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Review on forensic importance of myiasis: Focus on medicolegal issues on post-mortem interval estimation and neglect evaluation

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ABSTRACT

Myiasis is the infestation of live vertebrates by dipterous larvae that feed on living or necrotic tissues, liquid body substances or ingested food and develop in or on the vertebrate body. In both animals and humans, myiasis plays a fundamental role in forensic practice because of its implications in the evaluation of cases of neglect and in the mPMI (minimum *post-mortem* interval) estimation. The present study aims to provide a review of forensic issues related to myiasis. A retrospective analysis was conducted by exploring major electronic literature databases. Methodological evaluation of each study was performed according to the PRISMA (Preferred Reporting Items for Systematic Review and Meta-Analyses) standards. Sixty-one papers were included in this review. Entomology shows great potentiality in forensic investigations, mostly because of its contributory role in mPMI estimation. Such evaluation, however, requires a careful consideration of the possibility of an *ante-mortem* myiasis infestation, which could complicate the correct assessment of the mPMI. Hence, forensic pathologists should pay attention to the entomological fauna on a corpse, in both fresh and decomposed bodies. Moreover, in cases with poor nutritional or hygienic conditions, myiasis should be carefully evaluated as an indicator of neglect.

1. Introduction

Myiasis refers to the infestation of vertebrates by dipterous larvae that feed on living or necrotic tissues, liquid body substances or ingested food and develop in or on the vertebrate body [1–2]. This phenomenon is mainly caused by four families - Calliphoridae, Sarcophagidae, Oestridae, and Phoridae - but rarely some species of the families Muscidae and Psychodidae can be involved as well [3–4].

Depending on the biologic characteristics of colonizing flies, myiasis is traditionally classified into primary or secondary [5–6]. Fly species responsible for primary myiasis are obligate parasites, so they cannot survive without the living host; on the contrary, secondary myiasis is caused by facultative species that usually develop on decaying organic material but can take advantage of living hosts (especially in case of preexisting wounds) to complete their life cycle [7–12]. Although spread worldwide, myiasis is subject to seasonal variability related to the latitude and the life cycle of certain species of flies, being limited to summer period in temperate zones and present throughout the year in warm climate zones such as tropics, south-east Asia, and subtropics of Africa [4].

Colonization of living people by fly maggots represents a relevant problem of public health [13]. Specifically, according to the anatomical location of the infestation, myiasis are classified by the WHO ICD-10 as cutaneous, nasopharyngeal, ocular, aural, intestinal, and urogenital [14]. Considering the alarming consequences of global warming in terms of invasion of new fly species in Western Europe, the risk of unwanted human infestation by fly larvae is predictably expected to increase in proportion [13–15]. On the other hand, it must be retrieved that fly maggot colonization represents a useful tool in some clinical controlled settings. Medical implications of human myiasis, in fact, are largely known with reference to the so called “maggot therapy”, also known as maggot debridement therapy or larval therapy. This practice consists in the placement of sterile fly larvae sorted in a biobag, on a nonhealing wound for a defined period, in order to obtain the removal of

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necrotic tissue and wound disinfection, with a final effect comparable to microsurgery [13]. While partly reconsidered with the discovery of antibiotics, the role of maggot therapy has recently gained attention in some countries as an alternative to surgical treatment in specific cases [16].

Although less known, the study of human myiasis also plays a crucial role in forensic investigations. The main field of interest is represented by forensic entomology, which is the study of insects associated with human remains to obtain fundamental information about time and circumstances related to the crime [17]. One of the most challenging issues fly larvae allow to explore is the estimation of the so-called time of colonization, commonly considered equivalent to the minimum *post-mortem* interval (mPMI), in cases of advanced stage body decomposition which impedes the forensic pathologist from using the standard approach to establish time since death [4,17–20].

However, the possibility of an *ante-mortem* myiasis infestation could further complicate the correct assessment of the *minimum post-mortem* interval. In fact, mPMI in forensic entomology is assessed by calculating the age of the oldest larval instar collected from the corpse or its surroundings [4,21–22]. In a case of myiasis, female flies can lay eggs on living individuals in association with poor personal hygiene and evidence of necrotic tissues (e.g., gangrene, pressure ulcers, open wounds). In this scenario, the window of time between insect colonization of the corpse and its discovery clearly does not correspond to the time of death, being the mPMI significantly anticipated [22–25]. Thus, these circumstances may result in relevant and critical errors in the estimation of mPMI with serious consequences in the investigation process.

