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# Family Calliphoridae: An Integral Part of Postmortem Interval Estimation

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## Abstract

**Background**: The arrival and activity of family Calliphoridae on a deceased corpse indoors can aid in the quantitative measure of the Postmortem Interval. Family Calliphoridae is a group of necrophagous insects that have a proclivity for decomposing corpses and are found worldwide. Their prevalence and activity on a deceased corpse indoors makes it an integral component of estimating the postmortem interval.

**Methods**: A literary review was performed to support the hypothesis. Case control and retrospective studies were used. Study populations were not restricted to age, sex, or geographical locations. Human studies as well as animal models, due to ethical restrictions, were examined.

**Results**: Results showed that by paralleling known developmental timelines to Calliphoridae collected from a corpse, investigating pre-arrival interval, understanding external pressures on development, and considering post-feeding behavior can lead to PMI estimated with a higher degree of accuracy.

**Conclusion**: To disregard the role of Calliphoridae in human decomposition is to renounce a more accurate way of estimating the postmortem interval. More studies should be performed on a variety of Calliphoridae species, pupation in human corpses, and environmental stress on blowfly development to further reduce error in calculating PMI.

**Keywords**: Calliphoridae; Forensic entomology; Postmortem interval; Indoor death; Decomposition

## Introduction

Postmortem Interval (PMI) is critical in forensic and legal investigations and involves measuring time interval from death to the discovery of the corpse. For law enforcement and forensic pathologists, PMI provides essential information in homicide, suicide and suspicious death investigations, the inclusion or exclusion of persons of interest, the establishment of alibis, and the identification of missing persons. When insects colonize an undiscovered corpse, autopsy findings and police and eyewitness testimony may not provide the most accurate PMI estimation. Interestingly, entomology, not pathology, becomes the best way to determine PMI [1].

The standard model of human decomposition comprises of five stages: Fresh, discoloration, bloating, skeletonization, and skeletal decomposition [2]. Each stage has an average time-frame. Based on the stage in which a corpse is discovered an approximate PMI can be calculated. However, because of the limitless combination of environmental factors, each case is unique and the length of each stage of decomposition can vary greatly. Furthermore, decomposition is not necessarily a uniformly sequential process. Sometimes a single corpse can undergo multiple stages of decomposition simultaneously on different parts of the body and it is unclear as to which stage would provide the most accurate PMI. Therefore, using only the standard model of physical characteristics of human decomposition can lead to a flawed estimation of PMI. External factors such as temperature, climate, and predation can all lead to certain corpse parts decomposing at slower or faster rates. Any inaccuracy, no matter how small, could be detrimental to an investigation. For corpses found indoors, looking only at the stage of decomposition tends to underestimate PMI. In one case, the PMI was determined to be 5 days. However after an analysis of insect colonization, examiners realized that the PMI was actually closer to 7 days [3]. This error in PMI could lead to the creation of a faulty time-line for a criminal investigation, unintentionally include or exclude persons of interest, and be problematic for the corroboration of potential suspects' alibis.

To prevent the complications and discrepancies in determining PMI, investigators should resort to a simple solution: insects. Insects are quick to colonize a corpse, and at the 48 h postmortem, entomology takes over as the preferable field to determine a more accurate time-line of PMI [2,4]. Blowflies have in particular a predisposition to human decomposition [5]. Blowflies belong to the necrophagous family Calliphoridae, and are of special interest in indoor deaths. On outdoor corpses, Calliphoridae, Formicidae, Braconidae, Silphidae, Cleridae, Staphylinidae are commonly found. On indoor corpses, however, Calliphoridae are often the only insects that colonize [6]. Many insects fail to colonize a clothed portion of a corpse, whereas Calliphoridae colonizes bodies in various states of dress or undress. They also have the ability to colonize corpses at varying temperatures in diverse geographical locations. Subspecies like *L. sericata* can be found indoors in the colder climate of Finland [7], the milder climate of Spain, and in the tropical conditions of Malaysia. Thus, the ubiquitous Calliphoridae is invaluable in the estimation of PMI [8].

