CASE REPORT

PATHOLOGY/BIOLOGY

J Forensic Sci, November 2017, Vol. 62, No. 6
doi: 10.1111/1556-4029.13481
Available online at: onlinelibrary.wiley.com

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A Case of Insect Colonization Before the Death

ABSTRACT: Forensic entomology is a branch of forensic science in which insects are used as evidence in legal investigations relating to humans, domestic animals and wildlife. One of the theoretical pillars on which the discipline is based concerns the fact that flies colonize a body after death. However in cases of myiasis, maggots are present before death, with consequences in the correct estimation of the minimum postmortem interval (mPMI). We report here the case of a woman, largely colonized by fly larvae, who has lain alive in her garden for four days prior to being rescued. Larvae were found on the conjunctivae, the bronchi, the rectum and vagina. The woman's death, two months later, was caused by tetanus. The consequences of myiasis on mPMI estimation are here discussed. In fact, despite she was still alive larvae, indicated and estimated age of 1.5–2.5 days, based on environmental and body temperature.

KEYWORDS: forensic science, forensic entomology, forensic pathology, PMI estimation, Myiasis, Calliphoridae

Forensic entomology is the branch of forensic sciences in which insects are used as evidence in legal investigations related to humans, domestic animals and wildlife (1–3). The examination, identification and analysis of the insects associated with human remains, combined with the knowledge of insect biology and the meteorological parameters, especially temperature, can provide a further level of detail in addition to medical and anthropological data in reconstructing the events that occurred close to the death.

In particular, necrophagous insects are useful for the minimum postmortem interval (mPMI) estimation, the movement of the body after the death, for the detection of drugs or poisons if soft tissues are no longer available or too decomposed (1,4). Furthermore, insects found on a crime scene can be used for human DNA extraction for identification purposes, when no suitable tissues are available (5) or even no body was found on the crime scene because removed before the police arrival (e.g., postmortem body movement).

One of the theoretical pillars on which the discipline is based concerns the fact that flies colonize a body after the death and that the age of the insects can be estimated considering the temperature at which these organisms were exposed during their development. The estimation of the age of the first body colonizers allows the estimation of the so-called time of colonization, commonly considered as the mPMI (6).

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Received 4 Nov. 2016; and in revised form 1 Feb. 2017; accepted 2 Feb. 2017.

However in cases of myiasis, the infestation of fly larvae on living or necrotic tissues in vertebrates, maggots are already present on the body of the victim before his/her death.

Human myiasis can be classified into two groups: specific myiasis produced by flies that need a live host to develop their immature stages, and semispecific myiasis caused by flies that typically develop in decomposing organic matter but occasionally develop on/in living organisms, mostly in the presence of wounds.

The most common semispecific myiasis-causing species belong to the genera *Calliphora, Lucilia* (Diptera, Calliphoridae) and *Sarcophaga* (Diptera, Sarcophagidae). In humans, these species mainly develop in necrotic tissue, in cutaneous wounds with nonnecrotic tissue, and in body cavities with an accumulation of secretions and excreta with a high bacterial load that constitutes the source of food for the larvae (7–9).

In a case of myiasis, female flies can lay eggs on living subject in cases of poor personal hygiene as recorded in several homeless persons, in the presence of open wounds and gangrene like in diabetes patients (9), and in case of nonconscious or nonself-sufficient/dependent persons such as cases of abandoned children and neglected elders (10).

However, in cases of poor personal hygiene or abandoned children and neglected elderlies, the estimation of the colonization time can provide useful information about when the condition, allowing flies to lay eggs took place. This information can have a forensic interest in case of people neglected or in nosocomial myiasis to define legal responsibilities (9). In other cases like in the case here described, the colonization time can provide information about when the accident, responsible of the loss of consciousness of the "victim," took place.

However, in all the cases previously described, the time of colonization does not correspond to the mPMI, if the subject was found already dead. These circumstances may result in

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relevant and critical errors on the estimation of mPMI with investigative consequences.

In this paper is presented the case of a woman, in an unconscious state and largely colonized by fly larvae, who had lain alive in her garden for several days before being rescued.