Necrophagous fly maggots can also behave as facultative parasites, thus associated with cases of extreme human and animal neglect. Veterinary cases include forms of cruelty or neglect against domestic animals or livestock, being owners and farmers eventually prosecuted [21,23]. Instead, human neglect is a phenomenon most observed among non-self-sufficient people (abandoned children and elders) as well as debilitated or homeless people who are unable or unwilling to maintain acceptable levels of hygiene especially in presence of open wounds or bed sores [16,21,24]. Alcoholism, drugs, and peripheral vascular disease are considered risk factors for neglect [21,24–25]. As all insects develop following a predictable life cycle strictly related to species and temperature, in both humans and animals the age of the maggots can be estimated considering the exposure temperature during their development [4,16]. And ultimately, larval instar enables the forensic entomologist to determine when flies have colonized the body after death and the duration of neglect as well [1–2,21,24].

Starting from these assumptions, so far very few papers have been published on the consequences of myasis in neglect but mainly discussing mPMI estimation. The aim of this review is to illustrate the forensic implications of myiasis by focusing on those aspects in which the study of body infestation by fly larvae can play a decisive role in estimation of mPMI and minimum time since neglect.

2. Materials and methods

2.1. Eligibility criteria

The present systematic review was carried out according to the Preferred Reporting Items for Systematic Review and Meta-Analyses (PRISMA) standards [26–27].

2.2. Search criteria and critical appraisal

Both a systematic literature examination and a critical appraisal of the collected studies were conducted. PubMed, Science Direct Scopus, and Excerpta Medica Database (EMBASE) were used to perform an analysis, from the inception of these databases to August 2022.

The search terms were “myiasis”, “forensic”, and “neglect” in the title, abstract, and keywords. Bibliographies of all identified papers were reviewed and compared to identify additional relevant literature. Methodological evaluation of each study was conducted according to PRISMA standards, including assessment of bias. All researchers independently reviewed papers whose title or abstract appeared relevant and selected those that analyzed mPMI with “myiasis”. The study design included original articles, reviews, case reports, and case series. Disagreements on eligibility among researchers were resolved by a consensus process. No unpublished or gray literature was considered. One researcher (LF) performed the data extraction, and another researcher (VB) verified it.

2.3. Risk of bias

Highlights of this systematic review include number and breadth of the collected studies, which span the globe; the hand search and scan of reference lists for the identification of all relevant studies; and a flowchart that describe in detail the study selection process. Despite our efforts to fairly evaluate the existing literature, this review includes studies that were published in a time frame of 30 years; thus, these results should be interpreted considering that the accuracy of the scientific procedures may have changed over the years.

3. Results

An appraisal based on titles and abstracts, as well as a hand search of reference lists were conducted. The bibliography of all identified articles was reviewed to detect still hidden literature. A total of 106 studies relevant to present review were identified; of these, 9 were excluded due to unavailability and 4 because not in English.

After the screening phase, 45 publications were considered eligible for full-text assessment. The analysis of the cited references during the full-text reading of the selected articles allowed the inclusion of further 4 studies, for a total number of 49 articles. There were 23 original articles, 13 case reports, 10 reviews, 1 comparative study, 1 letter to the editor, and 1 technical note. No extensive reviews focused on forensic evaluation of myiasis, and subsequent implications were found. Fig. 1 (Fig. 1) summarizes the PRISMA chart.

The analysis of literature revealed that several authors described, mainly through original research, the most common activity of insects that produce myiasis and their implications. Table 1 lists all articles included in the review.

4. Discussion

The potential presence of insects in *ante-mortem* wounds should encourage the collaboration between pathologists and entomologists in the evaluation of each case study with implications in forensics. Indeed, forensic entomology is the branch of forensic science related with the arthropods and the law [28]. Medicolegal entomology focuses on the criminal component of the legal system and deals with necrophagous (or carrion-feeding) insects that typically colonize human and animal remains [29]. All decaying organic materials, including corpses, are an ideal habitat for the offspring of several arthropods. They use it as nourishment, breeding site, mating or hiding place. As the soft tissues decompose, they undergo a series of changes offering to different species with exactly the substrate they need [30]. Numerous succession studies were carried out [31–37] to understand the sequence in which species are attracted to the different stages of decomposition and to estimate the mPMI by successive changes over time [38]. Information provided by succession studies has disclosed the potential use of insect community sequence in forensic cases. However, every succession study is highly dependent on the habitat, the microclimate, and can be affected by several biotic and abiotic factors. Nevertheless, it is important to know the local fauna in order to evaluate the entomological findings in each single case [39].

