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Comparative Study of Forensic Insect Succession on Animal Carcasses in Arid Rural Environments

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ABSTRACT

Background: Forensic insects are considered of significant practical value in criminal investigations and serve as a vital biological tool in determining the post-mortem interval (PMI).

Methods: The field study conducted in an arid rural area of Wasit Province demonstrated clear differences in the succession of forensic insects between sheep and dog carcasses.

Result: Muscidae were recorded as the earliest colonizers during the fresh stage, with higher abundance on dogs compared to sheep, reflecting the influence of skin thickness and the presence of fur on the rate of colonization. During the bloating stage, Calliphoridae represented the primary and most abundant colonizers, with their highest prevalence on sheep carcasses, indicating their rapid sensitivity to gaseous odors. Throughout the active and advanced decay stages, Dermestidae were prominently observed, particularly on dog carcasses, due to their ability to feed on dried tissues and hair remnants. In the final stage (advanced decay), Acari were dominant, associated with the accelerated desiccation of tissues under solar radiation. Meanwhile, Formicidae were present across all stages as opportunistic colonizers, without a specific association with any particular stage. These findings highlight that host type (sheep/dog) and environmental factors (temperature, humidity and sunlight) were decisive elements in shaping the speed of succession and species diversity. The study emphasizes that understanding succession patterns in arid environments provides a crucial basis for developing accurate models to estimate the postmortem interval (PMI) in forensic investigations.

Key words: Acari, Animal carcasses, Calliphoridae, Dermestidae, Forensic entomology, Formicidae, Insect succession, Muscidae, PMI.

INTRODUCTION

Forensic insects are considered of significant practical value in criminal investigations and serve as a vital biological tool in determining the post-mortem interval (PMI). These insects are characterized by their ability to colonize corpses at various stages of decomposition, enabling forensic entomologists to accurately estimate the time elapsed since death (Peoples and Payne, 2018). However, environmental conditions, particularly in rural arid areas, greatly influence insect succession and diversity. Key factors affecting the rate of decomposition and colonization timing include the type of animal, morphological characteristics (such as skin thickness and the presence of fur) and the existence of wounds. For instance, Kökdener and Polat (2014) reported that dog carcasses in a region of Turkey were colonized by various insect species, with blow flies typically arriving first, followed by beetles.Summer arid environments are characterized by high temperatures and low humidity, which accelerate decomposition in the early stages but slow it down in later stages (Al-Mesbah *et al.*, 2017). This variation influences the timing of appearance of different insect species.

A study in Ghana revealed that carcasses reached complete decomposition within 16 days during the dry season compared to 24 days in the wet season, with notable differences in the insect species colonizing the carcasses in each season (Dadzie *et al.*, 2025). Similarly, research in Pakistan's semi-arid climate showed that fluctuations in temperature and humidity directly impacted insect activity, with blow flies and beetles being active during cooler, more humid hours, while extreme midday dryness limited their activity (Shahid *et al.*, 2014). Low humidity in arid environments reduces the activity of certain insects, such as flesh flies (Sarcophagidae) and blow flies (Calliphoridae), while populations of beetles (Coleoptera), which are more tolerant to dryness, may increase (Catta and Haskell, 1990). This shift in species composition alters the succession pattern of insects, as confirmed by studies in semi-arid regions of Brazil and arid areas of China, which documented significant changes in the diversity and abundance of necrophagous species such as *Lucilia sericata*, *Chrysomya megacephala* and *Phormia regina* (Guo *et al.*, 2022). This study contributes to improving forensic investigation methods, particularly in dry climates such as Iraq. By comparing insect succession patterns in

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arid and humid environments, more accurate models for estimating the post-mortem interval can be developed, thereby enhancing the effectiveness of forensic investigations

