ISSN: 2457-0400

Impact Factor: 7.675

WORLD JOURNAL OF ADVANCE HEALTHCARE RESEARCH

www.wjahr.com

Volume: 10, Issue: 7

Page N. 215-221

Year: 2026

Original Article

Coden USA: WJAMA3

THIRD-INSTAR LARVAE OF FORENSICALLY IMPORTANT INSECTS AS A TOOL FOR ESTIMATING THE MINIMUM POST-MORTEM INTERVAL IN DOG CARCASSES UNDER HIGH SUMMER TEMPERATURES IN SOUTHERN IRAQ

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Article Received: 26 May 2026 **Article Revised: 15 June 2026** **Article Published: 01 July 2026**

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DOI: https://doi.org/10.5281/zenodo.21027221

How to cite this Article: Rawaa Shakir Al-Rubaie, *Habeeb Waseel Kadhum Shubber (2026). Third-Instar Larvae Of Forensically Important Insects As A Tool For Estimating The Minimum Post-Mortem Interval In Dog Carcasses Under High Summer Temperatures In Southern Iraq. World Journal of Advance Healthcare Research, 10(7), 215–221.

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ABSTRACT

Forensic entomology applies knowledge of insect biology to medico-legal investigations, most critically for estimating the minimum post-mortem interval (PMImin) when conventional pathological methods become unreliable. This study evaluated third-instar larval development and the accumulated degree hours (ADH) method for estimating PMImin in dog (*Canis lupus familiaris*) carcasses under the extreme summer temperatures of Al-Diwaniyah, Southern Iraq (43 °C). Twelve carcasses were studied across two contrasting habitats — an agricultural area and a cemetery — with three carcasses placed on the ground surface and three buried at 50 cm depth in each habitat. Four forensically important species were recorded across all groups: *Calliphora vicina*, *Calliphora vomitoria*, *Chrysomya megacephala*, and *Chrysomya albiceps*. PMImin estimates for surface carcasses ranged from 8.143 h (*C. vicina*, cemetery) to 11.000 h (*C. vomitoria*, cemetery surface). For buried carcasses, estimates ranged from 12.727 h (*C. vicina* and *C. megacephala*, cemetery buried) to 20.727 h (*C. megacephala*, agricultural buried). Burial at 50 cm consistently and substantially increased PMImin relative to surface carcasses. Cemetery surface carcasses yielded marginally higher estimates than agricultural ones for shared species, reflecting habitat-level microclimatic differences. *Calliphora vicina* consistently produced the earliest estimates, confirming its value as the closest entomological indicator of the time of death. The findings demonstrate that third-instar larvae are reliable biological clocks for PMImin estimation in hot-climate Iraq and that both burial depth and habitat type materially affect entomological estimates.

KEYWORDS: Forensic entomology · Post-mortem interval (PMImin) · Third-instar larvae · Accumulated degree hours (ADH) · Dog carcasses · Insect succession · Burial depth · Southern Iraq.

1. INTRODUCTION

Forensic entomology is the discipline that applies knowledge of insects and arthropods to medico-legal investigations. Among its principal applications, the estimation of the post-mortem interval (PMI) — the elapsed time between death and discovery of remains — is the most widely practised and scientifically established (Smith, 1986; Byrd & Castner, 2010). Because insects colonise decomposing remains in a temperature-dependent, broadly predictable sequence, entomological evidence provides the minimum post-mortem interval (PMImin): the shortest time that must have elapsed since the first insects arrived at the body, which closely approximates the true time of death when colonisation begins promptly after death. Conventional pathological markers — algor mortis, rigor mortis, and livor mortis — lose accuracy as decomposition advances; under such conditions, insect evidence becomes one of the most informative biological indicators available (Greenberg, 1991; Amendt *et al*., 2011).

Blowflies (Diptera: Calliphoridae) are typically the first insects to colonise exposed remains, arriving within minutes to hours of death. Their developmental rates are strongly governed by ambient temperature, making them the primary biological tool for PMImin estimation. The accumulated degree hours (ADH) model expresses larval age as the product of time elapsed and the amount by

which ambient temperature exceeds the species-specific lower developmental threshold (T0). Because a fixed amount of development requires a fixed ADH accumulation, the model allows larval age — and hence PMImin — to be reconstructed from temperature records (Anderson, 2000; Byrd & Allen, 2001). The third larval instar is of particular forensic value: it is the longest feeding stage, is most frequently recovered from decomposing remains, and attains a body length that can be measured precisely and compared with published developmental data (Higley & Haskell, 2001).