Post-mortem interval estimation and neglect are burning issues in

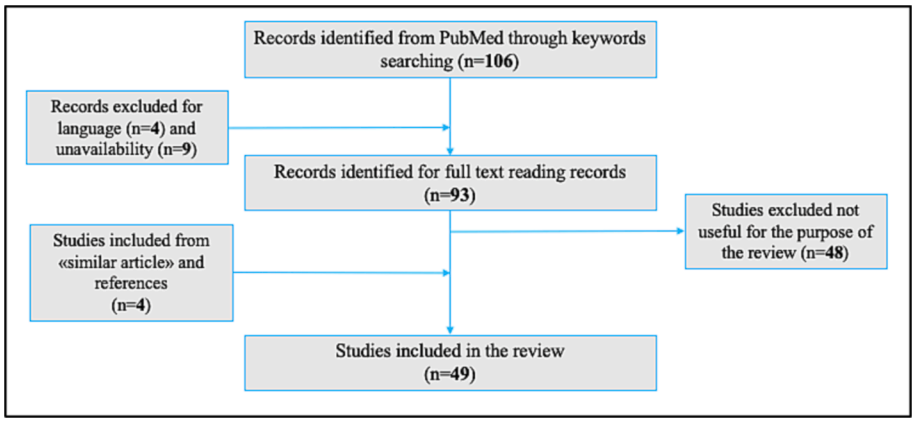

Fig. 1. Prisma chart for this review.

forensic sciences, especially in forensic pathology. In this context, human infestation by myiasis-causing larvae is particularly interesting and worthy of consideration, since it could negatively interfere with the mPMI estimation, which is strictly based on the evaluation by forensic entomologists of the age of immature insects collected on a death scene [20,22]. Infestation of myiasis might also raise issues about neglect, which is typically documented among very elderly or debilitated people, who are unable to provide for their basic needs such as personal hygiene, medications of wounds and treatment of ulcers [40]. Human myiasis is often encountered in situations of low economic and educational development [3]; thus, it has been defined as a neglected zoonosis attacking the poorest people and mutilating them in a serious or even permanent way [41,42]. However, myiasis is not limited to tropical and subtropical regions, since there are a few forensic papers – mostly case reports – describing conditions of serious neglect among fragile people in Western countries [4,40,43–45].

In the context of caregiving, neglect is a form of abuse where the perpetrator fails to take care for someone who is unable to self-caring. It can be a result of carelessness, indifference, or unwillingness and abuse. Neglect may include the failure to provide sufficient supervision, nourishment, or medical care, or the failure to fulfill other needs for which the victim cannot provide themselves. Accurate prevalence and incidence rates of neglect are difficult to obtain; yet, in 2015 the WHO estimated prevalence rates of elder abuse in community settings in high- or middle-income countries ranging from 2.2% to 14%, with estimates for neglect from 0.2% to 5.5% [46]. Every year, about 4–16% of children are physically abused and one in ten is neglected or psychologically abused [47]. To the authors' knowledge, forensic and medical literature does not have much concern about neglect, as the issue is limited to a very few publications.

Autopsy and medical findings indicative of neglect include malnutrition, dehydration, poor hygiene (urine/fecal staining, bedding, clothing), infected decubitus ulcers, necrotizing fasciitis/gangrene and wound infested by maggots (myiasis) [15,48]. Therefore, the development of parasitic infestation is conditioned by several predisposing factors such as poorly treated open wounds, poor social conditions, poor hygiene, psychiatric illness, alcoholism, diabetes, and severe handicap status [20,48]. Cases of myiasis have been also reported inside health care facilities, especially for patients in reanimation services using drains and tracheal cannulas, which are not adequately changed daily [40]. Myiasis acquired within nosocomial facilities is not uncommon [12], but it is probably underreported in many regions (e.g., Latin America and the Caribbean) since notification is not compulsory [40]; however, cases of nosocomial myiasis have been reported in Germany and Italy [44,49]. Therefore, in cases of medical liability, forensic entomologists may be called upon the definition of the period of infestation – similarly to mPMI – discriminating between domestic and nosocomial-acquired infestation [24]. In any case, medical staff in all geographic locations must be reminded of the possibility of myiasis during the relevant fly season, carrying out prevention strategies (e.g., installation of insect electrocuting device, close vent system, environmental sanitation).

As stated above, eight families of flies are involved worldwide in myiasis phenomenon: Calliphoridae and Sarcophagidae represent the most common families and subfamilies of insects responsible of secondary myiasis [24,50–53], whereas Phoridae, Oestridae, Hypodermatidae, Gasterophilidae, Muscidae and Psychodidae may cause myiasis more rarely [54–58]. Calliphoridae-associated myasis includes three important subfamilies: Chrysomyinae (*Chrysomya albiceps* [59], *Ch. megacephala* [60–62], *Ch bezziana* [63–64], *Ch marginalis* [65], *Ch putoria* [59], *Cochliomyia macellaria* [66], *Co. hominivorax* [25,48,64], *Ch saffranea* [67], *Ch rufifacies* [62,68–70]), Calliphorinae (*Calliphora vicina* [59]), and Luciliinae (*Lucilia cuprina* [71], *L. sericata* [4,12,72]). Table 1 (Tab. 1) detailly illustrates the entire list of fly-associated myiasis.