Case

In July 2015, an 84-year-old woman affected by hypertension and hyperthyroidism was found unconscious supine in the garden of her house in the suburbs of Florence (Central Italy) by the emergency rescue team. The woman was wearing an undershirt and underwear. Neighbors reported that she had been seen five days before her rescue. Medical observations carried out during the prehospital evaluation indicated that the patient was hypertensive, hypoglycemic and with a strong larvae infestation on the face, especially on the conjunctivae, the nasal choanae, the mouth and the external auricular canals. An ulceration was detected in the sacral region, potentially related to her lying position. This injury was later treated with escarectomy and debridement. External examination revealed the absence of traumatic injuries; however, it underlined the presence of sunburns I and II degree on exposed parts of the abdomen and thorax. An initial total body CT scan showed a left cerebral intraparenchymal-capsulo-lenticular hemorrhage causing only the compression of the left lateral ventricle. No other traumatic injuries were detected. The observed hemorrhage can be identified as the cause of the woman fall and loss of consciousness. Further investigation, based on fiberscopy, revealed that larvae were present in the bronchi, rectum, vagina and in the external auricular canals. In addition, it was observed that larval feeding activity was also responsible of multiple bilateral perforations of the tympanic membranes. A second CT scan reported a bilateral purulent ethmoidal-maxillary sinusitis that underwent a surgical drainage. No larvae were found in the affected sinuses. During the hospitalization, a bacterial pneumonia was detected. During the neurological rehabilitation, muscular spasms appeared and the woman died two months later. The cause of death was determined as tetanus based on the presence of trismus, stiffness in the joints and the column followed by immunologic tests. It is worth mentioning that at the hospital admission the woman was treated with an emergency booster injection of tetanus toxoid.

Larval samples were collected from different body regions, killed in hot water, fixed in 70% ethanol and sent to an entomologist following the standard and guidelines of the European Association for Forensic Entomology (1). Larvae were diaphanized using a hot solution of NaOH, and all the entomological observations were performed with a Leica M60 stereomicroscope and a Leica DME compound. Larvae, on the II/III instars, were identified as members of the Calliphoridae family. The oldest specimens showed posterior spiracles and cephaloskeleton sclerites of both the instars present at the same time, indicating the molting stage. Diagnostic features related to the shape of the oral sclerites allow the identification of the species as *Lucilia* cfr *sericata* (Meigen, 1826). (Diptera, Calliphoridae) Temperature obtained from the closest meteorological station collected during the days before the body rescue indicated a temperature average of 28°C with a minimum of 22 and a maximum of 35°C. This late temperature is close to the body average temperature and for this reason is more accurate in the estimation of the development in case of internal myiasis. The larval age was estimated being between 1.5 and 2.5 days using the data published by Grassberger & Reiter (11).

Lucilia sericata is a cosmopolitan species and one of the most common body colonizers especially in the warmer seasons. This species has been reported from several outdoors Italian cases (12–14), and in a general survey carried out in 2010 on over 200 cases from Northern and Central Italy, the species was found in more than 30% of the cases (15).

Lucilia sericata, as other species of the genus *Lucilia* (e.g., *Lucilia cuprina* (Wiedemann, 1830), *Lucilia silvarum* Meigen, 1826, *Lucilia bufonifora* Moniez, 1876, and *Lucilia illustris* Meigen, 1826, is known as a myiasigenic species in both human and other vertebrates (16–20).

Developmental data have been produced by several authors in a range between 10 and 34°C (for a general summery, see Amendt et al. (1)).

Conclusion

It is worth mentioning that, in cases like this, a later arrival of the rescue team would result with the finding of a dead woman. The estimation of the time since death based on the entomological and on the tanatho-chronological approaches clearly would give different results. For the first one, the estimation would be of 1.5–2.5 days, whereas applying the methods commonly used by the forensic pathologists (*e.g., algor, livor, rigor mortis*, electrolyte concentration in *vitreus humor*), the estimation would be of some hours. In fact, this case clearly demonstrates that in case of dead body the time of insect colonization could not correspond to the minimum time since death. For this reason, a close collaboration between forensic pathologist and forensic entomologist and a careful analysis of all possible variables are necessary, to answer at the investigative question about the time since death. In particular, in suspicious conditions, especially when the body is fresh, histological analysis of the colonized tissues can be helpful to evaluate whether the colonization started before or after the death of the victim (21) and to evaluate the time of a possible agony. A multidisciplinary approach has to be considered fundamental for a proper reconstruction of the peri-mortem events and for the mPMI estimation.

Acknowledgments

Authors want to thank Prof. GA Norelli, Prof. A. Peris and Dr M. Mangini for the information provided and the critical review of the manuscript. The authors are grateful to the anonymous reviewers and the editor for their suggestions.

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