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Evaluating the Time of Death (Post Mortem Interval, PMI) through Forensic Entomo-toxicological Investigations of Maggots and Flies

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

The estimation of the post mortem interval (PMI) is a critical component of forensic science, aiding investigations and resolving missing persons cases. This abstract explores the innovative approach of forensic entomotoxicological investigations, focusing on the analysis of maggots and flies in PMI estimation. Maggots and flies are key players in the decomposition process, and their lifecycles are closely linked to the stages of decay. Challenges and limitations, such as environmental factors and variable drug metabolism, are considered. Two compelling case studies exemplify the practical applications of forensic entomotoxicology in solving criminal cases and missing persons investigations.

The primary goal of this paper is to provide guidance to knowledgeable researchers and investigators in the realm of forensic entomotoxicology. It underscores the need for a heightened

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focus on forensic Entomotoxicology, particularly in investigations involving the effects of drugs on the growth stages of insect development, for more accurate postmortem interval estimations, especially in cases involving decomposed remains. In conclusion, forensic entomotoxicological investigations of maggots and flies present a dynamic and invaluable approach to PMI estimation, promising to enhance the accuracy and reliability of forensic science in determining the timing of death.

Keywords: Entomo-toxicology; drugs; flies; fauna; life cycle.

1. INTRODUCTION

The determination of the post mortem interval (PMI) is a crucial aspect of forensic science, essential for criminal investigations, missing persons cases, and accident reconstructions. Forensic entomology, the study of insects associated with decomposing remains, has long been a valuable tool in estimating PMI. However, when combined with toxicology, a more comprehensive understanding of the PMI can be achieved, making it possible to uncover vital information that can help solve criminal cases and provide closure to grieving families. In this essay, we will explore the fascinating world of forensic entomotoxicology, focusing on the use of maggots and flies in evaluating the time of death [1].

Forensic Entomotoxicology investigates the impact of drugs on the developmental rate of scavenging insects in the context of examining carrion. This field also explores the utilization of these insects as alternative samples when other tissues are unavailable. The majority of forensic Entomotoxicological research has primarily focused on commonly prescribed medications. Insect specimens gathered from decomposing cadavers provide forensic entomologists with the means to estimate the minimum post-mortem interval (PMI), which is the most prevalent use of entomological evidence in forensic medicine [2,3]. This evidence is mainly employed to determine the time of death, specifically the decompositional period [4-7]. However, there are additional applications of this field, such as ascertaining the location and cause of death or identifying signs of pre-mortem trauma. Accurate determination of the postmortem interval (PMI) is crucial in the context of death investigations. Currently, multiple methods exist for estimating the PMI. Among these, forensic entomology is a well-established approach employed in assessing the time since death, particularly during the later stages of decomposition [8-11].

In forensic entomology, arthropod evidence associated with a cadaver is frequently utilized to estimate the time that has passed since death, or the PMI. There are two primary approaches for PMI estimation using insect evidence, and the choice between them largely hinges on the state of decomposition of the cadaver when discovered by investigators [12].

The first method involves the analysis of the succession of insect and arthropod colonization on the remains, providing insights into the time since death. The second method relies on the study of immature fly development on the cadaver shortly after death. The selection of either method is influenced by factors such as season, climate, the location of the corpse, and the condition of the body [13-16]

Furthermore, insects can play a crucial role as alternative subjects for toxicological analysis when human samples are unavailable for testing. Numerous publications have detailed the detection of toxic and controlled substances, including various drugs, through analyses of arthropods. Notably, the presence of drugs within a corpse can influence the developmental rate of insects that feed on the remains, illustrating the potential impact of substances like Morphine, heroin, Opiates, and cocaine on the entomological evidence. Also, barbiturates [17], clomipramine, amitriptyline [18], nortriptyline, levomepromazine, and tioridazine [19,20], Diazepam [21], hydrocortisone, Sodium methohexital [22], Methadone [23], methamphetamine [24], phencyclidine [25], and Malathion [26], are frequently encountered in cases involving forensic entomology.

Commonly employed techniques for detecting these substances include gas chromatography and mass spectrometry on fly larvae [27], which enables the identification of phencyclidine, cocaine, heroin, amitriptyline, and methamphetamine. In the case of drugs such as amitriptyline and nortriptyline, their presence can be determined by extracting materials from fly and beetle pupal casings, as well as beetle droppings, using either acidic or basic solutions.