There are novel forensic studies about these species, which focus on the importance of a proper ultrastructure definition by using SEM especially for young instars [59,72–78] and the effect of ecological adaptation for cycle of growth [10,53,60,64,69,71,79–81]. Thus, Calliphoridae species causing myiasis are very similar, both adults and immature forms, which suggest paying careful attention to avoid misinterpretations in the forensic field [82]. On the other hand, several studies show different flies' behaviors, depending on local temperature and humidity: higher temperatures accelerate their development and ultimately increase the number of generations of flies due to the animals' alternating warm lifestyles [11–13,20,50]. Owings et al. [83] described the increase in pupae size of *Co. macellaria* collected at three different ecoregions in Texas. The authors proposed that environmental conditions could influence puparia and adult size. Furthermore, the study by Alvarez Garcia et al. [10] revealed that blowflies' oviposition from the urban area of Sincelejo (Colombia) did not differ temporally and did not correlate with meteorological variables but it was influenced by polluted streams and organic waste, which provided resources to help these flies proliferate. The influence of abiotic factors at immature development should also be carefully considered to calculate mPMI or *ante-mortem* colonization. Similar considerations have also been documented among *Ch. megacephala* [60–62], *Ch. rufifacies* [62,68–70], *Ch. bezziana* [62–63] and *L. sericata* [12]. Additionally, Boatright et al. [66] highlighted the importance to standardize rearing conditions, which best mimic growth on actual carrion to compare blowfly datasets for use in legal investigations in different regions of the country.

Conversely to Calliphoridae, Sarcophagidae species colonize dead bodies out and indoors, in sunny or shaded sites, as well as in wet or dry environments, and could be detected within the early and late stages of decomposition [51–52,74,84–85]. Flesh flies include different species of forensic relevance (e.g., *Sarcophaga (Liosarcophaga) dux* [73], *S. tibialis*

Table 1
The articles that have been included in the review and the list of fly-associated myiasis.

Type of article	Authors, year	Title	Type of myasis	Age of victims (y/o)	Anatomical region	Families and species of insects
Review	Amendt et al., 2011	Forensic entomology: applications and limitations	H	NA	NA	NA
	Hall et al., 2016	Traumatic Myiasis: A Neglected Disease in a Changing World	H	NA	NA	NA
	Ivorra et al., 2021	Review of *Synthesiomyia nudiseta* (Diptera: Muscidae) as a useful tool in forensic entomology	H	NA	NA	*Synthesiomyia nudiseta* (Diptera: Muscidae)
	Nasser et al., 2021	Evolutionary profile of the family Calliphoridae, with notes on the origin of myiasis	H	NA	NA	NA
	Martín-Vega, 2011	Skipping clues: forensic importance of the family Piophilidae (Diptera)	H	NA	NA	NA
	De Azeredo-Espin et al., 2006	Genetic approaches for studying myiasis-causing flies: molecular markers and mitochondrial genomics	H	NA	NA	NA
	Shang et al., 2019	*Boettcherisca peregrina* (Diptera: Sarcophagidae): A flesh fly species of medical and forensic importance.	H	NA	NA	*Boettcherisca peregrina* (Diptera: Sarcophagidae)
	Baumgartner et al., 1993	Review of *Chrysomya rufifacies* (Diptera: Calliphoridae).	H, V	NA	NA	*Chrysomia rufifacies* (Diptera: Calliphoridae)
	Bernhardt et al., 2019	Myiasis in humans-a global case report evaluation and literature analysis.	H	NA	NA	*Lucilia sericata* (Diptera: Calliphoridae)
	Suwannayod et al., 2013	Suwannayod S., Sanit S., Sukontason K., Sukontason K.L. *Parasarcophaga* (*Liopygia*) *ruficornis* (Diptera:Sarcophagidae): a flesh fly species of medical importance.	H	NA	NA	*Parasarcophaga* (*Liopygia*) *ruficornis* (Diptera: Sarcophagidae)
Original article	Bambaradeniya et al., 2019	Myiasis incidences reported in and around central province of Sri Lanka.	H	30–92 (average 59.4 years)	Cutaneous (lower limbs, scalp, umbilical) urogenital	*Chrysomya bezziana* (Diptera: Calliphoridae) *Chrysomya megacephala* (Diptera: Calliphoridae)
	Alahmed et al., 2020	Two new records of flies causing myiasis from Saudi Arabia with a survey of flies parasitizing goats and sheep in Jazan Region.	H	NA	NA	*Chrysomya albiceps* (Diptera: Calliphoridae) *Chrysomya bezziana* (Diptera: Calliphoridae) *Chrysomya megacephala* (Diptera: Calliphoridae) *Hemipyrellia pulchra* (Diptera: Calliphoridae) *Lucilia cuprina* (Diptera: Calliphoridae) *Lucilia sericata* (Diptera: Calliphoridae) *Sarcophaga* (*Liosarcophaga*) *esuberans* (Diptera: Sarcophagidae) *Sarcophaga (Bercaea) africa* (Diptera: Sarcophagidae) *Sarcophaga (Parasarcophaga) hirtipes* (Diptera: Sarcophagidae) *Wohlfahrtia nuba* (Diptera: Sarcophagidae) *Wohlfahrtia indigens* (Diptera: Sarcophagidae)
	Lutz et al., 2021	It is all about the insects: a retrospective on 20 years of forensic entomology highlights the importance of insects in legal investigations.	H	NA	NA	NA
	Bambaradeniya et al., 2019	Effect of Temperature and Tissue Type on the Development of the Forensic Fly *Chrysomya megacephala* (Diptera: Calliphoridae).	H	NA	NA	*Chrysomya megacephala* (Diptera: Calliphoridae)