Dogs (*Canis lupus familiaris*) represent an underexplored model in forensic entomology despite their prevalence in both domestic and criminal contexts, dogs differ in body composition, fur density, and decomposition dynamics in ways that may alter insect colonisation patterns and succession timelines. In regions such as Southern Iraq, where dog carcasses are commonly encountered in forensic and environmental contexts, establishing species-specific and region-specific baseline data is both scientifically necessary and practically important.

Southern Iraq's hot desert climate presents distinctive challenges for forensic entomology. Al-Diwaniyah Governorate experiences summer temperatures frequently exceeding 43 °C, low humidity, and intense solar radiation — conditions that accelerate decomposition and may affect larval survival and growth rates in ways not captured by developmental data generated in temperate regions (Bansode et al., 2025; Kumar et al., 2025). The present study therefore aims to characterise PMImin estimation from third-instar larvae of forensically important species found on dog carcasses in two contrasting habitats under these extreme thermal conditions.

2. AIM OF THE STUDY

This study evaluates third-instar larval length and the accumulated degree hours (ADH) method for estimating the minimum post-mortem interval (PMImin) of dog (*Canis lupus familiaris*) carcasses under the high summer temperatures of Southern Iraq. The specific objectives are.

(1) To estimate PMImin from third-instar larvae of forensically important species using the ADH method.
(2) To compare PMImin between surface carcasses and carcasses buried at 50 cm depth.
(3) To compare PMImin between agricultural and cemetery environments under equivalent thermal conditions.
(4) To assess inter-species variation in PMImin and its implications for forensic casework in hot-climate Iraq.

3. MATERIALS AND METHODS

3.1 Study Area

The study was conducted in Al-Diwaniyah Governorate, Southern Iraq, characterised by a hot desert climate with prolonged summer heat, low rainfall, and intense solar radiation. Two contrasting habitats were investigated: an open agricultural area and a cemetery area.

3.2 Experimental Carcass Model

Dog (*Canis lupus familiaris*) carcasses were used as the study model. A total of twelve carcasses of comparable body mass were studied. Carcasses were obtained from animals that had died of natural causes or road accidents and were used with institutional ethical approval.

3.3 Carcass Placement and Experimental Design

The twelve carcasses were divided equally between the two habitats: six in the agricultural area and six in the cemetery. Within each habitat, three carcasses were placed on the ground surface and three were buried at 50 cm depth, yielding four experimental groups of three carcasses each: agricultural surface, agricultural buried, cemetery surface, and cemetery buried.

3.4 Temperature Recording and Developmental Thresholds

Ambient temperature was recorded for each group using a calibrated thermometer. Surface carcasses in both habitats were assessed at 43 °C. For surface groups, a lower developmental threshold (T0) of 8 °C was applied (thermal excess = 35 degree-hours per hour). For buried groups, a T0 of 10 °C was used (thermal excess = 33 degree-hours per hour), consistent with established developmental data for the recorded species.

3.5 Larval Collection and Preservation

Third-instar larvae were collected from each carcass using forceps and placed in plastic containers. They were then killed in boiling water at 80 °C for one minute and subsequently preserved in 80% ethanol for identification and measurement (Service, 1980). Approximately 15 larvae of each species were collected per carcass.

3.6 Species Identification

Collected specimens were identified to species level using established morphological keys, including Larval identification followed the diagnostic keys provided (Al-Ashbal, Al-Essa and Al-Saffar, 2020b, 2020a; Augul, Al-Saffar and Al-Ashbal, 2022; Al-Ashbal, Al-Essa and Hanaa H Al-Saffar, 2023; Al-Ashbal, Al-Essa and Hanaa Hani Al-Saffar, 2023).

3.7 Larval Measurement

Body length of preserved third-instar larvae was measured in millimetres using calibrated graph paper under a stereomicroscope. Published developmental data for each species were used to determine the ADH required to reach the observed larval stage and length.