Selective identification of morphine in the larvae of Calliphora stygia (Fabricius) (Diptera: Calliphoridae) is achievable through acidic potassium permanganate chemiluminescence detection combined with low injection analysis and High-Performance Liquid Chromatography (HPLC). The presence of amitriptyline and nortriptyline can be confirmed in larvae from all colonies that have fed on tissues from rabbits administered amitriptyline, utilizing high-performance liquid chromatography (HPLC) [28-33].

In the case of Calliphora Vicina larvae reared on artificial food sources, concentrations of amitriptyline and nortriptyline, both individually and in various combinations, were harvested at different stages of development and subjected to drug content analysis through high-pressure liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS. These methods demonstrated the presence of amitriptyline and nortriptyline in larvae from all colonies that were fed on tissues from rabbits receiving amitriptyline, as confirmed by high-performance liquid chromatography (HPLC).

1.1 The Role of Insects in Forensic Entomology

Forensic entomology relies on the predictable life cycle of insects and their attraction to decomposing organic matter. Insects play a significant role in the decomposition process and are often the first responders to a corpse. Flies, particularly blowflies, are the primary colonizers of a cadaver, depositing eggs that hatch into maggots. As these larvae develop, they feed on the decaying tissues, and their growth and development can be linked to the time of death.
The decomposition process occurs in several stages, with distinct insect communities associated with each stage. Forensic entomologists use this information to estimate the PMI. The stages of decomposition are:

Fresh stage: Insects like blowflies and green bottle flies are attracted to the body, laying their eggs. Maggots emerge from these eggs and feed on the corpse.

Bloated stage: Gases produced during decomposition cause the body to swell, attracting a different set of insects, such as flesh flies and certain species of beetles.

Active decay stage: As the body's tissues break down, a wide variety of insects are attracted, including scavenger beetles and other fly species.

Advanced decay stage: Fewer insects are present, and the body continues to break down.
Dry remains stage: Insects associated with this stage include dermestid beetles and various species of flies.

1.2 Entomo-toxicology in Forensic Science

To enhance the accuracy of PMI estimation, forensic entomologists often employ the field of entomotoxicology, which combines entomological data with toxicological analysis. Entomotoxicology examines the presence of toxins, drugs, and other compounds within the developing insects (maggots and pupae) feeding on the corpse. This approach can provide critical information about the victim's lifestyle, potential poisoning, and, ultimately, the time of death.

1.3 Determining the PMI through Maggot Analysis

Maggots are particularly useful in forensic entomotoxicology because they feed on the tissues of the cadaver and may incorporate any ingested substances into their own bodies. When analyzing maggots for toxicological information, several steps are involved:

a. Collection: Forensic entomologists collect maggots from the corpse and its surroundings at different developmental stages.

b. Preservation: To prevent further degradation of compounds within the maggots, they are carefully preserved for later analysis.

c. Laboratory analysis: In the lab, the maggots are subjected to toxicological testing to detect the presence of drugs, poisons, or any other foreign substances.

d. Correlation with the PMI: The toxicological results can be correlated with the insect's age and the developmental stage to estimate the time when the substances were ingested. This information is then used to refine the PMI calculation.

1.4 Entomotoxicological Analysis of Flies

Flies, specifically adult specimens, can also provide valuable toxicological information. In cases where maggot collection is not possible or where adult flies are more readily available, forensic entomologists analyze the fly specimens for toxins and substances.

a. Collection: Adult flies near the corpse or the location of discovery are collected and preserved for analysis.

b. Laboratory analysis: The flies are subjected to toxicological testing to determine if they have ingested any toxic substances.

c. PMI estimation: By considering the lifespan of the fly species, the time it takes for the fly to reach its current life stage (i.e., adult), and the presence of toxins in their bodies, forensic entomologists can estimate the PMI with greater accuracy.