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Table 1 (*continued*)

Type of article	Authors, year	Title	Type of myasis	Age of victims (y/o)	Anatomical region	Families and species of insects
	Anderson et al., 2004	Myiasis in pet animals in British Columbia: the potential of forensic entomology for determining duration of possible neglect.	V	NA	Cutaneous (oral cavity)	*Lucilia illustris* (Diptera: Calliphoridae) *Phaenicia sericata* (Diptera: Calliphoridae) *Cuterebra jellisoni* (Diptera: Oestridae)
	Talebzadeh et al., 2020	Molecular Species Identification of Six Forensically Important Iranian Flesh Flies (Diptera).	NA	NA	NA	NA
	Bambaradeniya et al., 2021	Effect of Type of Tissue on the Development of *Chrysomya rufifacies* (Diptera: Calliphoridae) in Sri Lanka.	NA	NA	NA	*Chrysomya rufifacies* (Diptera: Calliphoridae)
	Luz et al., 2020	Diversity of Calliphoridae and Mesembrinellidae (Diptera: Oestroidea) in a Mangrove, Restinga, and Forest Landscapes From a Lagoon Complex on an Atlantic Forest Coastline (Rio de Janeiro, Brazil)	NA	NA	NA	Calliphoridae and Mesembrinellidae (Diptera: Oestroidea)
	Williams et al., 2019	Spatial and Seasonal Distribution of Forensically Important Blow Flies (Diptera: Calliphoridae) in Makhanda, Eastern Cape, South Africa.	NA	NA	NA	NA
	Feng et al., 2013	Pupal age estimation of forensically important *Megaselia spiracularis* Schmitz (Diptera: Phoridae)	H	NA	NA	*Megaselia spiracularis* Schmitz (Diptera: Phoridae)
	Mozaffari et al., 2020	Geographical Distribution, Biodiversity, and Species Richness of Medically Important Necrophagous Flies in Central Iran	H,V	NA	NA	*Calliphora vicina* (Diptera: Calliphoridae) *Lucilia sericata* (Diptera: Calliphoridae) *Musca domestica* (Diptera: Muscidae) *Wohlfahrtia nuba* (Diptera: Sarcophagidae) *Chrysomya albiceps* (Diptera: Calliphoridae)
	Alvarez Garcia et al., 2019	Spatial and Temporal Variation of the Blowflies Community (Diptera: Calliphoridae) From an Urban Area in Northern South America.	H	NA	NA	*Cochliomyia macellaria* (Diptera: Calliphoridae) *Lucilia cuprina* (Diptera: Calliphoridae) *Chrysomya albiceps* (Diptera: Calliphoridae) *Chrysomya megacephala* (Diptera Calliphoridae)
	Bambaradeniya et al., 2018	Temperature and Tissue Type Impact Development of *Lucilia cuprina* (Diptera: Calliphoridae) in Sri Lanka.	H, V	NA	NA	*Lucilia cuprina* (Diptera: Calliphoridae)
	Mendonça et al., 2014	Ultrastructure of immature stages of *Lucilia cuprina* (Diptera: Calliphoridae) using scanning electron microscopy.	H, V	NA	NA	*Lucilia cuprina* (Diptera: Calliphoridae)
	Mendonça et al., 2012	Ultrastructure of larvae and puparia of the blowfly *Chrysomya megacephala* (Diptera: Calliphoridae).	H	NA	NA	*Chrysomya megacephala* (Diptera: Calliphoridae)
	Thyssen et al., 2014	Rates of development of immatures of three species of *Chrysomya* (Diptera: Calliphoridae) reared in different types of animal tissues: implications for estimating the postmortem interval	H	NA	NA	*Chrysomya albiceps* (Diptera: Calliphoridae) *Chrysomya putoria* (Diptera: Calliphoridae)
	Mukandiwa et al., 2012	Evaluation of plant species used traditionally to treat myiasis for activity on the survival and development of *Lucilia cuprina* and *Chrysomya marginalis* (Diptera: Calliphoridae).	H	NA	NA	*Lucilia cuprina* (Diptera: Calliphoridae) *Chrysomia m arginalis* (Diptera: Calliphoridae)
	Boatright et al., 2010	Effects of temperature and tissue type on the development of *Cochliomyia macellaria* (Diptera: Calliphoridae).	H	NA	NA	*Cochliomyia macellaria* (Diptera: Calliphoridae)

