

Entomotoxicology in burnt bodies: a case of maternal filicide-suicide by fire

V. Bugelli^{1,2} · L. Papi³ · S. Fornaro³ · F. Stefanelli³ · S. Chericoni³ · M. Giusiani³ · S. Vanin⁴ · C. P. Campobasso⁵

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Abstract One of the most common methods of maternal filicide is by fire. In this case study, a 40-year-old female and her children were found completely burned in a burnt out car. All bodies showed a degree of destruction by fire consisting to a level 3 of the Crow-Glassman Scale (CGS) and early stage of insect activity. Toxicological analyses were performed on soft tissues and body fluids still available. The results were positive for diazepam and its metabolites only for children with blood concentrations consistent with therapeutic doses of benzodiazepines. Home video surveillance cameras confirmed sedation prior to death recording the mother while administering some drops of sedative drugs in a soft drink to the children just a couple of hours before setting fire to the car. Based on autopsy findings, all victims were still alive at the time of fire. The cause of death was determined as carbon monoxide poisoning and fatal thermal injuries by fire. This case study has a special focus on the entomotoxicology and the potential role

of insects in death investigations of burnt bodies, supposed to be an inadequate substratum for insect colonization. It demonstrates that in burnt bodies, arthropod colonization can be quite immediate after fire is extinguished. Toxicological analyses performed on larvae actively feeding on the children's bodies were positive for diazepam and its metabolites in small amount compared with blood concentrations, whereas the larvae collected from the mother's body were totally negative. These data, according to the autopsy findings and the toxicological results from the victim's blood and tissues, supported the suspect of a non-lethal sedation prior to death, which is a common behaviour in maternal filicide.

Keywords Entomotoxicology · Filicide-suicide · Burnt bodies · Forensic entomology · *Lucilia sericata* · Benzodiazepines

Highlights • One of the most common methods of maternal filicide is by fire, and sometimes victims are sedated prior to death.

- In burnt bodies, Diptera colonization can occur quite immediate, soon after fire is extinguished.
- Entomological specimens may serve as reliable alternate specimens for toxicological analysis supporting death investigation.

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✉ C. P. Campobasso
CarloPietro.Campobasso@uminacampania.it;
carlocampo67@gmail.com

¹ Department of Medicine & Health Sciences, University of Molise, via De Sanctis, 86100 Campobasso, Italy

² Department of Medicine and Health Sciences, Forensic Science Section, University of Florence, largo Brambilla 3, 50100 Florence, Italy

³ Department of Forensic Pathology, University of Pisa, via Roma 55, 56126 Pisa, Italy

⁴ Department of Biological Sciences, School of Applied Sciences, University of Huddersfield, Queensgate, Huddersfield HD1 3DH, UK

⁵ Department of Experimental Medicine, University "L. Vanvitelli" of Campania, via Santa Maria di Costantinopoli, snc., 80138 Naples, Italy

Introduction

Filicide is the murder of a child over the age of 12 months by a parent [1]. It is a quite rare event, which differs from neonaticide (killing of a child on the day of birth) and infanticide (killing of a child under the age of 12 months where only the mother can be the killer). The official filicide rate ranges from 2.4 to 7.0 per 100,000 inhabitants [1, 2]. Most of the literature suggests that filicide is predominantly a female-perpetrated crime [3].

Filicide-suicide is the second most common form of murder-suicide after intimate partner murder-suicide [4]. Usually, mothers who kill their children do that based on causal relationship between pregnancy, childbirth and subsequent maternal illness such as postpartum disorders [5, 6].

Mothers who kill their children aged more than a year old use greater levels of aggression in their offences, e.g. shaking, throwing to the ground, hitting, strangling, stabbing, but also poisoning and suffocating [7–9]. Maternal filicide by fire is one of the most common methods of filicide [10] with a wide range in literature between 3 and 37% depending on studies [11, 12]. Mothers who commit filicide sometimes try to avoid any further pain to their victims caused by fire. In this respect, they want to be sure that children are asleep or sedated before setting the fire [12]. This is what happened in the present case study where a mother killed her two children by fire after administering them some sedative drugs. The study has also a special focus on the potential role of insects in death investigations of burnt bodies. In fact, charred remains are really a challenge for pathologists and investigators to reconstruct peri- and post-mortem events as well as to collect evidence for personal identification of the victims [13]. In this kind of bodies, arthropod specimens may be the only tool useful in the estimation of time since the fire was extinguished which is often strictly related to the minimum post-mortem interval (PMI_{min}) and cause of death [14, 15]. In fact, Dipteran larvae feeding on intoxicated human tissues introduce into their own metabolism drugs and toxins taken by the person when still alive. Therefore, insects may serve as reliable alternate specimens for toxicological analysis where more traditional sources (such as blood, liver, urine) are not available [16]. We describe herewith the findings observed in the present interesting case study dealing with a maternal filicide-suicide and the information derived from the insects associated with burnt bodies concerning the pattern of colonization and drug intoxication.