The well-known development stages of Calliphoridae, our understanding of the effects of external factors on its development, and the ability of insect to detect human decomposition across long distances make it an integral component of calculating PMI. By examining the arrival of an insect and its proliferation on a corpse, the investigator can conclude the minimum postmortem interval ($PMI_{min}$). The $PMI_{min}$ has its limitations, as it only measures the time by the

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growth and development of Calliphoridae and does not account for the Pre-Arrival Interval (PAI). New studies have shown the potential for calculating PAI by examining Volatile Organic Compounds (VOC) such as Dimethyl Disulfide (DMDS) and butan-1-ol by Two-Dimensional Gas Chromatography-time-of-Flight Mass Spectrometry (GCxGC-TOFMS) and Calliphoridae reaction to these compounds by Electroantennography (EAG) [9,10]. Varying temperatures, as well as illicit substances such as cocaine, heroin, and barbiturates all play important roles in either accelerating or delaying different parts of development. Calliphoridae participate in oviposition in temperatures ranging from 5°-38°C, but rarely lays eggs below 10°C [11]. Understanding how temperature affects development and oviposition and knowledge of ambient temperature where corpse was discovered can aid in the PMI determination [12]. Cocaine and ketamine were found to accelerate development [13,14], while morphine, heroine, and barbiturates delayed development [4,13]. PMI examines the development of the oldest larvae or pupae found on the corpse, but the post-feeding interval occurs when Calliphoridae migrate off the corpse [6]. If an investigator fails to notice traveling larvae, PMI could be underestimated.

The arrival and activity of family Calliphoridae on a deceased corpse indoors can aid in the quantitative measure of the PMI. A mathematical analysis was performed using Accumulated Degree Days (ADD) and Total Corpse Score (TBS). ADD is a standard to measure and estimate decomposition rates by calculating thermal energy needed for the chemical and biological reactions for decomposition to occur. TBS is also a measurement of decomposition but is used to quantify when an amount of a reaction equals the thermal energy consumed by a corpse. A plot of the log of ADD versus TBS yielded a statistical significance ($p$=0.001) suggesting that the greatest effect on rates of decomposition was due to the presence or absence of insects regardless of the environment [15]. By examining PAI, effects of external factors on the known rates of development, and post-feeding interval, PMI could be determined with greater accuracy. Continued research into more subspecies of family Calliphoridae, VOC on PAI, further studies on pupation, and additional training and education of medico-legal personnel will further reduce the PMI estimation error.

## Methods

The databases of choice used for the literature review were Pubmed and Medline. When Pubmed was used, MeSH terms were enrolled. Key terms that were used in the literary search were "Calliphoridae", "Postmortem Interval", "Indoor Death", and "Decomposed Bodies." Most articles were limited to being published in the last 10 years. Narrowing the timeline of articles was to prevent the use of outdated information that has been replaced with a plethora of research in recent years.

Further limits were placed on the articles by focusing on family Calliphoridae and indoor deaths. Bodies found outdoors are exposed to the elements and are susceptible to external exposures that vary greatly from one geographical location to another. Focusing on indoor deaths will allow for a closer look at the role of Calliphoridae without effects of weather, temperature and scavenging animals to alter the postmortem interval.

In addition, there were no restrictions on age, sex or geographical location in regards to the study populations. Human case studies were reviewed but most were done internationally, due to legal and ethical restrictions in the United States. The most thorough studies involving humans in the United States are performed through the Anthropology Department at the University of Tennessee, commonly known as the Body Farm. When restrictions were in place, many studies used domestic pigs (*Sus scrufa*) as human analogs.

