CASE REPORT

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Three Case Studies in Forensic Entomology from Southern Italy

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ABSTRACT: Three cases of forensic interest regarding the estimation of postmortem interval (PMI) by entomological data are presented. The three cases concerning criminal investigations were performed in Southern Italy by the Entomological Laboratory of the Institute of Forensic Medicine at the University of Bari. For each case the authors present a detailed description of the remains as observed at the crime scene and a description of the arthropods collected from the remains. The PMI estimation was based on comparison of data from autopsy reports (rate of decay), local environmental conditions (temperature, humidity, rainfall) and development times for the immature stages of each species of local arthropod and succession patterns. The collection of insects was performed at the discovery site and during autopsy procedures. In the first case a PMI of 5 to 8 days was established based on the presence of adult specimens of *Saprinus aeneus* (family Histeridae), and mature larvae of *Chrysomya albiceps* and *Sarcophaga carnaria* (3rd instar). In the second case, on the charred remains of a corpse, larvae of *Sarcophaga haemorrhoidalis* (3rd instar) and *Protophormia terraenovae* (2nd instar) were observed in different developmental stages, as indicated, giving a PMI of 3 to 4 days based on entomological data. In the third case a PMI of 36 to 48 hours was defined from the evidence of *Calliphora vicina* 2nd instar on the two burnt bodies. In all cases the entomological evidence alone led to conclusions on PMI.

KEYWORDS: forensic science, forensic entomology, postmortem interval determination, blow flies, maggot age

An accurate determination of postmortem interval (PMI) is of primary importance in any forensic investigation of a death (homicide, suicide, accident, unattended death due to natural causes). Autopsy reports usually provide information regarding the manner and cause of death, degree of corpse decomposition, location and extent of any insects present. The comparison of all this information may help the medical examiner to estimate the time of death (1).

The potential usefulness of the sarcosaprophagous insects and their succession patterns as forensic indicators is well documented in entomological literature (2–4). Two basic steps should be applied by the forensic entomologist (5): the first is the correct collection and identification of the arthropods feeding on the corpse, both at the crime scene and at autopsy; the second is the application of knowledge of the maggot's life-cycle in local fauna to determine the age of the larvae taking into account major environmental factors influencing the rate of development (season, temperature, rainfall, lighting, humidity).

In Southern Italy, blow flies (*Calliphoridae*) and flesh flies (*Sarcophagidae*) predominate in the earliest stages of decomposition and, because of their short developmental cycles, they are very useful for PMI determination during the first three–four weeks following death (3,4,6,7). In these three cases a reliable PMI estimation was based on interpretation of stages of the decay process and on entomological specimens collected at the crime scene and during autopsy. Entomological data were obtained from Lab rearings and from experimental reconstruction of arthropod life-cycles in a growth cabinet (8,9). Using this apparatus, it is possible to program on an hourly schedule some fundamental microclimatic parameters, such as temperature and humidity, to reproduce the environmental conditions recorded in the field. In previous studies, our results have shown that statistically there is no difference between life cycles of specimens reared in the field and those reared in the laboratory under artificial, field-like conditions (10).

Case 1

The remains of a 20-year-old white male were recovered in the month of September in a suburban area of the City of Bari (Southern Italy). The head was mostly skeletonized; some tissue of the scalp and neck muscles were still present with the upper ribs, sternum and clavicles being partially exposed. The skin of the body was blackened and covered by feeding larvae, flesh of creamy consistence with exposed dark green parts; some skin blisters on the lower surfaces of the trunk and thighs, marbling of veins and cutis peeling with further spread of stains into inferior limbs were observed. Lividity, rigor and body temperature were no longer useful for a correct determination of time since death. Examination of the remains during autopsy revealed a bullet entrance wound in the right retroauricolar surface associated with skull fractures at the base; the bullet (cal. 38 Special) was found within the cranial cavity. Most of the brain, lung and heart tissue were absent, and crawling larvae were observed on the remains. The stomach was empty. Arthropods and larvae were collected from the body surface and from the body cavities; no puparia were observed following removal of the remains. Two main groups of maggots were found infesting the decaying body: the larvae not more than 16 mm long