Case report

During the summer month of July, in Pisa (Tuscany, Central Italy), fire brigades were alerted at 15:38 because of the presence of a large fire. Firefighters were soon on the scene and

extinguished the fire 20 min after the alert, finding a car completely charred. The car was parked in a clearing at the end of a secondary road in a hilling area in the Pisa outskirts. Inside the car, three burnt bodies were discovered. When forensic pathologists arrived on the death scene at 18:50, the fire was already extinguished after more than 2.5 h. All the burnt bodies were located on the back seats. The body of an adult was in a sitting position just in the middle with the bodies of two children at each lateral side (Fig. 1). The vehicle was soon identified as that one belonging to a lady, 40 years old and mother of two children: a girl, 10 years old and a boy, 3 years old. They had been seen alive all together for the last time at a gasoline station close to the crime scene at 15:08, just 30 min before fire brigades alert. Some video surveillance records helped to reconstruct the events just preceding the fire. An empty can of gasoline was found close to the car suggesting that an accelerant could be used and planning in the offence. Soon after the removal of the bodies from the car, lividity, rigour and body temperature were no longer useful for a correct estimation of the time since death. All the bodies showed the pugilistic attitude with the skullcap free from any soft tissue, but not calcinated. The head was present in all cases although identification was not evident because flesh was largely devoid due to soft tissues of the face having been burnt away or partially charred. The Crow-Glassman Scale (CGS) [17] was used for describing the extent of burns to remains. At the external examination, all bodies showed a degree of destruction by fire consisting to a level 3 of the CGS [17]. In fact, in all the burnt bodies, major portions of the arms and/or legs



Fig. 1 The remains of three burnt bodies found on the back seats of the burnt out car

were still articulated with only hands and/or feet missing. The body cavities became visible with the internal organs exposed and some early stage of insect activity was noted. In fact, some blackened tissues were already colonized by clusters of fly eggs and larvae not older than a couple of hours after the firefighters' intervention. The ambient temperature recorded on the scene was 25 °C. The bodies were transferred in the local morgue and stored at 4 °C late at night (approximately at 23:00). Before autopsy, a CT scan was performed for each body. The autopsies were performed about 42 h after the discovery of the bodies and toxicological analysis took place to determine the vitality of the victims at the time of the fire based on CO levels in haemoglobin (COHb). Although the degree of destruction of all the bodies was consistent with level 3 of the CGS, several soft tissues (liver and kidneys) and some body fluids like blood and urine were still available for toxicological analysis. The burnt bodies of the two children are illustrated in Fig. 2, available as electronic supplementary material. Samples of the liver and kidney were collected from all bodies as well as samples of blood and urine. Relevant autopsy findings common to all the bodies were also represented by the soot particles in the airways as a sign of vitality and active inhalation at the time when the fire broke out, providing evidence that all the individuals were still alive when the fire began. Based on autopsy findings and toxicological results, the cause of death for all bodies was determined as carbon monoxide poisoning and fatal thermal injuries by fire. Personal identification was later made using dental comparison of ante-mortem and post-mortem teeth features. In fact, although most of the anterior teeth had burst apart, some posterior teeth were still present and partially damaged by the fire. Furthermore, DNA analysis showed no incongruities between the missing individuals and the DNA collected from burnt bodies confirming the previous suspect. Autopsy findings and toxicological results for each body are the following:

Adult body no. 1

The mother's body was found in the posterior seat, between the two children. The female external genitals were still preserved and sex determination was easy. The head was still articulated and extended as the result of the contraction of massive muscle mass at the back of the neck. Portions of the chest wall were burnt away exposing the viscera. The arms and legs were skeletonized with the extremities fractured by the heat thus resembling the posture of a boxer. The right upper arm was partially flexed just below the head of the little boy as in the position of holding him tightly. The abdomen wall was completely destroyed with exposure of the intestine. Abdominal organs appeared colonized by maggots actively feeding on them. Larvae were no longer than approximately 1.5 cm. In fact, although the remains were stored in a

refrigerator at 4 °C for 24 h approximately, the larvae in the abdomen developed some heat due to their frenetic feeding activity and a remarkable internal thermal elevation by more than 18 °C above the surroundings was recorded with a larval mass temperature of 22 °C approximately. The COHb analysis performed on blood samples by a spectrophotometric method revealed high levels of carbon monoxide (up to 55.13%) consistent with inhalation in life (as suggested by soot lining the airways). Screening test and toxicological analysis of all other tissue samples (kidney, liver and urine) were totally negative.

Daughter's body no. 2

The 10-year-old girl was found in the posterior seat, seated on the left side of the mother. The external genitals were still well preserved. The vault of the skull was completely free of soft tissues. Portions of the brain were exposed and of creamy consistence because of an early-stage larvae colonization (5–8-mm long). The internal thermal elevation was pretty small as the larval mass temperature recorded in the brain was only 7 °C. No other anatomical regions or internal organs were colonized by insects, although the chest and abdomen walls were burnt away exposing muscles and portions of internal organs. During autopsy, the stomach analysis revealed the presence of a semi-fluid substance containing corns and tomato fragments. The analysis of COHb revealed very high levels of carbon monoxide up to 95.7%. The COHb saturation level recorded in the blood reflected the amount of CO inhaled in life. The qualitative toxicological analysis of other tissues (kidney, liver and urine) was positive for diazepam and its main metabolites such as nordiazepam and oxazepam. The blood concentration of diazepam was 169.1 ng/ml just a little bit below the therapeutic doses of benzodiazepines usually in the range between 200 and 600 ng/ml, according to main references in specific literature [18–21].

Son's body no. 3

The 3-year-old boy was found in the posterior seat, seated on the right side of the mother's body. The external genitals were shrunken and blackened but still recognizable. The scalp was burnt with brain tissues exposed. Portions of the chest and abdominal walls were mainly burnt away exposing the viscera. However, the internal organs were less affected by flames and colonized by a large larval infestation. Larval masses were observed in the chest and in the abdomen. Although maggots in the body cavities, collected during the autopsy, were no longer than approximately 1.5 cm, a remarkable heat generated from the larval aggregation was recorded. A thermal elevation by more than 15 °C above the surroundings was recorded with a larval mass temperature of 20 °C approximately in the thorax and 16 °C in the abdomen. The COHb analysis showed an elevated blood

carboxyhemoglobin saturation (78.9%). The toxicological analysis of other specimens sampled from the kidney, liver and urine was positive for diazepam and its main active metabolites such as nordiazepam and oxazepam. The blood concentration of diazepam was 589.7 ng/ml, consistent with therapeutic doses of benzodiazepines, according to previous studies [18–21].

Entomotoxicological study

Larvae associated to the remains of each body were collected mainly for the identification of the species and age estimation. Some of them were also used as alternative substrates for toxicological analysis and stored in a freezer at $-20\text{ }^{\circ}\text{C}$ in order to compare the results with those obtained from the human tissues. In fact, in pre-skeletonized bodies or bodies in advanced decay where no traditional sources for toxicological analyses are available, insect specimens feeding on human remains can be used as alternative substrates as they introduce into their own metabolism drugs and toxins taken by the victim when still alive [16].

Species identification

Immature specimens collected at the death scene and at the autopsy were reared to the adult stage under controlled and monitored conditions of temperature and humidity according to best practice guidelines [22]. Specific morphological identification keys were therefore used for identification of both immature and corresponding adult samples. The eggs and larvae collected at the death scene were therefore identified as Diptera eggs of *Lucilia sericata* (Meigen) (Diptera: Calliphoridae) and larvae of first instar of *Sarcophaga crassipalpis* (Macquart) (Diptera: Sarcophagidae) (Table 1). The entomological samples collected at the autopsy also confirmed that only two main Diptera species colonized the burnt remains. The most mature specimens were represented by larvae third instar of *L. sericata* (Meigen) (Diptera: Calliphoridae) and larvae third instar of *S. crassipalpis* (Macquart) (Diptera: Sarcophagidae) for larvae feeding on