## Results

### Development

In order to use Calliphoridae to determine $PMI_{min}$, examiners should be familiar with the known sequence of development. The Calliphoridae family is one of the most studied and has its development mapped almost down to the hour. Currently two methods of Calliphoridae development measurement are employed: Accumulated Degree H (ADH) and a newly developed computational model called ExLAC. ADH is an older and more widely accepted method for measuring development and provides a linear relationship between development and temperature. However, ExLAC is a new method that is proving to be more accurate. It utilizes exponential functions of data points of larval development that is dependent on time and temperature [16]. ExLAC includes the calculation for Root Mean Square (RMS), a statistical measurement used similarly to standard deviation. In a study by Reibe-Pal and Madea, developmental data for *Lucilia sericata* is measured against a known control timeline of 508 h. The ExLAC method results in a $PMI_{min}$ of 505.8 h with a RMS of ± 2.2 h (Table 1) [16].

### Identification

In order to apply a known developmental timeline to determine PMI, an investigator must be able to quickly and reliably identify family Calliphoridae. A recent study demonstrates that the 1300 base pair region of mtDNA coding for Cytochrome Oxidase I (COI) gene is essential for distinguishing Calliphoridae and its subfamilies based on molecular phylogenies. Phylogenic trees are created following PCR amplification, cloning, sequencing and comparing minor nucleotide variants of COI gene. When the genera are clearly separated, the subfamily grouping matches the morphological findings of the confirmed species [1].

### Collection

Applying sequence of development to an identified specimen source is worthless unless proper sample collection procedure of Calliphoridae is followed. When Calliphoridae colonize a corpse, they first congregate in openings most often in the eyes, nose and mouth. If the corpse is found in a state of undress, the anus and vagina are also colonized [17]. After collecting pupae and larvae samples, the investigator should halt the development process with the hot water kill method, and then place the larvae in 70% ethanol and pupae in 90% ethanol. This method creates the optimal pupal preservation for histology [18].

### Pre-Arrival Interval (PAI)

The pre-Arrival Interval (PAI) is defined as the period of time from the moment of death to the landing of the first necrophagous insect [11]. The calculation of $PMI_{min}$ and knowledge of PAI aids in the comprehensive figuring of PMI. Volatile Organic Compounds (VOC) and temperature play an important role in determining PAI. A decomposing corpse releases thousands of VOCs. However, studies show that Dimethyl Disulfide (DMDS) and butan-1-ol are of special interest regarding Calliphoridae. The most accurate tool to measure these VOCs involves Two-Dimensional Gas Chromatography Time-of-Flight Mass Spectrometry (GCxGC-TOFMS). DMDS and butan-1-ol are VOCs that are released in the earliest stages of decomposition.

| Calliphoridae Development | | |
|---|---|---|
| **Stages of Development** | | **Timing** |
| Eggs |  | 1 day |
| Larva -1st Instar |  | 1.8 days |
| Larva–2nd Instar |  | 2.5 days |
| Larva–3rd Instar |  | 4-5 days |
| Pre Pupa |  | 8-12 days |
| Pupa |  | 14-18 days |
| Adult |  | Emerges from pupa case after 4-5 days |

**Table 1:** Stages of development of Calliphoridae.

DMDS is a methionine based sulfur compound while butan-1-ol is an alcohol found in lipids and carbohydrates [19]. These compounds are measured by GCxGC-TOFMS [9]. Electroantennography (EAG) is used to calculate depolarization in relation to Calliphoridae response to certain VOC. The more well-known VOCs are cadaverine and putrescine, yet they have no discernible effect on depolarization recorded by EAG [10]. DMDS and butan-1-ol exhibit the largest depolarization for female *L. sericata* at 0.5 µg, but not 100 µg. DMDS displays large depolarization, regardless of *L. sericata* sex [10]. Studies also show that the PAI in regards to Calliphoridae is closely related to temperature. The PAI of adult flies is the interval from time of death to the first arrival of adult Calliphoridae, while PAI of oviposition is the interval from time of death to the first eggs laid [11].