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with spines along the entire length of the body were identified as *Chrysomya albiceps* (3rd instar, family Calliphoridae) and those between 19 and 21 mm in length with characteristic posterior spiracles situated in a deep cavity as *Sarcophaga carnaria* (3rd instar, family Sarcophagidae) by examination of their spiracular slits and cephalopharyngeal skeleton. The identification was confirmed by rearing of representative larvae to the adult stage in the Entomological Laboratory of the Institute of Forensic Medicine (University of Bari). Beetles collected at the crime scene were identified as adult *Saprinus aeneus* (family Histeridae). The remains were identified from dental record comparisons. The cause of death was craniocerebral injuries due to a gunshot wound to the head; the manner of death was homicide. The PMI was estimated to be 5–8 days based on the stage of decomposition and entomological conclusions. The degree of decomposition observed (moderately advanced decay) for remains found outdoors in summer is typical of a PMI of approximately 6 to 10 days when no insects are present. However, fly larvae (maggots) attracted to decomposing remains in this case played an active role in the decay process and were responsible for the advanced cleaning of the corpse's soft tissues. The heavy maggot infestation and the destruction of skin with numerous maggot holes and sinues made easier the spread of proteolytic enzymes secreted by larvae as well as other environmental bacteria that speed up degradation of the body (11). The correct estimation of the time of death was finally determined based on entomological evidence related to weather data collected from the nearest meteorological station (Bari airport, approx. 7 km from the scene). In Southern Italy flesh flies (*Sarcophaga carnaria*) are frequently associated with decomposing remains, especially in summer; the females are viviparous and deposit active first-instar larvae at approximately the same time as Calliphoridae. *Chrysomya albiceps* (Wieldemann) is also common in Southern Italy, especially in the hot season; it extends from the southern portion of the Palearctic Region (North Africa eastwards to north-west India and Southern Europe) throughout Africa. It is an aggressive colonizer of remains found outdoors and oviposition occurs from the first hour after death during the early to mid decomposition period. It does not form dense maggot masses and the larvae feed on other Diptera larvae present on the remains as well as the decomposing tissue. Adult *Saprinus aeneus* (family Histeridae) occurs usually during the early to mid decomposition period or longer. In our practical experience, they have been found during the bloated decay and early parts of the dry stage and therefore they were not useful in this case for an accurate determination of PMI. For this purpose experimental laboratory rearing of *Chrysomya albiceps* and *Sarcophaga carnaria* was performed in a growth cabinet used to recreate the climatological conditions to which the body was exposed in that period of September (time elapsed between when the victim was last seen and the discovery of the corpse). Emergent third-instar larvae of *Sarcophaga carnaria* were observed after 5 days in most specimens from the laboratory rearings at fluctuating field temperature (min T = 11°C; max T = 35°C; mean T = 24°C) reproduced in the growth cabinet (warmer during the day and cooler at night according to data collected from the meteorological station). The 3rd instar larvae persited for another 36 hours before leaving the rearing medium (beef liver) to pupate in soil and decrease in size from 24 mm (max length of larvae) to 12–13 mm (max length of pupae). Most specimens of *Chrysomya albiceps* from the experimental rearings under the same laboratory conditions reached the 3rd instar stage (Fig. 1) of larval development 6 days after the egg hatching period (18–24 h). From entomological data the PMI was estimated at 5–8 days considering a mean interval

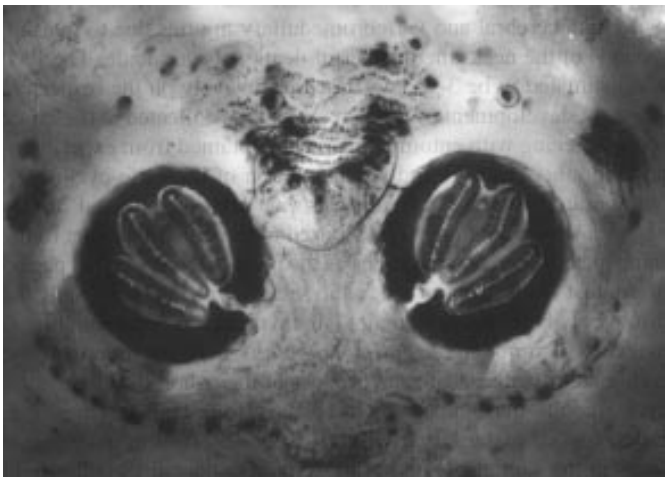

FIG. 1—*Posterior spiracles of third instar larva of* Chrysomya albiceps.

of time elapsed between attraction to the corpse and the oviposition and larviposition by colonizing adults of blowflies and fleshflies. In this case the victim had not been moved after the murder and circumstantial evidence confirmed that he had been assassinated in the same place where his remains were found. The PMI estimate fit well with the last time the victim had been seen alive: 8 days prior to discovery of the body.