MATERIALS AND METHODS

The study was conducted in Wasit Province, Al-Ahrar District, Al-Intisar Village, during the period from March to October 2025. Carcasses of sheep (25-35 kg) and dogs (12-18 kg) (Fig 1), which had died of natural causes, were placed in open areas along roadsides near the village, with a total of 12 carcasses for each species. The carcasses were monitored from the time of placement both in the morning and evening for eight consecutive days and subsequently once daily until day 32, covering the final stage of decomposition. This protocol followed classical studies conducted on various animal models such as pigs and dogs (Payne, 1965; Reed, 1958).Data loggers were installed at a height of 80 cm to record temperature and relative humidity every 40 minutes, along with wind speed measurements, in line with standard forensic decomposition studies for precise monitoring of environmental conditions (Gill, 2005; Charabidze *et al.*, 2019). Decomposition stages were classified as fresh, bloated, active decay, advanced decay and advanced decay, based on visible indicators such as odor, discoloration, fluid exudation and mass loss. Daily photographic documentation was carried out using a digital camera, consistent with the five-stage decomposition model described in the literature (Payne, 1965). For insect collection, sweep nets were employed around the carcasses with a standardized sampling effort (5 minutes/ carcass/visit) to capture flying adult insects. In addition, pitfall traps were placed around the carcasses (three traps arranged in a triangular layout at 0.5-1 m distance) to capture ground beetles and migrating larvae, while sticky traps were installed at a height of 1 m to collect adult flies during the bloating and active decay stages (Reed, 1958; Als-Journal, 2019). Taxonomic identification of Diptera (Calliphoridae, Muscidae, Sarcophagidae) and Coleoptera (Dermestidae) was performed using standard keys commonly applied in previous studies (Payne, 1965).A database was designed in Microsoft Excel, with each record coded according to site, host, decomposition stage, date, time, collection method and sample code.

Temporal period of forensic insect succession according to animal carcass decomposition some stages

The duration of insect succession was calculated as the number of hours/days from the beginning to the end of each decomposition stage for each carcass. Additionally, the time of first and last appearance for each species/ family within each stage was recorded. Comparisons of these times between sheep and dogs, as well as between study sites, were performed using generalized linear mixed models (GLMM), as recommended by recent studies for analyzing complex forensic data (Amendt *et al.*, 2007).

Percentage of insect presence by decomposition stage

The presence or absence of each species or family during each stage was calculated across all visits using the following formula:

$$\% \text{ Presence} = \frac{\text{Number of visits in which the species was recorded}}{\text{Total number of visits during that stage}} \times 100 \text{ (Payne, 1965)}$$

Frequency of family occurrence on sheep and dogs

The occurrence frequency of each family was computed as the number of records per visit for each host type, standardized by sampling time and number of traps using the formula:

$$\text{Frequency Rate} = \text{Number of records for the family} / \text{Total visits for the host (Reed, 1958)}$$

Temporal distribution of key insect taxa during decomposition

Key insect taxa were identified based on abundance/ frequency thresholds. For each species, a temporal series of occurrence and developmental stage (eggs/larvae/ adults) was extracted across the days and decomposition stages (Payne, 1965; Gill, 2005).

Statistical analysis

The data for the studied traits were statistically analyzed using SPSS software and the significant differences between mean scores were evaluated using a t-test. A p-value of less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Temporal period of forensic insect succession according to animal carcasses decomposition some stages

The results presented in Table 1 indicated a clear relationship between the different decomposition stages and the recorded insect taxa, as well as the influence of various environmental and biological factors on the characteristics and rate of insect colonization of carcasses.During the fresh stage of sheep carcasses, Muscidae was recorded within the first hours (0-13 hours) (Fig 1). These species are among the earliest insects attracted to odors emitted from bodily fluids at the onset of decomposition. Previous studies have reported that members of the family Muscidae, including Muscidae, may appear in the early stages of decomposition alongside blowflies (Calliphoridae) and flesh flies (Sarcophagidae) (Amendt *et al.*, 2007; Anderson, 2010). Environmental factors such as temperature and humidity play a critical role in the speed of arrival and activity of these insects (Mann *et al.*, 1990; Gill, 2005). Table 1 also showed that during the bloating stage of sheep carcasses, Calliphoridae were present between 20-72 hours. These flies are well known for their ability to detect carcasses from long distances and are considered key indicators for

estimating the postmortem interval (PMI) (Catts and Haskell, 1990; Byrd and Castner, 2010). The presence of open wounds was another influencing factor, as it accelerates insect colonization by facilitating larval access to internal tissues, consistent with previous studies showing that injuries or natural openings promote egg-laying and tissue penetration (Goff, 2000). The results also indicated that during the active decay stage in dogs (4-12 days), Dermestidae beetles were attracted, which aligns with established knowledge of insect succession. Dermestid beetles tend to appear in relatively late stages, feeding on dry tissues and animal hair (Hedges and Mellini, 1984; Martín-Vega *et al.*, 2011).

Skin thickness and the presence of fur were factors that delayed the arrival of some fly species, allowing beetles to become the dominant colonizers later (Kashyap and Pillai, 1989). During the advanced decay stage in dogs (11-32 days), mites (Acari) were recorded. These minute arthropods typically appear in the final stages of decomposition, feeding on advanced decay or other insect larvae (Perotti *et al.*, 2009). Solar radiation was also an influencing factor, as sunlight accelerates tissue desiccation, creating favorable conditions for the proliferation of small arthropods such as mites (Mann *et al.*, 1990; Gill, 2005).