## External Effect on Development

Toxicology is one of several factors that can change PMI calculation by altering the development and arrival of Calliphoridae. Cocaine and ketamine have been found to accelerate development. Cocaine generally accelerates larval development by 36-72 h post hatching. However, a more conclusive study depicted even greater specific developmental acceleration for *Chyrsomya albiceps* and *Chrysomya putoria*. Carvalho, et al., find that at 24 h post contact with cocaine, no differences are observed in either *C. albiceps* or *C. putoria*. However, at 30-42 h, an increase in development for *C. putoria* was observed, but not for *C. albiceps*. At 54 h, both species exhibit increased larval development, but *C. putoria* doubled in weight compared to the controls [20]. Ketamine is a cyclohexanone derivative that is typically used as an anesthetic, but is also used as a recreational drug. Examining the effects of ketamine on *L. sericata* show that at half the lethal dose, full larval development was documented at 96.5 h in contrast to the control group that developed in 108 h. When larvae are exposed to ketamine, their length and weight differ from baseline measurements, at a statistically significant *p* value of 0.05. Barbiturates, morphine, and heroin have shown the ability to delay Calliphoridae development. All these compounds have been shown to slow the rate of larval development [14]. Heroin is especially stable in tissue over time, even in its legal form of codeine. *L. sericata* that feed on tissue containing heroin remain in pupation for an additional 6-28 h [4].

Ambient temperature is another factor that alters PMI calculation. Recording ambient temperature where a corpse is found is pertinent in determining PMI, especially with bodies found indoors where the temperature is much less likely to fluctuate [21]. It is accepted practice to place a corpse into a morgue refrigerator post discovery and prior to autopsy or examination. This quick drop in temperature has the potential to force Calliphoridae to go into quiescence [22]. In Malaysia, a corpse was found decomposed indoors, and was placed into a morgue cooler. The ambient temperature in the morgue refrigerator was 4°± 3°C, and *Chrysomya megacephala* was not collected until after the corpse was left for a few days in the refrigerator. $PMI_{min}$ was calculated to be between 2.9 and 3.9 days. Later, however, the individual was confirmed to have been deceased for at least 12 days [22]. Additional studies show that higher temperatures also alter development, by increasing *Lucilia cuprina* larval development at peak temperatures of 24°-27°C [21].

A restrictive environment can also alter PMI. For example, victims of homicides who are excessively wrapped in a carpet, tarp or blanket could delay insect arrival and oviposition [23]. Another common scenario in the forensic field is to find a corpse inside a vehicle, often due Carbon Monoxide (CO) poisoning. A study by Voss et al. shows that Calliphoridae are the first colonizers on the corpse found in a

vehicle, but eggs are not observed until the late morning of day 1. Its outdoor counterpart has evidence of oviposition on day 0. That same study demonstrates that unlike other substances, CO did not alter the development of Calliphoridae. Ultimately, it was discovered that the corpse decomposes 3-4 days more quickly in the enclosed space of the vehicle even though Calliphoridae arrival is delayed 16-18 [23].

### Post-feeding Interval

The post-feeding interval is the period of time between late larval period and pupal stage. During this post-feeding interval larvae have the ability to travel. This interval is expected to vary depending on the amount of time and distance traveled. It has been found that *L. sericata* pupae disperse from bodies at much greater distances indoors than outdoors [6]. Indoor larvae have been found to travel distances of up to 30 m, which can delay the pupal stage [24]. Studies on *L. sericata* based on length of vein and corpse size show a decrease in size with increase in time spent in the post-feeding interval.