Case 2

The charred remains of a 25-year-old white male were recovered in the month of August, in his burned-out car, in a rural area of the City of Brindisi (Southern Italy) close to agricultural fields. The arms were partially skeletonized with the extremities fractured by the heat thus resembling the posture of a boxer. The skull was largely devoid of flesh due to soft tissue having been burnt away and some brain tissue was exposed and had numerous Diptera larvae feeding on it. A protruding tongue and portions of partially burnt flesh on the neck were observed. The murder victim had been burnt to conceal the true cause of death. Examination of the remains during autopsy revealed two bullet entrance wounds on the neck. The bullets (cal. 38 Special) were shown by postmortem radiographs of the skull; the first within the oral cavity close to the left angle of the mandible, the second in the cranial cavity (*cerebellar fossa*), inside of which was a blood's clotlike structure. Soft tissues, including muscles, were greatly charred; the skin was burnt away and the underlying muscles were exposed and ruptured due to heat. Portions of the chest and abdominal walls were burnt away exposing the viscera; the internal organs were less affected by flames and contained a large larval infestation (particularly the lungs and heart; the liver was partially cooked). The stomach contained a quantity of partially digested foodstuffs amongst which were a few tomato skins, remains of vegetables and meat. The last meal had been eaten 3 to 5 hours before the fatal assault occurred. Larvae collected were identified as *Sarcophaga haemorrhoidalis* (3rd instar) and *Protophormia terraenovae* (2nd instar) by examination of their spiracular slits and cephalopharyngeal skeleton. The diagnosis was confirmed by rearing representative larvae to the adult stage in the Entomological Laboratory of the Institute of Forensic Medicine (University of Bari). No puparia were observed at the scene or during the autopsy. The remains were identified from dental record comparisons. The cause of death was craniocerebral and vertebromedullary injuries due to gunshot wounds of the

neck; the manner of death was homicide. The PMI was estimated to be 3–4 days based exclusively on the respective stages of development of the two insect taxa collected at the crime scene agreeing with entomological data obtained from experimental laboratory rearings and reconstruction of their life-cycles.

Laboratory rearings of *Protophormia terraenovae* and *Sarcophaga haemorrhoidalis* were exposed in the growth cabinet to the same meteorological conditions as the body had been in that period of August (time elapsed since the victim was last seen until the discovery of the charred remains). In favorable summer conditions, maggots of *Sarcophaga haemorrhoidalis* may be found on the corpse shortly after death and because of their short developmental cycles adults may appear in 8–10 days following larviposition. Emergent 3rd instar larvae (Fig. 2) were observed in most specimens in the samples reared after 72 hours under fluctuating field temperature (min T = 14°C; max T = 36°C; mean T = 26°C) reproduced in the growth cabinet (warmer during the day and cooler at night based on data collected from the Brindisi airport meteorological station, approx. 10 km from the scene). The 3rd instar larvae between 20 and 22 mm in length persisted for another 24 hours before leaving the growth medium (beef liver) to pupate in soil and decrease in size. Regarding *Protophormia terraenovae*, it was known from the Kamal experiments (12) that the minimum duration of its total development is 71% of that of *Calliphora vicina;* this was confirmed in our experimental rearings. Most specimens of *Protophormia terraenovae* in the samples reared reached the 2nd instar stage of development within 40 hours (48 h for specimens of *Calliphora vicina*) after the hatching period (12–18 h) under laboratory conditions identical to those where the charred-body had been found (mean T = 26°C). Emergent 3rd instar larvae of *Protophormia terraenovae* were observed after 24 hours. Subsequent investigations verified the entomological findings and our estimate: the victim had last been seen alive with some friends, eating salad and white meat, in a restaurant 4 days before the discovery of the charred remains. In our experience, forensic cases involving charred bodies are not infrequent (ritual suicide, arson to cover up a murder inside a building, a car, etc.), these events challenge forensic pathologists because there are no useful elements for the estimation of the time since death (hypostasis, cadaveric rigidity, body temperature). Stomach emptying as a measure of time since death is a method too uncertain to have any validity because of different physiological factors (11). Nevertheless the state of digestion of gastric content can be considered a reliable indicator of the time elapsed between the last meal and death. Only entomological evidence can help the forensic pathologist to determine an accurate PMI. In charred-bodies the viscera is frequently exposed due to thermic consumption of the chest and abdominal walls. The protected irregularities of internal organs are skillfully sought by blowflies and fleshflies and in these suitable sites the females can lay eggs or deposit larvae. However, in charred-bodies found in burned-out cars the discovery of the remains by sarcosaprophagous flies occurs only after the fire has gone out and the body has cooled enough to reach favorable microclimatic conditions of temperature and humidity for oviposition or larviposition.