Percentage of insect presence by decomposition stage

The results presented in Table 2 indicated a marked variation in the occurrence of forensic insects between sheep and dog carcasses across different decomposition stages. During the fresh stage, Musca spp. (housefly) accounted for 25% of occurrences on sheep carcasses compared to 64% on dogs, suggesting that dogs are more attractive to these species in the early hours postmortem. This may be attributed to the relatively thin skin of dogs compared to sheep, as well as the presence of fur, which can retain local moisture and attract flies more rapidly. Muscidae is considered an early colonizer of carcasses in open conditions, particularly in urban and semi-urban environments (Amendt *et al.*, 2007; Anderson, 2010). Table 2 also showed that during the bloating stage, the highest presence was recorded for the family Calliphoridae,

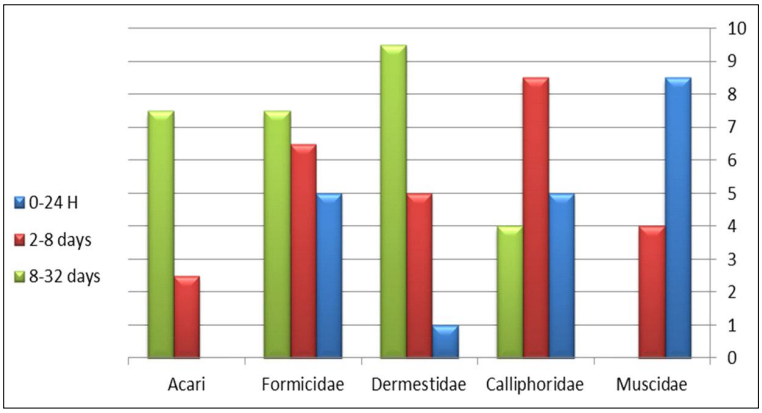


Fig 1: Temporal distribution of key insect taxa during decomposition stages of animal carcasses.

Table 1: Temporal duration of forensic insect succession according to animal carcasses decomposition some stages.

Animal	Decomposition stage	Recorded insects	Time (hours/days)	Influencing factors
Sheep	Fresh	Muscidae	0-13 hours	Temperature, humidity
Sheep	Bloat	*Calliphoridae*)	20-72 hours	Presence of an open wound
Dogs	Active decay	*Dermestidae*)	4-12 days	Skin thickness, presence of fur
Dogs	Advanced decay	Mites (*Acari*)	11-32 days	Sunlight exposure

Table 2: Frequency of forensic insect family occurrence on sheep and dog carcasses.

Insect type	Animal		Stage of carcass decomposition
	Dogs (%)	Sheep (%)	
Muscidae	64.00	25.00	Fresh
Calliphoridae	55.00	73.00	Bloating
Dermestidae	79.00	10.00	Dry decomposition
Formicidae	5.00	32.00	All stages
L.S.D	144.30	121.26	Interaction
			133.23

reaching 73% on sheep carcasses compared to 55% on dogs. This reflects the high ability of these flies to detect gases emitted within the body, a key indicator of the onset of active decay. The higher prevalence on sheep may be associated with the faster decomposition of their soft tissues compared to dogs. According to Anderson (2010) and Byrd and Castner (2010), Calliphoridae are primary and abundant colonizers, making them important indicators.

For estimating the postmortem interval (PMI).During the dry decomposition stage, Table 2 revealed a notable increase in Dermestidae occurrences on dog carcasses (79%) compared to sheep (10%). This may be due to the thicker skin and fur of dogs, which provide a suitable environment for dermestid beetles to feed on dried tissues. Other studies have similarly noted that Dermestidae are key insects in the late stages of decomposition, especially in the presence of fur or hair (Goff, 2000; Martín-Vega *et al.*, 2011).Furthermore, Formicidae (ants) were recorded in all stages, with a 32% occurrence on sheep carcasses, whereas their presence on dogs was (5%). This may be attributed to the opportunistic behavior of ants, which are not tied to a specific decomposition stage but appear in search of food, particularly larvae or eggs. In this context, Campobasso *et al.* (2001) noted that ants can influence crime scene interpretation, as they may cause injuries or modifications on the carcass that could be mistakenly interpreted as ante-mortem wounds.