## Discussion

The arrival and activity of family Calliphoridae on a deceased corpse indoors can aid in the quantitative measure of the PMI. The proper identification, collection, evaluation of PAI, measurement of development, and analysis of post-feeding stages of Calliphoridae provides a comprehensive picture that reduces error in the estimation of PMI. In order to use Calliphoridae for deducing PMI, an investigator must first have a quick and reliable means to identify the species of interest. The most reliable method is to examine the mtDNA from a COI gene, but this method can be time consuming and leads to a higher rate of error if performed by an inexperienced individual. Another technique of determining PMI involves the use of a 3D Micro-computed tomography for examining the oldest stage of pupae from the Calliphoridae collected [25]. Both techniques provide the investigator with the identity of the Calliphoridae of interest, however, limitations do exist. By only focusing on a single, small fragment of DNA, in this case the single COI gene, it may oversimplify the phylogenic grouping by failing to recognize genetic variability [1]. In the case of the 3D Micro-computed tomography, measurements were only performed on a single species of Calliphoridae, namely, *Calliphora vicina* [25]. To eliminate the minutest possibilities of identification error, both mtDNA sequencing of COI gene and 3D Micro-computed tomography should be performed on as many species found in the Calliphoridae family as possible. When Calliphoridae is found and identified, existing phylogenic maps and morphological charts of various species can be used for comparison.

An examiner should not limit the identification process at just the family level of Calliphoridae since certain species differ in oviposition, behavior, and geographical location. Determining only the family ignores these variables, and possibly alters the PMI calculation. Therefore, identifying the genus and species is also important. Calliphoridae presence may be global, but *Chrysomya rufifacies* is predominant in Asian countries [26]. In Malaysia, *C. rufifacies* have been found to delay egg lying until after the arrival of *Chrysomya megacephala* [27]. Predatory behavior of certain species must also be considered. For example, *Chrysomya albiceps* eliminate early corpse colonizers [3]. If an examiner just finds the remnants of *C. albiceps* without knowing its predatory behavior, he or she could mistakenly deduce that this was the first corpse colonizer and underestimate PMI. Standard protocol should be initiated for investigators to identify the species of interest to reduce PMI error as much as possible.

Larval and pupal development can be used to calculate $PMI_{min}$, however this measurement fails to account for the amount of time it takes for an insect to arrive. By examining $PMI_{min}$ and taking into account the PAI, an investigator is able to put together the most comprehensive and accurate PMI. An examiner should confirm the species and analyze the ambient temperature. Temperature was found to affect the PAI of certain species differently. While the minimum amount of time for arrival varied little, maximal arrival periods differed more substantially. *Lucilia caesar* was found to have the shortest maximal adult arrival time and *Calliphora vomitaria* to have the longest maximal arrival time [11]. If ambient temperature of the scene is unavailable, or if the examiner fails to measure it, information can be collected through weather station data [12]. However, temperature fluctuation is minimal indoors because of insulation and use of thermostats. The study on PAI and temperature relation does have its limitations, and was only performed on three different species of Calliphoridae, namely, *C. vomitaria*, *L. caeser*, and *Lucilia sericata* [11]. Future studies relating PAI to temperature should extend to as many Calliphoridae species as possible. By understanding this relation, an investigator can conclude the most accurate PAI possible, and therefore estimate a more definite PMI. PAI is not solely dependent on temperature, for VOC also play an important role. Identification of VOC by GCxGC-TOFMS and electrical depolarization measurement by EAG has found that DMDS and butan-1-ol are of special interest for Calliphoridae. These two compounds are not exclusive to human decomposition in fact they are usually generated during the decay of most organic matter. However, the combination of 3-methylthio-1-propanol, methyl (methylthio) ethyl disulfide, diethyl disulfide, pyridine, ethyl propionate, propyl propionate, propyl butyrate, and ethyl pentanoate during decomposition is unique to humans and domestic pig (*Sus scrufa*) [28]. This finding confirms the ability to use domestic pig (*Sus scrufa*) as a human analogue in further studies of VOC released during human decomposition [9]. Ultimately, to best understand and document the PAI with greater accuracy, one must have knowledge of the species of interest, temperature, and Calliphoridae behavior in regards to specific VOC.