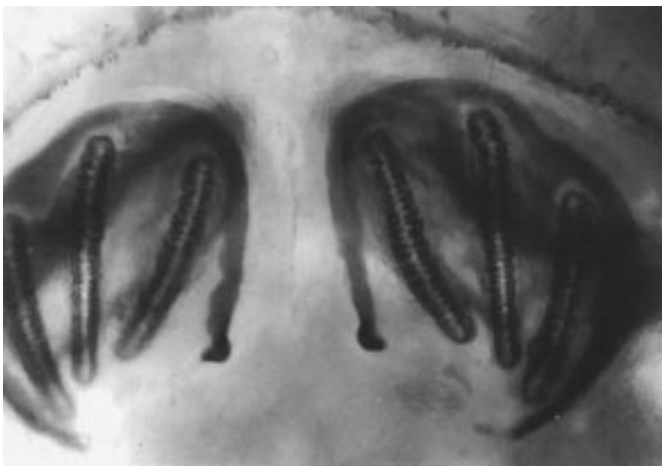

FIG. 2—*Posterior spiracles of third instar larva of* Sarcophaga haemorrhoidalis.

Case 3

The charred remains of a woman and a man both 25 years of age were discovered in their car which was completely destroyed by fire in the month of August. The charred bodies and the car were at the same ambient temperature. The man was found on the right front seat; the woman supine, lying on the back seat. The arms were skeletonized with extremities fractured by the heat. Soft tissues, including muscles, were mainly burnt. Frontal teeth were charred, the scalp was completely burnt and the skull exposed with characteristically curved fractures due to the high temperatures reached by fire. Portions of brain tissue were exposed and shrunken due to the heat. Portions of the chest and abdominal walls were burnt away exposing the viscera. Some early stage larval activity was noted within the chest and abdomen of each body, but it was found to be less than that in the cranial cavity of the man. Examination of the remains during autopsy revealed two different causes of death: for the man, a bullet exit hole in the residual occipital bone was observed; for the woman, only a blood forming clot pressed out of the lung tissue into the alveoli and airways suggesting a blunt force injury with blood inhaled during life. The stomach of each body contained a quantity of partially digested foodstuffs amongst which vegetable residues were recognizable. The last meal had been consumed within 2 to 4 hours before death. However no information regarding circumstances of the last meal was available. Obviously after the victims were murdered the bodies were left in their car where they were burnt. The assassin set fire to the car in the hope of destroying the corpses or to conceal the identity of the victims by extensive disfigurement and destruction of the remains. Identification was made on dental evidence. Diptera larvae between 6 and 11 mm in length were collected during the autopsy and identified as *Calliphora vicina* (2nd instar). This species identification was confirmed by rearing representative larvae to the adult stage in the Entomological Laboratory of the Institute of Forensic Medicine (University of Bari).

The stage (instar) of development was quickly determined by examination of the spiracular slits and internal skeletal structures. *Calliphora vicina* is the most common species of Calliphorid found on corpses in Europe. Oviposition on carrion can occur often within a few hours and under the right conditions (season and temperature), almost immediately after the death of a victim. Blowfly females have an excellent olfactory sense and are very skillful in seeking suitable oviposition sites such as the internal organs of charred bodies. Rearing of eggs and larvae of *Calliphora vicina* to adult flies, under laboratory conditions at a mean temperature of 28°C and 50% humidity corresponding to those to which the remains were exposed (min T = 18°C, max T = 39°C) showed