the remains of the mother and little boy. Only larvae collected from the daughter's body were still in a very early stage of development corresponding to first and second instar larvae of *L. sericata*. The stage of development of immature specimens was made according to the main morphological features observed at the posterior spiracles and cephalo-pharyngeal skeletons, and the corresponding identification keys for such species. The advanced stage of larval development observed at autopsy of the mother and the little boy was not consistent with the ambient temperatures to which maggots were really exposed equating 408 accumulated degree hours (ADH) approximately. In particular, the thermal history for this case study can be summarized as follows: $25\text{ }^{\circ}\text{C}$ at the death scene for 8 h approximately equating 200 ADH, $4\text{ }^{\circ}\text{C}$ in the refrigeration units for 34 h approximately equating 136 ADH and $18\text{ }^{\circ}\text{C}$ at the autopsy for 4 h approximately equating 72 ADH. The discrepancy between larval stage of specimens at autopsy and ADH recorded can be explained as the effect of the remarkable heat produced by maggot masses once in coolers as the refrigeration did not stop the feeding activity of maggots clustered on the remains. In fact, *L. sericata* larvae need from a minimum of 1.050 ADH at $30\text{ }^{\circ}\text{C}$ (35 h) to a maximum of 1.225 ADH at $25\text{ }^{\circ}\text{C}$ (49 h) to reach the third instar, based on relevant developmental data by Grassberger and Reiter [23]. Sarcophagid larvae need from a minimum of 720 ADH at $30\text{ }^{\circ}\text{C}$ (24 h) to a maximum of 1.200 ADH at $25\text{ }^{\circ}\text{C}$ (48 h) to reach the third instar, based on developmental data published by Nishida [24].

Toxicological analysis

The material and methods used for the toxicological analysis were the following. Frozen larvae of *L. sericata* (about 200 mg) were repeatedly washed with distilled water to remove any external contamination and then homogenized by Precellys® (an homogenizer produced by Bertin Technologies) placing the sample in a tube with ceramic beads, 2 ml of phosphate buffer and deuterated internal standard (flunitrazepam-d7, 200 ng/g). A rapid shaking of vials causes the lysis of the larvae. Then, 2 ml of hexane was added to the solution and after mixing and centrifugation, the

Table 1 Larval stages of insects collected on the bodies at the death scene and at the autopsy (36 h later approximately) and larval length in millimetres (mm)

| Taxon | Mother | | Daughter | | Son | |
|---|-------------|----------------|-------------|-------------|-------------|----------------|
| | Death scene | Autopsy | Death scene | Autopsy | Death scene | Autopsy |
| <i>Lucilia sericata</i> (Meigen) (Diptera: Calliphoridae) | E | LII, 5–8 mm | E | LI, 2–4 mm | E | LII, 5–8 mm |
| | | LIII, 9–15 mm | | LII, 5–8 mm | | LIII, 9–15 mm |
| <i>Sarcophaga crassipalpis</i> (Macquart) (Diptera: Sarcophagidae) | LI, 2–4 mm | LII, 5–9 mm | | | LI, 2–4 mm | LII, 5–9 mm |
| | | LIII, 10–14 mm | | | | LIII, 10–14 mm |

E eggs, LI larvae first instar, LII larvae second instar, LIII larvae third instar

supernatant was discarded and 0.5 ml of saturated solution of Na_2CO_3 was added. Subsequently, a liquid-liquid extraction with 3 ml of diethyl ether was performed; the organic phase was collected in a clean tube and evaporated under a gentle stream of nitrogen at room temperature. The residue was re-suspended with 0.5 ml of hexane saturated with acetonitrile followed by addition of 50 μl of acetonitrile itself. After mixing and centrifugation, the acetonitrile was collected in a clean tube and added with 50 μl of BSTFA for derivatization (70 °C for 30 min). The analyses were performed by GC/MSMS. The method was validated using both blood and larvae spiked with diazepam, oxazepam and nordiazepam, evaluating linearity, precision (expressed as percent variation coefficient, CV%), accuracy (expressed as bias %) of limit of detection (LOD) and lower limit of quantification (LLOQ).