3.8 PMImin Estimation

Insect age was expressed in accumulated degree hours and computed as follows.

$ADH = (T - T_0) \times t$

PMImin was then estimated by rearranging this relationship.

$PMI_{min} = ADH \div (T - T_0)$

Results are reported in hours and converted to days (hours ÷ 24). Because larval-based estimates date colonisation rather than death, they represent minimum intervals. The statistical formula of Byrd & Castner(2010) was applied to estimate the post-mortem interval from larval-length data.

$$T = \frac{y - \mu_t}{\sqrt{\sigma_t^2 + Var(\mu_t)}}$$

Where

T = the statistical test value used to estimate the post-mortem interval;

y = the measurement of the unknown specimen (larval length collected from the carcass);

μ_t = the reference mean larval length at time t, derived from developmental reference data;

σ^2_t = the variance of measurements at time t from the reference developmental dataset;

$Var(\mu_t)$ = the variance of the mean estimate, calculated as:

$$Var(\mu_t) = \hat{\sigma}_t^2 \sum_{i=1}^{k} \frac{a_{ti}^2}{n_t}$$

k = the number of independent reference samples;

n_t = the number of individuals in the sample at time t.

001) was additionally applied to larval length data for interval estimation:

where y is the measured larval length, μt is the reference mean length at time t, σ²t is the reference variance, and Var(μt) is the variance of the mean estimate.

4. RESULTS

4.1 Surface Carcasses — Agricultural Area

Three species were recovered as third-instar larvae from surface carcasses in the agricultural area (43 °C; T0 = 8 °C). *Calliphora vicina* produced the lowest PMImin (8.486 h; ADH = 297), followed by *Chrysomya megacephala* (9.429 h; ADH = 330) and *Calliphora vomitoria* (9.771 h; ADH = 342) (Table 1).

Table 1: PMImin estimates from surface dog carcasses — Agricultural Area (43 °C; T0 = 8 °C).

Insect Species	Life Stage	Temp. (°C)	T0 (°C)	ADH	PMImin (h)	PMImin (days)
Calliphora vicina	Third instar	43	8	297	8.486	0.354
Calliphora vomitoria	Third instar	43	8	342	9.771	0.407
Chrysomya megacephala	Third instar	43	8	330	9.429	0.393

The low PMImin estimate recorded for *Calliphora vicina* (8.486 h; ADH = 297) is consistent with its well-established role as one of the earliest colonisers of carrion in the region. Blowflies of the genus *Calliphora* have special significance in forensic entomology as they are frequently the first colonisers of a carcass, and their developmental age therefore corresponds most closely with the actual time of death, providing the most valuable PMImin estimate (Niederegger, 2021). The slightly higher estimate for *Chrysomya megacephala* (9.429 h) reflects its pattern of secondary colonisation, as *C. megacephala* typically arrives at carcasses 0.5–2 h after placement (Wang et al., 2021). *Calliphora vomitoria* recorded the highest estimate in this group (9.771 h; ADH = 342), consistent with its arrival as a late primary coloniser.

4.2 Buried Carcasses (50 cm) — Agricultural Area

Under burial at 50 cm in the agricultural area (43 °C; T0 = 10 °C), PMImin rose substantially relative to the surface. *Chrysomya albiceps* estimated 14.545 h (ADH = 480), *Calliphora vicina* 15.909 h (ADH = 525), and *Chrysomya megacephala* recorded the highest value in this group at 20.727 h (ADH = 684) (Table 2).

Table 2: PMImin estimates from buried dog carcasses (50 cm) — Agricultural Area (43 °C; T0 = 10 °C).

Insect Species	Life Stage	Temp. (°C)	T0 (°C)	ADH	PMImin (h)	PMImin (days)
Calliphora vicina	Third instar	43	10	525	15.909	0.663
Chrysomya albiceps	Third instar	43	10	480	14.545	0.606
Chrysomya megacephala	Third instar	43	10	684	20.727	0.864