Frequency of forensic insect family occurrence on sheep and dogs carcasses

The results presented in Table 3 indicated that Calliphoridae were the primary colonizers during the bloating stage, with very high frequency on sheep and high frequency on dogs. This is consistent with previous studies showing that Calliphoridae, as primary colonizers, arrive within the first hours or day, from the fresh stage to the bloating stage and lay eggs rapidly, even in indoor environments, often within the first 24-48 hours (Martín-Vega *et al.*, 2017; Laabusi, 2022). The observed differences in frequency between dogs and sheep and the higher occurrence on sheep, may reflect microclimatic factors created by dense wool coverage (retaining moisture and heat), which accelerate the release of attractive odors and facilitate detection by Calliphoridae. Carcass size and placement also influence detection speed and initial colonization intensity (Zhang *et al.*, 2022).

Table 3 further recorded a high frequency of Muscidae during the fresh stage, with very high occurrence on sheep and above-average occurrence on dogs. In this context, McIntosh *et al.* (2017) highlighted the importance of local thermal conditions in accelerating life cycles, thereby increasing their early-stage abundance and hastening the appearance of Muscidae during early decomposition stages.The table also indicated a very high frequency of Dermestidae on dog carcasses compared to low frequency on sheep during the late stages. Colonization of Dermestidae on dry tissues in arid or indoor environments is a biological characteristic consistent with their ecological and life-history traits (Mayer and Vasconcelos, 2013; Seay, 2021).Meanwhile, Formicidae exhibited low frequency on dogs and moderate frequency on sheep, which is an important factor in estimating the postmortem interval (PMI). Experiments on fire ants have shown that their presence can reduce fly larval density, delay insect succession and cause effects on bones and tissues (Al-Ahmad *et al.*, 2-22; Tembe and Mukaratirwa, 2021; Yadav *et al.*, 2024).

Temporal distribution of key insect taxa during decomposition stages of animal carcasses

Fig 1 indicated that insects do not appear randomly on carcasses but rather follow a consistent ecological succession, with each taxon associated with a specific decomposition stage. This pattern is crucial in forensic entomology, as it is used to estimate the postmortem interval (PMI) by observing insect taxa and their abundance (Shahid *et al.*, 2014).

The results in Fig 1 showed that Calliphoridae appear from the early hours at moderate levels, then increase significantly during the intermediate stage (2-8 days) before declining later. This aligns with the study by Sharif and Qamar (2021), which reported that blue/green blowflies are the first to reach the carcass, peaking when soft tissues begin to decompose. Muscidae, on the other hand, exhibit high abundance from the start but decrease rapidly and disappear in the later stages, reflecting their role as secondary colonizers appearing immediately after Calliphoridae (Sharma *et al.*, 2025; Sharma *et al.*, 2022; Al-khshemawee *et al.*, 2024). The table also indicated that Dermestidae beetles are rare during early stages, become moderate during the intermediate stage and peak in the late stage (8-32 days). This behavior reflects their feeding preference for dry tissues and hair, making them indicators

Table 3: Insect occurrence percentages across decomposition stages of animal carcasses.

Insect families	Frequency on sheep	Frequency on dogs	Observations
Muscidae	High	Above average	High presence during the fresh stage
Calliphoridae	Very high	High	First colonizers during the bloating stage
Dermestidae	Low	Very high	Abundant during late decomposition
Formicidae	Moderate	Very low	High predation in all stages
Acari	Rare	Moderate	Dependent on dry carcass residues

* Very high(80-90%) , *High (65-70%), * Low (20-30 %), *Very low (15-19), * Moderate (50-55 %) ,* Rare (10-5%).

of an extended postmortem period (Byrd and Castner, 2010). Acari were absent during the early stage, appeared in low numbers during the intermediate stage and were consistently present during the late stage, which aligns with observations reported by Goff (2000). Fig 1 further showed that ants (Formicidae) exhibit opportunistic behavior, acting as predators of other insects or directly benefiting from carcass remains. They were moderately abundant in the early stage, slightly increased during the intermediate stage and continued to be present in the late stage (Amendt *et al.*, 2007).

CONCLUSION

The field study conducted in rural areas revealed clear differences in the succession patterns of forensic insects between sheep and dog carcasses, underscoring the critical role of host type in determining both the rate of succession and species diversity. Muscidae were identified as the earliest colonizers during the bloating stage, while Calliphoridae exhibited the highest abundance on sheep carcasses, reflecting their rapid response to gaseous odors. In the advanced stages, Dermestidae became predominant due to their ability to consume desiccated tissues, whereas Acari dominated the final stages as a result of extreme desiccation. The consistent presence of Formicidae across all stages highlights their opportunistic nature, independent of any particular stage. These findings emphasize that host type and environmental factors (temperature, humidity and solar radiation) are critical variables that must be considered when developing accurate models for postmortem interval (PMI) estimation in arid environments.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments were approved by the Committee of Experimental Animal care and handling techniques were approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No. funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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