second-instar larvae after one and a half days (13). Blue bottle fly eggs typically hatch after 18–24 hours or more depending on temperature. In our experimental rearings at a fluctuating temperature (mean T = 28°C) reproduced in the growth cabinet, the hatching period (from oviposition until the emergence of the first instar larve) was 18 hours and most specimens in the samples reared reached the 2nd instar stage of development (6–11 mm in length with two slits in each posterior spiracle) after a further 18–24 hours. The PMI estimate for this case was 36–48 hours which was consistent with the last sighting of the deceased. With regards to burned remains, it has been observed in a study by Meek in Lousiana (unpublished data) that there is such a delay in colonization. Approximately a one-week delay in fly strike occurred on pig carrion contained inside an automobile that was set afire (4). Nevertheless in this case the insect evidence confirmed that the two victims had been murdered during the hours of darkness of the day before their remains were found. Blowflies do not normally lay eggs at night; in this case oviposition took place after sunrise of the day after the murder when the fire had gone out, the bodies cooled enough and the temperature inside the burnt car stabilized. The exposed internal organs, less affected by flames, begin to emit odors. Females of *Calliphora* readily detect the odor of flesh and begin to oviposite in protected irregularities of the corpse (inside the cranial cavity, inside the body cavities behind the exposed internal organs). The estimate of PMI was confirmed by the confession of a suspect who was a relative of the young woman. He had been devasted by her engagement to the male victim.

Discussion

All three cases deal with human remains discovered during the early postmortem period (from 36–48 hours in Case 3 up to 5–8 days in Case 1). The basis for the estimate of PMI in these three cases was that the most mature larva of the insect species collected is indicative of the longest period of association with the remains. In fact the age of the larvae found on a corpse can be very helpful for forensic pathologists in estimating the minimum time since death (3,4,7,14,15).

In Case 1 a reliable PMI determination was based primarily on data from the degree of decomposition (moderately advanced decay: head partially skeletonized and covered by feeding larvae with remains of the scalp and of the neck muscles, skin blackened with numerous maggot holes, skin blisters on surface of inferior limbs with marbling of veins, cutis peeling, etc.). Lividity, rigor and body temperature were no longer useful for a correct determination of time of death. The PMI estimate was based upon a second step in accordance with data from experimental laboratory rearings of Diptera larvae collected at the crime scene. A PMI of 5–8 days was established based on mature larvae of *Chrysomya albiceps* and *Sarcophaga carnaria.*

In charred bodies (Cases 2 and 3) arthropod specimens were the only tool (supported by climatological data collected from the weather recording stations) used in the determination of the time of death. Insects can provide correct estimates relative to the interval of time that a body has been exposed to arthropod activity. In charred bodies hypostasis, cadaveric rigidity and body temperature are elements not available to forensic pathologists. Gastric content can be helpful to determine how long before death the last meal was eaten for verification of circumstancial evidence but is not useful for PMI estimation.

In Case 2 entomological data for determination of PMI were obtained from experimental reconstruction of two blow fly species life-cycles in the growth cabinet used to recreate the meteorological conditions to which the remains were exposed and the time elapsed from when the victim was last seen until the discovery of the charred remains. A PMI of 3–4 days was estimated on different developmental stages of *Protophormia terranovae* (2nd instar) and *Sarcophaga haemorrhoidalis* (3rd instar). In Case 3 we were able to determine a PMI of 36–48 hours based on 2nd instar larvae of *Calliphora vicina* 6–11 mm in length collected at the crime scene. Laboratory rearings at a fluctuating temperature (mean T = 28°C) corresponding to that to which the remains were exposed produced similar sized larvae.

In the literature there are very few cases concerning burnt bodies (3,16), but our forensic experience involving charred remains shows that when a human body is not completely combusted or carbonized and decomposing exposed internal organs are present (brain, lung, liver, etc.), blowflies can be very skillful in seeking suitable oviposition sites. However, oviposition can occur only after the flames have been extinguished (especially for bodies burnt in cars) and when the decline in body temperature has reached favorable microclimatic conditions for larval development. Neither the colonization time and the rate of development of carrion insect fauna on burnt and charred bodies nor the effect of burning on arthropod succession patterns have been fully investigated previously.

Any medical examiner intending to use entomological methods on a regular basis should familiarize themselves with the blowflies and other carrion insects occurring in the local geographical area; however, forensic workers should establish a careful documentation and collection of entomological data as a standard routine in forensic investigation and then call upon the forensic entomologist for analysis and conclusions (3). The comparison of data concerning information from autopsy reports (decay rates of human remains, pattern of decomposition, dismembered or charred bodies), local environmental conditions (season, temperature, rainfall), development times for the immature stages of each local species (reproduced preferably in a growth cabinet) can be a reliable technique for the determination of an accurate PMI when interpreted by a qualified forensic entomologist.

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