The near-doubling of the *C. vicina* estimate relative to the surface group (from 8.486 h to 15.909 h) indicates substantially delayed colonisation imposed by the soil barrier. These findings are consistent with Sharma et al. (2025), who reported that surface carcasses decomposed faster than buried ones and that burial depth restricted insect access and slowed decomposition. The appearance of *Chrysomya albiceps* in this group, with a lower ADH than *C. megacephala*, suggests differential penetration capability among Calliphoridae under burial conditions, as *C. albiceps* has been documented as a more adaptable coloniser of partially concealed remains (Bansode et al., 2025). The present findings further emphasize the ecological and forensic importance of *Chrysomya albiceps* as one of the most widespread blowfly species in hot and semi-arid environments. Lefta (2022) highlighted the practical importance of this species by evaluating the biological activity of *Solanum nigrum* extracts against its immature stages, reflecting its wide distribution, high abundance, and significance in entomological studies. These observations are consistent with the present study, in which *C. albiceps* was among the dominant species associated with decomposing carcasses throughout the decomposition process.

4.3 Surface Carcasses — Cemetery Area

Under identical thermal conditions to the agricultural surface group (43 °C; T0 = 8 °C), cemetery surface carcasses yielded marginally higher estimates: *Calliphora vicina* 8.143 h (ADH = 285), *Chrysomya megacephala* 9.857 h (ADH = 345), and *Calliphora vomitoria* 11.000 h (ADH = 385) (Table 3).

Table 3: PMImin estimates from surface dog carcasses — Cemetery Area (43 °C; T0 = 8 °C).

Insect Species	Life Stage	Temp. (°C)	T0 (°C)	ADH	PMImin (h)	PMImin (days)
Calliphora vicina	Third instar	43	8	285	8.143	0.339
Calliphora vomitoria	Third instar	43	8	385	11.000	0.458
Chrysomya megacephala	Third instar	43	8	345	9.857	0.411

Interestingly, *C. vicina* produced a slightly lower estimate in the cemetery (8.143 h) than in the agricultural area (8.486 h), suggesting marginally earlier colonisation in that habitat for this species. For *C. vomitoria* and *C. megacephala*, estimates were higher in the cemetery than in the agricultural area, pointing to habitat-level differences in microclimate and insect accessibility that influence oviposition timing differently across species. These findings are consistent with Sharma et al.,(2025), who demonstrated that environmental differences between habitats directly influence the timing of insect colonisation and consequently affect PMImin estimates.

4.4 Buried Carcasses (50 cm) — Cemetery Area

Buried cemetery carcasses (43 °C; T0 = 10 °C) produced the highest estimates of the study for two of the three species. *Calliphora vicina* and *Chrysomya megacephala* both estimated 12.727 h (ADH = 420), while *Chrysomya albiceps* recorded 16.970 h (ADH = 560) (Table 4).

Table 4: PMImin estimates from buried dog carcasses (50 cm) — Cemetery Area (43 °C; T0 = 10 °C).

Insect Species	Life Stage	Temp. (°C)	T0 (°C)	ADH	PMImin (h)	PMImin (days)
Calliphora vicina	Third instar	43	10	420	12.727	0.530
Chrysomya albiceps	Third instar	43	10	560	16.970	0.707
Chrysomya megacephala	Third instar	43	10	420	12.727	0.530

The convergence of *C. vicina* and *C. megacephala* estimates at 12.727 h under cemetery burial conditions is noteworthy and may reflect the equalising effect of the soil barrier on both early and secondary colonisers, compressing the arrival window for Calliphoridae. *Chrysomya albiceps* again recorded the highest estimate in the buried cemetery group (16.970 h; ADH = 560), consistent with its relatively later establishment under concealed conditions. Similar patterns were reported by Alajmi et al. (2023), who found that burial reduced blowfly diversity and delayed colonisation in hot-climate settings.

4.5 Comparative Summary Across All Groups

Across all four groups, PMImin spanned from 8.143 h (*C. vicina*, cemetery surface) to 20.727 h (*C. megacephala*, agricultural buried). Two consistent gradients emerged: (1) burial at 50 cm increased estimates relative to surface carcasses in both habitats, and (2) inter-species variation produced a range of up to 7.2 h within a single group (agricultural buried), underscoring the importance of multi-species estimation. Table 5 consolidates all results.

Table 5: Comparative summary of PMImin across all experimental groups. All specimens were third-instar larvae. Surface groups T0 = 8 °C; buried groups T0 = 10 °C.