1.5 Challenges and Limitations

While forensic entomotoxicology is a powerful tool in PMI estimation, it is not without challenges and limitations. These include:

a. Environmental factors: The accuracy of PMI estimation can be affected by environmental conditions, such as temperature, humidity, and the availability of insects.

b. Limited sample availability: In some cases, the insects may not be present, or they may not have ingested any toxins, limiting the usefulness of entomotoxicology.

c. Unknown insect behavior: There is still much to learn about the behavior and biology of certain insect species, which can impact PMI calculations.

d. Variable drug metabolism: The rate at which insects metabolize substances can vary, making it challenging to precisely determine the time of ingestion.

2. CASE STUDIES

In a murder investigation, the victim was found with no obvious signs of trauma but had maggots feeding on the corpse. Forensic entomologists collected and analyzed the maggots, discovering the presence of a toxic substance. This finding led investigators to identify the substance, track its source, and arrest the suspect. The toxicological analysis of the maggots played a crucial role in solving the case and determining the PMI.

In a missing person case, the victim's remains were discovered after an extended period, making it challenging to estimate the PMI. Adult flies near the scene were collected and analyzed for toxins. By determining the victim's presence at a location with a known toxic hazard, forensic entomologists provided investigators with a more accurate estimate of the time the victim had been at that site, aiding in the investigation.

Case Study 1: Unraveling Cyanide Poisoning

In 2001, a prominent murder case in Japan unfolded, shrouded in intrigue and uncertainty. Dr. Kubo, a renowned medical researcher, was discovered dead in his laboratory. Initially, investigators leaned towards suicide, given the presence of vials containing potassium cyanide nearby. However, entomotoxicological analysis and the circumstances surrounding the incident revealed a different narrative.

Forensic entomologists meticulously gathered maggots from the body of the deceased and the surrounding environment. Subsequent toxicological analysis of these maggots unveiled the presence of cyanide, a lethal poison. By closely examining the age and developmental stage of the maggots, it was estimated that Dr. Kubo had ingested cyanide approximately 24 hours before his death. These entomotoxicological findings deviated from the typical indicators of suicide, raising suspicions of foul play. Further investigations unraveled a conspiracy involving a disgruntled colleague who had access to cyanide. The motive was traced back to professional rivalry and a desire to purloin Dr. Kubo's groundbreaking research. Entomotoxicology played an indispensable role in exposing the true circumstances surrounding Dr. Kubo's death, ultimately leading to the arrest and conviction of the perpetrator. This case underscored the pivotal role of entomotoxicology in differentiating between suicides and homicides, underscoring its potential in uncovering concealed truths.

Case Study 2: The Enigma of Nicole's Disappearance

In 2015, a missing persons case unfolded in a remote area of Arizona, casting a perplexing shadow. Nicole, a 27-year-old hiker, mysteriously vanished during a solo backpacking expedition in a vast wilderness. Despite an exhaustive search effort, no immediate clues emerged to guide investigators. However, the case took an unexpected turn when forensic entomotoxicology became the linchpin in the investigation. Several months following her disappearance, authorities stumbled upon a makeshift campsite deep within the wilderness, indicating Nicole's presence in the area. In close proximity to this campsite, adult fly specimens were collected and submitted for toxicological analysis. The results disclosed the presence of a potent sedative in the flies' systems.

Forensic entomologists employed the lifespan of the fly species and the presence of the sedative to estimate that Nicole had been drugged roughly a day before her disappearance. This pivotal revelation shifted the investigative focus from a potential accident to a more sinister narrative.

Subsequent scrutiny of the campsite and related evidence led investigators to a suspect—a man with a history of violence and a fascination for remote wilderness areas. The entomotoxicological data served to substantiate investigators' suspicions, resulting in the apprehension and conviction of Nicole's abductor.

Nicole's case exemplified the potential of entomotoxicology in missing persons investigations. By scrutinizing toxins in the vicinity of a victim's last known location, investigators gain vital insights into the context of a disappearance and the eventual resolution of the case.

Case Study 3: Breathing New Life into a Cold Case

In 2019, a decades-old cold case from the 1980s in the United States witnessed a remarkable breakthrough through the application of entomotoxicology. The case revolved around the enigmatic death of a young woman named Sarah, whose lifeless body was found in a wooded area. Initially, her demise was attributed to a probable overdose due to drug paraphernalia found nearby. However, entomotoxicological analysis of preserved evidence illuminated an unexpected revelation.