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Table 1 (*continued*)

Type of article	Authors, year	Title	Type of myasis	Age of victims (y/o)	Anatomical region	Families and species of insects
	Abd-AlGalil et al., 2016	First record of *Chrysomya saffranea* (Diptera: Calliphoridae) of forensic importance in India.	V	NA	NA	*Chrysomya saffranea* (Diptera: Calliphoridae)
	Sukontason et al., 2003	Larval ultrastructure of *Parasarcophaga dux* (Thomson) (Diptera: Sarcophagidae).	H	NA	NA	*Parasarcophaga dux* (Thomson) (Diptera: Sarcophagidae)
	Paños-Nicolás et al., 2015	*Sarcophaga* (*Liosarcophaga*) *tibialis* Macquart 1851 (Diptera: Sarcophagidae): micromorphology of preimaginal stages of a fly of medical and veterinary interest.	H, V	NA	NA	*Sarcophaga* (*Liosarcophaga*) *tibialis* Macquart 1851 (Diptera: Sarcophagidae)
	Pezzi et al., 2016	Ultrastructural Morphology of the Antenna and Maxillary Palp of *Sarcophaga tibialis* (Diptera: Sarcophagidae).	H	NA	NA	*Sarcophaga tibialis* (Diptera: Sarcophagidae).
	Mendonça et al., 2013	Ultrastructure of immature stages of *Peckia* (*Euboetcheria*) *collusor* (Diptera: Sarcophagidae).	V	NA	NA	*Peckia* (*Euboetcheria*) *collusor* (Diptera: Sarcophagidae)
Case report	Dutto et al., 2013	Nosocomial myiasis in a patient with diabetes	H	81	Cutaneous (right foot)	*Sarcophaga* (*Bercaea*) *africa* (Diptera: Sarcophagidae)
	Baumjohann et al., 2011	Maggots reveal a case of antemortal insect infestation	H	80	Cutaneous (face), genital	*Lucilia Sericata* (Diptera: Calliphoridae)
	Sanford et al., 2014	Human wound colonization by *Lucilia eximia* and *Chrysomya rufifacies* (Diptera: Calliphoridae): myiasis, perimortem, or postmortem colonization?	H	60	Cutaneous (lower left leg), genital	*Lucilia eximia* (Diptera: Calliphoridae) *Chrysomya rufifacies* (Diptera: Calliphoridae)
	Thyssen et al., 2012	Record of oral myiasis by *Cochliomyia hominivorax* (Diptera: Calliphoridae): case evidencing negligence in the treatment of incapable	H	95	Oral	*Cochliomyia hominivorax* (Diptera: Calliphoridae)
	Ahadizadeh et al., 2015	Human Cutaneous Myiasis by the Australian Sheep Blowfly, *Lucilia cuprina* (Diptera: Calliphoridae), in Oklahoma.	H	53	Cutaneous	*Lucilia cuprina* (Diptera: Calliphoridae)
	Sukontason et al., 2005	First report of human myiasis caused by *Chrysomya megacephala* and *Chrysomya rufifacies* (Diptera: Calliphoridae) in Thailand, and its implication in forensic entomology	H	53	Cutaneous (lower right leg)	*Chrysomya megacephala* (Diptera: Calliphoridae) *Chrysomya rufifacies* (Diptera: Calliphoridae)
	Benecke et al., 2001	Child neglect and forensic entomology	H	Unspecified (child)	Cutaneous (face), anal-genital	*Muscina stabulans* (Diptera: Muscidae) *Fannia canicularis* (Diptera: Fannidae) *Calliphora vomitoria* (Diptera: Calliphoridae)
	Vanin et al., 2017	A Case of Insect Colonization Before the Death.	H	84	Conjunctivae, bronchi, rectum, vagina	*Lucilia sericata* (Diptera: Calliphoridae)
	Trombetta et al., 2009	Cutaneous myiasis due to *Cochliomyia hominivorax* in a drug user.	H	32	Cutaneous (scalp)	*Cochliomyia hominivorax* (Diptera: Calliphoridae)
	Durão et al., 2017	A rare case of digital myiasis.	H	41	Cutaneous (second finger of left hand)	*Cochliomyia hominivorax* (Diptera: Calliphoridae)
	El- Dib et al., 2017	Case Report of Human Urinary Myiasis Caused by *Clogmia albipunctata* (Diptera: Psychodidae) with Morphological Description of Larva and Pupa.	H	24	Urogenital orifices	*Clogmia albipunctata* (Psychodidae)
	El-Dib et al., 2020	Human intestinal myiasis caused by *Clogmia albipunctata* larvae (Diptera: Psychodidae): First report in Egypt.	H	36	Intestinal	*Clogmia albipunctata* larvae (Diptera: Psychodidae)
	Bernhardt et al., 2018	Multispecies blow fly myiasis combined with hypothermia in a man assumed to be dead.	H	Unspecified	Cutaneous (feet)	*Phormia regina* (Diptera: Calliphoridae) *Lucilia sericata* (Diptera: Calliphoridae) *Calliphora vomitoria* (Diptera: Calliphoridae) *Lucilia caesar* (Diptera: Calliphoridae)