Entomotoxicological results

The quantitative results detected in each body fluid and tissue sample are reported in Table 2. As observed already from toxicological analysis of soft tissues and body fluids, also the larvae collected from the mother's body resulted totally negative to drug screening. On the other side, larval samples collected from the children were positive to benzodiazepines and, in particular, to diazepam and its main active metabolites (oxazepam and nordiazepam). In particular, the first and second instar larvae of *L. sericata* from the daughter's body were positive for diazepam, nordiazepam and oxazepam at concentrations of 9.1, 4.4 and 7.8 ng/g, respectively (Table 2). The benzodiazepines observed in the second and third instar larvae of *L. sericata* collected from the son's body were much higher, with concentrations of 71.5, 43.1 and 46.3 ng/g, respectively, for diazepam, nordiazepam and oxazepam. The mass chromatogram of tissues collected from the daughter's body is depicted on Fig. 3, available as electronic supplementary material.

Discussion

In the present case study, the contextual reconstruction (including the final position of the human remains, the autopsy

and the toxicological findings) strongly suggested that the manner of death was a maternal double filicide-suicide. Video surveillance records also confirmed the reconstruction of events prior to death. The soot lining the airways and high COHb levels in all burnt bodies revealed that all victims were alive at the time of fire. The cause of death for all of them was therefore determined as carbon monoxide poisoning along with thermal injuries by fire. However, the toxicological analysis performed on tissues still preserved by heat and fire showed positive results for benzodiazepines only for the remains of the children consistent with therapeutic doses usually in the range between 200 and 600 ng/ml, according to previous studies [18–21]. In fact, the quantitative analysis of tissues showed enough evidence that the children were sedated prior to death. Home video surveillance camera recorded events preceding the death and occurred just 2 h (at 13:30 approximately) before the fire alert occurred at 15:38. Mother with sons and former husband had lunch together consistent with corns and tomato fragments found in the bodies of the mother and daughter. Soon after lunch, the video camera recorded the mother while administering some drops of sedative drugs (diazepam) in a soft drink to their child in order to be sure that the children were asleep or sedated before setting the fire to the car.

This is consistent with the behavioural analysis of maternal filicide made by Meyer et al. [12] who reported that mothers who commit filicide by fire may be more likely to use passive approaches in their offence (e.g. administering some sedative drugs) as this makes it easier for them to distance themselves from the crime and the consequences. Furthermore, usually, they want to be sure that their sons be sedated before setting the fire so they do not hear them cry [12]. In this regard, benzodiazepines have consistent sedative effects as many other central nervous system-depressant drugs. Therapeutic doses of benzodiazepines produce sedation which typically impairs most aspects of performance in a dose-dependent manner [25]. In the present case study, although of the degree of destruction of the remains (classified as level 3 of the CGS), several internal tissues such as the liver and kidneys and also body fluids (blood, urine) were still available for toxicological analysis. However, in burnt or charred bodies as well as in

Table 2 Concentrations of drugs in victims' tissues and insects

| | Diazepam (ng/ml) | | Nordiazepam (ng/ml) | | Oxazepam (ng/ml) | |
|------------------|------------------|-------|---------------------|-------|------------------|-------|
| | Daughter | Son | Daughter | Son | Daughter | Son |
| Whole blood | 169.1 | 589.7 | 65.5 | 242.1 | 12.2 | 211.9 |
| Hydrolyzed urine | 389.5 | 323.9 | 59.2 | 69.3 | 113.7 | 96.4 |
| Larvae | 9.1 | 71.5 | 4.4 | 43.1 | 7.8 | 46.3 |
| Kidney | 38.9 | 78.4 | 21.9 | 30.1 | 13.5 | 12.8 |
| Liver | 74.4 | 218.6 | 23.7 | 36.1 | 18.5 | 11.2 |

Values are expressed in ng/ml or ng/mg

bodies in advanced decomposition, the traditional substrates, such as blood, urine or internal organs are not always available. In these cases, if bodies are colonized by microfauna, insects may serve as reliable alternate specimens for toxicological analyses [16, 26].