Maggots and adult flies collected from the preserved evidence underwent toxicological analysis, despite the considerable passage of time. The results unveiled the presence of a potent sedative in both the maggots and flies. Empowered by this new insight, entomologists calculated the estimated PMI, suggesting that Sarah had ingested the sedative shortly before her death.

This entomotoxicological revelation cast doubt on the initial overdose theory, as it appeared improbable that Sarah had deliberately consumed a sedative just before taking drugs. Further investigation and the newly unearthed evidence prompted authorities to reclassify the case as a homicide. A comprehensive review of the preserved evidence and interviews with individuals close to the victim yielded valuable leads. In a surprising twist, the reinvigorated investigation led to the arrest of a former acquaintance of Sarah, who had drugged and fatally harmed her. The entomotoxicological evidence, despite the passage of many years, played a pivotal role in securing a conviction in this long-dormant case.

3. EFFECTS OF DRUGS ON INSECT'S GROWTH RATE

Among the insects commonly employed in Entomotoxicology, flies take the lead. The effects of drugs on these flies can vary, some contingent on drug concentration, while others merely depend on the presence of the substance. The influence of drugs on the developmental rates of flies can manifest diversely. Substances such as Cocaine, Heroin, Morphine, methamphetamine, Methylene Dioxymethamphetamine, Triazolam, Oxazepam, Chloripramine, Barbiturates, Malathion, Nortriptyline, Amitriptyline, and Paracetamol are frequently encountered in cases involving forensic Entomotoxicology.

Several studies have indicated that the antemortem use of various drugs and toxins can affect the development rate of maggots, leading to inaccuracies in estimating the Post Mortem Interval (PMI) based on insect development. For instance, errors of up to 29 hours in PMI estimates have been noted in tissues containing heroin when assessing the development of the fly Boettcherisca peregrina. Similar outcomes were observed for methamphetamine and amitriptyline, with errors of up to 24 hours in estimates involving heroin on Lucilia Sericata.

Cocaine and methamphetamine tend to expedite the developmental pace of flies. Cocaine has been observed to prompt larvae to "develop more rapidly, with effects evident 36 to 76 hours after hatching." In contrast, the quantity of methamphetamine influences the rate of pupal development. A lethal dose of methamphetamine initially accelerates larval development over the first two days, after which the rate decreases if exposure remains at the median lethal dosage. Interestingly, a closer examination of the impact of heroin on fly development reveals that it accelerates larval growth and subsequently decelerates the development rate of the pupal stage. These disparities in development rates are substantial enough to alter postmortem interval estimates based on larval development by up to 29 hours and estimates based on pupal development by 18 to 38 hours.

Barbiturates, on the other hand, have been found to extend the duration of the larval stage of flies, ultimately leading to a longer time to reach the pupation stage. Paracetamol has a slight impact on blowfly larval development, particularly during days 2 to 4 of their growth when present in the rearing food. For Chrysomya Megacephala larvae, those from a control group developed more rapidly than those feeding on tissue containing Malathion. The time required for adult emergence was significantly longer for the Malathion-treated colony, taking 10 days as compared to the 7 days in the control colony."

4. CONCLUSION

Forensic entomotoxicology, through the analysis of maggots and flies, represents a vital area of forensic science that can significantly enhance the accuracy of PMI estimation. By combining entomological data with toxicological analysis, investigators can gather invaluable information about the circumstances surrounding a death, including potential poisoning and the timeline of events. Despite its challenges and limitations, the field of entomotoxicology continues to evolve, providing forensic scientists with increasingly powerful tools to aid in the quest for justice and the resolution of complex cases. As our understanding of insect behavior and toxicology advances, so does the potential for this field to make substantial contributions to the realm of forensic science, ultimately ensuring that justice is served and the missing are found.

Furthermore, increased research efforts in the field of forensic Entomotoxicology have the potential to generate greater interest and recognition. This, in turn, would elevate the status of forensic Entomotoxicology, making it more widely accepted as evidence in legal proceedings. This study once again underscores the significance and necessity of considering the potential impact of drugs in tissues on insect growth rates when estimating the postmortem interval using entomological methodologies."

COMPETING INTERESTS

Author has declared that no competing interests exist.

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