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Table 1 (*continued*)

Type of article	Authors, year	Title	Type of myasis	Age of victims (y/o)	Anatomical region	Families and species of insects
						Protophormia terranovae (Diptera: Calliphoridae)
Others (comparative study, letter to the editor, technical note)	Dian-Xing et al., 2012	Morphology of immature stages of *Megaselia spiracularis* Schmitz (Diptera: Phoridae)	NA	NA	NA	*Megaselia spiracularis* Schmitz (Diptera: Phoridae)
	Kleine et al., 2014	Cutaneous myiasis in a patient with seborrhoeic eczema.	H	55	Cutaneous (scalp)	*Chrysomya bezziana* (Diptera: Calliphoridae)
	De Jong, 2014	Field study on the attraction and development of insects on human meconium and breast-fed-infant feces.	H	Newborn	Urogenital	*Lucilia sericata* (Diptera: Calliphoridae)

NA: not applicable.
V: veterinary myiasis.
H: human myiasis.

[74–75], *Parasarcophaga (Liopygia) ruficornis* [76], *Peckia (Euboettcheria) collusor* [77], *Boettcherisca peregrina* [86], which are associated to human myiasis, especially involving the anogenital region. However, the literature review resulted in fewer papers based on forensic entomology research [86–87]. However, poor literature exists discussing human myiasis implications in mPMI estimation and neglect evaluation.

Among Calliphoridae, De Jong [88] demonstrated that meconium is not attractive to flies, while *Lucilia sericata* is able to locate breast-fed human infant feces within minutes if exposed and within hours even if enclosed. Moreover, its cephalopharyngeal mouth hooks are not developed enough in first instars to invade healthy skin. As a consequence, observation of urogenital myiasis in breast-fed infants and otherwise located myiasis in newborn infants in the first days postpartum should suggest the presence of wounds and require further investigations to exclude the hypothesis of child abuse. In addition, in case of dead infants, forensic implications of myiasis even multiply, considering that *Lucilia sericata* is a common blowfly species in the first wave of colonization and plays a crucial role in mPMI estimation [88].

Potentially confounding role of myiasis in estimation of time since death is discussed by Vanin et al (2017). The authors present a case of an elderly woman found unconscious and largely colonized by fly larvae four days after she had fallen in her garden; she died two months later of tetanus. Since the woman was still alive when conducted to the emergency department, larvae infestation was easily evaluated and treated as human myiasis. However, if the emergency rescue team had arrived later and found the woman already dead, a lack of proper knowledge of myiasis phenomenon would have led to a wrong interpretation of *Lucilia sericata* larval age, following an overestimation of mPMI [24].