The most controversial topic in entomotoxicology is still the correlation of results between human and insect tissues [26, 27]. In this respect, toxicological analyses of larvae actively feeding on remains were performed. The results show concentrations of benzodiazepines in maggots significantly lower than those observed in blood samples for both children accordingly with previous comparative studies of drug analysis in insects and human tissues [27, 28]. The drug concentrations were in pretty small amount compared with blood concentrations of benzodiazepines. This decrease can be explained by diverse distribution of drug in different human soft tissues as well as by the metabolization and possible bioaccumulation of benzodiazepines by maggots rather than excretion during different stages of development [27]. Only the nordiazepam and oxazepam concentrations in the maggots collected from the remains of the little boy were slightly higher than those in the kidney and liver samples. This contrasting result could be related to the different stages of development of maggots analysed (pretty young as first and second instars) or to a different feeding substrate according to insect preference or site-by-site variability of drug concentration especially in the skeletal muscle [29]. Additional explanations of such different toxicological patterns could be found in the lack of knowledge in pharmacokinetic of drugs in insects as well as in minors [26, 27].

It is quite clear that no better quantitative extrapolations can be made actually from entomological substrates in order to discriminate between lethal and non-lethal concentrations of benzodiazepines. However, it is worth mentioning the fact that previous studies on benzodiazepines and its metabolites (such as nordiazepam and oxazepam) were performed on *Calliphora vicina* species, from larval stage to pupal stage and adult [30–32], showing a predictable pattern of drug distribution during the developmental stages. The present results on *L. sericata* confirm the potential value of entomotoxicology in death investigations not only to determine the cause of death but also to confirm events and circumstances before death as the sedation of victims prior to death. Unfortunately, the entomotoxicological results alone cannot be considered evidence enough to discriminate between lethal and non-lethal concentrations of drugs. Autopsy findings (such as the deposition of soot particles in the airways observed in this case study) and toxicological results from the victim's blood or tissues, if available, are still more reliable tools compared with insects. According to Gosselin et al. [26], further research is still needed in this entomotoxicology focusing, in particular, on physiological process during feeding and post-feeding stage, drug metabolism and accumulation-excretion mechanisms, drug distribution in different human

tissues used as feeding substrate by insects and post-mortem drug stability in insects and tissues, and other factors affecting toxicological analysis. However, entomological specimens, even if only as qualitative specimens, can be considered as reliable samples for detection of drugs, supporting death investigation in the reconstruction of manner and cause of death.

This case is worthy of illustration because most of the circumstances related to this maternal filicide-suicide have been recorded and documented by video surveillance cameras, giving a precise reference frame of the time of death and the time of drug administration. From a forensic entomology point of view, this is quite unusual and it has permitted to verify some basic assumptions reported in literature related to burnt bodies and insect colonization as well as on entomotoxicology. The lady and two children were seen alive for the last time at 15:08, just 30 min before fire brigades alert, at a gasoline station pretty close to the death scene. An additional video surveillance camera belonging to the gas station also recorded this circumstantial data.

As already mentioned, the use of fire is a quite common method of filicide [3]. Interestingly, failed relationship and the use of fire in filicide are frequently mentioned as relevant risk factors in studies describing filicide-suicide suggesting that individuals who commit filicide by fire may also make a suicide attempt at the same time. Friedman et al. [33] found that in cases of filicide-suicide, the perpetrators used the same method to kill themselves and their children, which may offer some explanation to why filicide by fire and suicide have been linked as both the victim and the perpetrator can die within temporal proximity.

For years, the burnt bodies are supposed to be an inadequate substratum for insect colonization due to the poor protein content coagulated by heat and to dehydration of burnt and charred tissues. In the beginning of the century, Wardle [34] remarked that freshly cooked meat, however moist, is not attractive to blowflies. Catts and Goff [35] suggested first that oviposition could be prevented in burned bodies depending on the level of burning or incineration. In this respect, Glassman and Crow [17] developed the CGS (from levels 1 to 5) mainly to standardize the description of the remains for reporting purposes, focusing on the extent of burns to the remains of fire victims based on increased exposure to fire temperature and duration. In the present case study, all bodies showed a degree of destruction consistent with level 3 of the CGS. That means the bodies were exposed to >800 °C for 30 min approximately according to the time schedule of the fire brigades (15:38 time alert and 16:00 fire extinguished) and findings described by Bohnert et al. [36]. In fact, based on this previous study [36], after 20 min, all bodies showed the calvaria free from any soft tissue and fissures of the tabula externa, the destruction of prominent parts of the face, the body cavities mostly visible with internal organs exposed. Only after 40–50 min, the internal organs can be severely shrunken showing a net-like or sponge-like structure less attractive by blowflies.

