemann, 1819) (Calliphoridae) and *Musca domestica* (Linnaeus, 1758) (Muscidae). Among these, the collected larvae were in the following developmental stages: 1st instar (*C. albiceps*), 2nd instar (*M. domestica*, *C. putoria* and *C. albiceps*), 3rd instar (*C. megacephala*) and post-feeding (*L. eximia*).

ADH from August 6th to 5th was 1216 (Tab. 1). However, ADH remained at a minimal 287 ADH for *C. megacephala* to complete its cycle, making it necessary to divide this remaining value by the mean temperature of the following day, in this case 287 ADH / 26.6°C (±4.5) (mean temperature on 9/4/2017), thereby obtaining 10.8 h. Therefore, it was concluded that the first oviposition by flies of this species happened around 1:00 pm on September 4, 2017 (Fig. 1).



**Figure 1.** Timeline showing the events. Date of body discovery; Probable date of death based on minimum Post-Mortem Interval estimation.

**Table 1.** Calculation of Accumulated Degree Hour (ADH) of the case under study for the species *Chrysomya megacephala* during the developmental stages.

Date	Hours/ Mean temperature of the days		ADH
9/6/2017	14h x 32 °C (±1)	=	448
9/5/2017	24h x 32 °C (±1)	=	768
<b>Total ADH</b>		=	1216

**4. DISCUSSION**

The use of insects in criminal investigations in Brazil is still uncommon; which this being the first case reported in the State of Mato Grosso do Sul. By entomological data in this study, we were able to estimate the PMI<sub>min</sub> and infer the probable cause of death. To the best of our knowledge, we present, for the first time, a case study of fly species of forensic interest collected in a human corpse in the central-western region of Brazil. Other experimental research performed in this same region demonstrates the potential use for forensic medical examiners, especially to determine post-mortem interval [24-26].

The 48.8 hours PMI<sub>min</sub> is congruent with reports of witnesses who saw the victim for the last time on September 3rd, i.e., 3 days before body discovery. However, it is worth mentioning that the body was found indoor, so even with the windows open this could have delayed colonization. On the other hand, [27] discusses the reliability of climate data, such as temperature, obtained from weather stations near the place of death to estimate the PMI. According to this author, the accuracy of these data can be questioned since they vary between microhabitats that are affected by many other factors. Thus, the ambient temperature would not accurately represent the

condition to which the larvae would be exposed on the body [4, 22]. Larval masses showed higher temperatures than those found at the crime scene since metabolism during feeding increases their temperature [28]. Therefore, had we used only the average temperature of the environment, the estimate of the PMI<sub>min</sub> would have been higher than the forensic expert data indicate. In addition, the oviposition would have occurred in the evening, which is not common for Calliphoridae flies [29].

This estimate was based on data found regarding *C. megacephala* cycle in studies developed in China [23], as it is a more current and more detailed study on the development of this species, even though it is from a population that is not from Brazil. This demonstrates the need for basic studies on the biology of species of potential forensic importance, which would allow a more agile and accurate response in real cases.

In addition to *C. megacephala*, larvae from 4 other fly species were collected on the corpse, showing the richness of the diversity associated with decomposing corpses in the region, even though the body was inside a residence restricting their access. This factor may have affected the richness of species collected in this case, which could be higher. In cases where bodies are found indoors, the diversity of species colonizing the body are, generally, relatively sparse [13, 14]. It is noteworthy that the only access point to the body by insects was a window that was partially open. This is an especially important factor in indoor cases, as it can change the colonization pattern of the carrion entomofauna and decrease the occurrence of insects [11]. All the species found in this case are common in urban environments, and since the house where the case occurred was not close to forest fragments, we believe that this is probably the reason why insects of other orders were not found.

The entomological evidence found at the scene also helped to infer the cause of death. Investigations at crime scenes in the State of Mato Grosso do Sul are performed in two steps, similar to what happens in the State of São Paulo [30]. In the first step, an examination at the crime scene is performed by an expert in search of evidence, also known as perinecropsy. The second step occurs at the Instituto de Medicina Legal (IML) where the coroner performs the autopsy. In this case, the probable cause of death was inferred during the first step as injury by sharp object to the neck. It was possible to observe a large number of larvae in this body part. It was the first evidence, in this case, justifying that it was not a natural death. After that, the perinecropsy examination was made by a forensic expert, which confirmed the injury. The autopsy report performed by the coroner confirmed the cause of death due to a deep and incised lesion in the anterior region of the neck.

Immature flies feed on the soft tissues of the body because they are unable to feed on the more rigid tissues

since their oral apparatus is somewhat sclerotized [31, 32]. Thus, in the event of injury to the corpse, oviposition will occur near these regions [4]. Authors, such as [33], have already discussed the use of the feeding pattern of fly larvae in decaying cadavers to locate regions where injuries are present. Also, in the study case of Oliveira-[34], the authors pointed to the cause of death as being natural since entomological evidence was only found close to the natural orifices.

Therefore, using forensic entomology, it was possible to reach important conclusions regarding this case, including the probable time of death and the nature of the death. As demonstrated in other studies performed in Brazil, entomological evidence may assist in criminal investigations [14, 30, 35, 36]. However, the climatic differences of each region of the Brazilian territory make forensic entomology research necessary because of the great diversity of necrophagous insects found [14] in different regions of the country.

## 5. CONCLUSION

This is the first case report in the state of Mato Grosso do Sul, with a partnership between the forensic expert and the University whose collaboration using entomological data based on the age of the larvae found in the body allowed the calculation of the PMImín, helping to clarify when the death of the victim happened. In addition, due to the feeding pattern of the larvae observed in the body, it was possible to infer that it was not a natural death. These results demonstrate the need to collect entomologic data from different geographical regions of a diverse country in terms of climatic conditions, since populations of the same species may present significant differences regarding the development time, which can significantly affect estimates [37]. Moreover, further studies on taxonomy and biology of species with forensic potential in Brazil are also needed to expedite the use of this data in criminal investigations.

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