Calliphoridae is an integral part of PMI estimation due to its affinity for corpses and the extensive documentation of its development. In the past ADH or ADD were the accepted measurements of development. This method is limited, for it does not include a calculation for error and was only performed on 1 species at a constant temperature of 22°C [29]. The newer method of ExLAC uses RMS to allow for some standard deviation. A control PMI of 508 h was compared to an ExLAC PMI calculation of 505.8 h with a RMS of ± 2.2 h. This calculation was found to be more accurate than the 515 h estimation that was concluded using ADH [16]. The ability of ExLAC to calculate PMI with a greater accuracy argues that it should be employed over the outdated ADH or ADD method and investigators should be trained in its use. The known timeline of Calliphoridae development must be paired with toxicology results since some substances alter development rates and therefore affect PMI estimates. Studies have already demonstrated that cocaine and ketamine have the ability to increase rates of development, while morphine was shown to delay it. It should be taken into consideration that due to ethical restrictions placed on human studies and the limited availability of human subjects, animal models are often employed. Animal models other than domestic pig (*Sus scrufa*) have been used, however since different animals metabolize substances differently, the effects on Calliphoridae development could differ within them. The

study undertaken to examine the effects of ketamine on development were performed on *L. sericata* using rabbits [14]. Morphine, and its ability to delay development on *C. albiceps* and *C. putoria,* was studied using minced kangaroo meat and lamb hearts [4]. The cocaine studies were only performed on *C. albiceps* and *C. putoria*. There is evidence to conclude that ingestion of certain substances can change development rates, but more studies need to be performed. Future studies should experiment with a wider array of Calliphoridae to ensure that changes in developmental rates are recorded for various species. Furthermore, fewer studies have been performed using Calliphoridae samples collected from human subjects who have metabolized illicit substances and pharmaceuticals. Investigators should collect Calliphoridae from suspected subjects of overdose at the time the corpse is discovered to compare with later toxicology results. In this manner, a comprehensive database of Calliphoridae and the substance's ability to delay or accelerate development can be compiled.

In order to calculate a comprehensive PMI using Calliphoridae, investigators should be aware of the myriad factors that can effect development. One must consider temperature, oviposition behavior, and corpse location. *L. sericata* were found to inhabit areas that have more sunlight [30]. If a corpse is discovered in a dark environment, *L. sericata* might be slower to colonize than if the corpse was present in a bright location. This darkness could be natural or artificial, as in nighttime or in a windowless room. One investigation showed that higher temperatures have the ability to stimulate *L. sericata* to participate in nocturnal oviposition in 57 instances in the field and indoors [31]. This nocturnal ovipository behavior is most common in areas of higher nighttime ambient temperatures, such as the Southern United States and especially Florida. Conversely, a drop in temperature has been found to cause Calliphoridae to go into quiescence. When *C. megacephala* was collected off a corpse in a morgue refrigerator, whose ambient temperature was 4° ± 3°C, PMI was underestimated due to development going dormant. This knowledge of temperature would lead an investigator to match the development to a timeline based on that ambient temperature. But behaviors of maggot masses need to be taken into consideration as well. Maggot masses, despite existing in 4° ± 3°C, were found to have a temperature of 12°C [22]. Therefore if an investigator fails to collect Calliphoridae prior to placing the corpse in a morgue refrigerator, temperature of not only the refrigerator should be noted, but also the temperature of the sampled maggot masses. When examining the larvae and pupae to make the $PMI_{min}$ determination, investigators must consider that ongoing, multitudinous oviposition of multiple species will continually take place [27]. To ensure the investigator has truly collected the oldest pupae or larvae, an investigator should take multiple samples from different parts of the corpse.