Avila and Goff [37], using pig carcasses, found that burned carcasses at CGS level 2 were more attractive for calliphorids

presumably due to the cracked skin and easy access to flies of the body cavities. They also observed on the burnt flesh of animal carcasses significant oviposition by flies of Calliphoridae family 1 day earlier than on the unburnt carcass. An early colonization of burnt remains was confirmed by Italian forensic pathologists [14] in three case studies dealing with burnt bodies in burnt out cars showing that Diptera colonization can occur frequently and quite immediate. Introna et al. [14] observed that adult calliphorids are attracted in large numbers by the smell of the inner viscera, widely exposed by heat consumption of the thorax and abdomen walls quite soon after the fire is extinguished and the heat slow down to that attractive for blowflies. Depending on the extent of burn injury, Diptera can have easy access to the body's inner cavities and are able to deposit eggs directly on the surface of internal organs or tissues exposed or between the uneven internal recesses still hot.

This can help to establish the maggot mass sooner and sometimes prior to the remains being placed in the coolers, as in the present case study, where a remarkable heat generated by larval aggregation produced an accelerated stage of larval development at the autopsy. The potential immediate colonization of the remains by Diptera is confirmed in the present case where the bodies were examined on site after 2.5 h approximately the fire was extinguished. At the first examination of the remains, soon after removal of the bodies from the back seat, groups of eggs of *L. sericata* and early stage of *S. crassipalpis* larvae were already present.

Anderson [38, 39] also demonstrated the more rapid colonization of the burned flesh on a remarkable animal experiment using pigs as arthropods are strongly attracted to fire-modified tissues where fluids and odours are released from the remains. However, the fire can not only speed up the colonization rates but also changes the decomposition patterns [40]. In fact, understanding the effect of burning on the pattern and rate of decomposition can be crucial in PMI estimation [40]. An experimental study on animal models [40] noted differences in the regional rate and pattern of decomposition in a charred group of pig carcasses when compared to those of uncharred pigs mainly related to the level of charring in each region. An additional study on burned pig carcasses [15] showed that at CGS 2–3 insects from different waves arrive on the same time on the cadavers and that the “classic” insect succession model cannot be applied as it differs from unburnt carcasses. Different conclusions have been recently illustrated by Mahat et al. [41] but on smaller animal carcasses (rabbits) compared with those used by Vanin et al. [15] and Avila and Goff [37]. In fact, these authors have observed that the colonization of burned rabbit carcasses in CGS 1–2 is similar to that of the control (un-charred), while in CGS 3 carcasses, there is a 1-day delay. Unfortunately, rabbits or rodents cannot provide reliable animal models to be compared with humans due to the many differences in terms of size and biochemical and physiological processes.

The maternal filicide-suicide case study demonstrates that in burnt bodies with degree of destruction at CGS 3, arthropod colonization can be really fast and quite immediate. When forensic pathologists arrived at the crime scene, the colonization was already started and it stopped only because it was getting dark and nocturnal oviposition by blowflies in Central Europe under natural conditions is not common [42]. In this scenario, colonization took part in 2.5 h after firefighters' intervention. Based on these events, human remains heavily colonized by Dipteran larvae have to be considered as sort of “emergency” for forensic pathologists as well-established maggot masses do not stop the feeding activity once the body has been placed in the coolers [43]. The autopsy should be performed as soon as possible because Diptera larvae could easily destroy the residual part of physical evidence useful for personal identification or the determination of cause of death.

Conclusion Burnt bodies are a real challenge for forensic pathologists and entomologists. No classical post-mortem changes can be used for PMI estimation as lividity, rigor or body cooling are no more detectable. Depending on the extent of burn injury or degree of destruction of human remains, adult Calliphoridae can colonize the remains quite early and soon after the fire extinguished. Forensic practitioners need to realize that (a) burnt bodies seem to offer an extraordinary habitat for fly development and (b) insects may serve as reliable alternate specimens for toxicological analyses as in burnt or charred bodies more traditional sources, such as blood, urine or internal organs cannot be available [43]. This can be of great help in death investigations not only to determine the cause of death but also to confirm events and circumstances before death as the sedation of victims prior to death. Unfortunately, quantitative correlation of drugs in humans and in insects is still controversial without further circumstantial evidence of the events prior to death, caution is requested in the interpretation of toxicological results. Autopsy findings and toxicological results from the victim's blood or tissues, if available, are still more reliable tools compared with insects in death investigation.

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