Soulsby [89] similarly described a case of vaginal myiasis in an elderly woman found unconscious in her garden. According to the neighbors she had spent 2 days lying in her garden, this period resulting consistent with that requested by *Protophormia* larvae found in the labia to reach the second instar stage of development. Again, if the woman had been found dead, an incorrect interpretation of infestation as *post-mortem* colonization would have led to wrong estimation of mPMI. Goff [21] interestingly described two cases of self-neglect further discussing forensic implications of human myiasis. The first one was about remains of a 58-year-old female with a history of stroke and minimal contact with health care professionals. In this case, the presence of third instar larvae of *Phaenicia sericata* in a necrotic area on the low back penetrating into the abdominal cavity suggested an important general lack of care. Furthermore, the involvement of *P. sericata* in myiasis in Hawaii let the forensic pathologist conclude for a shorter mPMI than expected and consistent with her family's account. The second case concerned the death of an elderly woman who lived with her daughter in poor hygienic conditions and social isolation. While the daughter told the investigators that she had seen her mother alive the day before, the presence of multiple decubitus lesions with third instar larvae of *Lucilia sericata* suggested a period of insect activity of 4–5 days. As the development of bedsores may have required less than 4–5 days but more than the 6–12 h of estimated PMI based on thanatological findings, forensic pathologist concluded for an *ante-mortem* colonization with myiasis suggestive for the duration of neglect [21].

Despite few cases of myiasis are reported in literature [90–92], it is worth mentioning that we have not included *Musca domestica* in our results. Despite largely abundant, in fact, its relevance as causative agent of myiasis is extremely limited. Indeed, the common housefly is typically found in dung and decaying refuse, moreover if present on remains its appearance occurs later in the decaying process, so its role in *post-mortem* colonization of neglected corpse makes it very seldom likely to be confused with myiasis.

For all these reasons, there must be a close cooperation between forensic practitioners: forensic pathologists should know first the pathology of myiasis, which is very often underrated especially in Western countries [12,93]. Hence, they should pay more attention to the entomological fauna on a dead body, considering the evaluation of maggots' morphology and distribution as an integral part of *post-mortem* examinations. At autopsy, the presence of infested lesions should be carefully evaluated, and maggots properly collected, and the wounds' vitality always characterized by histopathology. Microscopic examination, in fact, could show vital red blood cells as well as inflammatory cells, which clearly demonstrate that the wound occurred before death [94]. Similarly, forensic entomologists are called to obtain complementary information identifying the colonizing species and their development in relation to temperature and environmental conditions. As previously mentioned, ultrastructure definition of early maggots by SEM as well as the use of molecular techniques [95–97] are very useful for entomologists in the forensic field: the evidence of different species of fly larvae or pupae in different evolutive instars does not necessarily mean that all maggots developed *post-mortem*. Thus, it is essential to bear in mind and then to identify possible "contamination" factors [98], including *ante-mortem* myiasis [1,16,21–24].

Lastly, myiasis may involve pet and farm animals, occurring when a wound is left untreated or when neglect results in the accumulation of feces or urine, which then attracts flies [9]. In cases of deliberate abuse or neglect, it is unlikely that owners would consult a veterinarian; however, in many cases, animal myiasis is probably the result of ignorance rather than deliberate neglect or abuse, and owner education may go a long way to alleviating the problem. As for humans, lack and type of housing and poor hygiene measures are the major risk factors for the exposure and occurrence of myiasis among pet animals, with the highest rates for puppies and unhouse animals [23].

5. Conclusion

While myiasis is extensively described in clinical and entomological literature, *ante-mortem* colonization should be further discussed among forensic pathologists. A deep knowledge of this phenomenon may

provide the forensic pathologist with additional elements to estimate in a more accurate way the time since death and evaluate possible cases of neglect; thus, an incorrect interpretation of myiasis, in fact, could led to an overestimation of mPMI and misinterpretation of colonized skin defects indicative of untreated wounds. This work also provides an overview of the diversity of entomological species involved in myiasis to facilitate their knowledge in forensic practice. The field of interest includes both human and veterinary forensic pathology. This work would like to get a general idea of which insects can be found in each case of myiasis, helping to guide forensic investigations when firm information is not available to assess the time of death or to identify neglect. Finally, the authors would suggest a more careful and deep approach in the analysis of wounds, which aims to improve the collaboration between different and complementary professional roles in forensics investigations.

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Valentina Bugelli: Conceptualization, Resources, Writing – review & editing, Supervision. **Ilaria Tarozzi:** Investigation, Writing – original draft. **Nicola Galante:** Formal analysis, Investigation, Resources, Writing – original draft. **Sara Bortolini:** Investigation, Resources, Writing – review & editing. **Lorenzo Franceschetti:** Conceptualization, Methodology, Data curation, Writing – review & editing, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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