The state of the corpse and its location must be properly noted to determine PMI. A corpse that is excessively covered or wrapped, such as homicide victim bundled in tarps, carpets, sheets and blankets, is problematic for two reasons. A swaddled corpse provides not only a physical barrier for Calliphoridae arrival, but could potentially stifle the release of VOC, thereby potentially delaying arrival and manipulating the PAI. The investigator should also take into account the surroundings and state of the indoor corpse. Surfaces around the corpse and the condition or position of the corpse change the behavior of Calliphoridae. For example, during post-feeding interval, larvae can travel greater distances over hard surfaces such as wood or linoleum floors, but travel shorter than normal distances on thick carpets and rugs or if there is a physical barrier such as a bathtub. Calliphoridae development and succession is also affected by the condition or position of the corpse in some cases, such as a burnt corpse or one that is suspended as seen in hanging suicides. Further studies need to be undertaken in order to examine how such cases affect the arrival and development rates of Calliphoridae. In an indoor scenario an investigator should note any and all openings such as windows, doors, ventilation ducts, or anything that would provide insects access from the outdoors. Most homes and indoor environments are not completely sealed, with gaps in doorways and small tears in screens being frequent. In an insulated house, with doors shut tight and windows closed PMI has the potential to be underestimated. In one case where an individual was found deceased indoors, *L. sericata* was used to determine PMI. The individual was completely sealed indoors and was estimated to have been deceased for 3 days, when in reality the individual had been deceased more than 5 days earlier [32]. In rare instances, a secure indoor inhabitance could also potentially prevent the arrival of Calliphoridae; sometimes their large size can prevent them entering from the smallest of spaces. In such cases, the smaller Phoridae family could be better indicators of PMI. One case that examined 3 corpses in a locked home, *L. sericata* and *Megaselia scalaris* (family Phoridae) were examined to determine PMI. The smaller *M. scalaris* was determined to be a better indicator of PMI because *L. sericata* underestimated PMI by 10 days [16].

Knowledge regarding the post-feeding behavior of Calliphoridae is essential in the PMI estimation. $PMI_{min}$ determination is performed using the oldest larvae or pupae, if an investigator is not aware that larvae have the ability to travel, one might make the assumption that the younger feeding maggots are the oldest insects present. By only examining the maggots on the corpse, a gross underestimation of PMI could be made. It should be noted the certain species like *L. sericata* have the ability to travel long distances, and that hard surfaces commonly found indoors such as hardwood and linoleum further aid the distant dispersal [6]. Depending on the surface on which a corpse is found, investigators may have to search not only the immediate area, but other rooms of the house to check for the maximum distance the larvae have traveled. The farther Calliphoridae larvae travel, the longer they will spend in the post-feeding interval. This delay in pupation was found to inaccurately calculate PMI by a delay of about 29 h when the larva spent a total of 48 h in the post-feeding interval [24]. With knowledge of factors that alter the PAI, in addition to $PMI_{min}$, investigators can begin to piece together the postmortem interval.

To overlook the role of insect succession in human decomposition is to relinquish a more reliable way of determining time of death. Calliphoridae is essential in investigations that employ forensic entomology. It has been documented that the greatest effect on rates of decomposition is the presence or absence of insects, regardless of environment [15]. The arrival and activity of family Calliphoridae on a deceased corpse indoors can aid in the quantitative measure of the postmortem interval. To confirm Calliphoridae importance in indoor deaths, *L. sericata* and *Calliphora vicina* were found in greater number in indoor deaths and were more prevalent in urban areas [30,33]. Calliphoridae will also preferentially inhabit a corpse in a rural area. This eliminates them as markers to examine if a corpse has been moved. But fortunately, it makes them essential in determination of PMI due to their ability to colonize in vast geographical locations [34]. In addition to estimating PMI, Calliphoridae can be used to perform toxicology tests when human tissue is no longer available [35]. Gas chromatography and mass spectrometry on collected Calliphoridae can be used to test for common overdose drugs such as phencyclidine, cocaine, heroin, amitriptyline, and methamphetamine [13]. Knowledge of proper collection and identification of Calliphoridae, the well documented sequence of its pre-arrival interval, development, and post-feeding interval, and understanding of external pressures on its development

gives any medico-legal investigator an effective methodology to accurately determine the postmortem interval [36].

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