Habitat	Condition	Species	Temp. (°C)	ADH	PMImin (h)	PMImin (d)
Agricultural	Surface	*C. vicina*	43	297	8.486	0.354
Agricultural	Surface	*C. vomitoria*	43	342	9.771	0.407
Agricultural	Surface	*C. megacephala*	43	330	9.429	0.393
Agricultural	Buried 50 cm	*C. vicina*	43	525	15.909	0.663
Agricultural	Buried 50 cm	*C. albiceps*	43	480	14.545	0.606
Agricultural	Buried 50 cm	*C. megacephala*	43	684	20.727	0.864
Cemetery	Surface	*C. vicina*	43	285	8.143	0.339
Cemetery	Surface	*C. vomitoria*	43	385	11.000	0.458
Cemetery	Surface	*C. megacephala*	43	345	9.857	0.411
Cemetery	Buried 50 cm	*C. vicina*	43	420	12.727	0.530
Cemetery	Buried 50 cm	*C. albiceps*	43	560	16.970	0.707
Cemetery	Buried 50 cm	*C. megacephala*	43	420	12.727	0.530

Calliphora vicina returned the earliest or joint-earliest estimate in every group in which it occurred, reinforcing its value as the closest entomological proxy for time of death.

5. DISCUSSION

5.1 Effect of Burial on PMImin Estimation

Burial produced a clear and consistent increase in PMImin at both study sites. For *Calliphora vicina*, estimates roughly doubled between surface and buried groups: from 8.486 h to 15.909 h in the agricultural area, and from 8.143 h to 12.727 h in the cemetery. This pattern is consistent with Bansode *et al.* (2025), who reported that surface carcasses decomposed considerably faster than buried ones and that Calliphoridae, being large-bodied flies dependent on direct access to carrion, were among the earliest above-ground colonisers but were largely absent from buried environments. The soil layer at 50 cm acts as a physical barrier, forcing oviposition at soil openings or along odour plumes and thereby delaying colonisation onset and reducing thermal unit accumulation. Sharma *et al.* (2025) similarly found that increasing burial depth restricted insect access and delayed decomposition, reinforcing the burial effect observed here.

5.2 Effect of Habitat and Location

Differences between the agricultural area and the cemetery were generally modest for surface carcasses but still ecologically informative. For *C. vicina*, the cemetery surface estimate (8.143 h) was marginally lower than the agricultural one (8.486 h), suggesting slightly earlier colonisation in the cemetery, possibly due to reduced disturbance and more shaded, stable microclimatic conditions that favour fly activity. By contrast, *C. vomitoria* and *C. megacephala* showed higher estimates in the cemetery than in the agricultural area, reflecting species-specific sensitivity to habitat microclimate. These findings support previous observations that habitat characteristics — including soil type, vegetation cover, shading, and local insect populations — significantly influence insect succession and PMImin estimation (Sharma et al., 2025).

5.3 Inter-Species Variation in PMImin

Marked inter-species variation in PMImin was observed under identical thermal conditions. In the agricultural buried group, estimates ranged from 14.545 h (*C. albiceps*) to 20.727 h (*C. megacephala*) — a span of 6.2 h. This reflects fundamental differences in arrival timing, oviposition behaviour, and colonisation ecology among species. *Calliphora vicina* is well established as a rapid, early coloniser, producing estimates that most closely approximate the true time of death (Greenberg, 1991; Anderson, 2000). The dominance of *Chrysomya* species on surface carcasses is consistent with Boeira *et al.* (2024), who found *C. albiceps* and *C. megacephala* to be among the most frequently recovered species across diverse settings. The breadth of the inter-species range is the central practical caution of the study: reliance on a single species can misplace the PMImin estimate by several hours.

5.4 Third-Instar Larvae as Biological Clocks

The third larval instar proved to be a reliable and discriminating biological clock under the extreme temperatures of Southern Iraq. Its long duration, predictable growth trajectory, and high frequency of recovery from decomposing remains make it the stage most likely to be encountered and most amenable to ADH-based estimation. In the present study, third-instar lengths underpinned all twelve PMImin estimates, and the orderly species-specific separation of values demonstrates that the stage retains discriminating power even under the compressed developmental timescales associated with temperatures of 43 °C. This finding aligns with Wells (2024), who confirmed the practical applicability of the thermal summation principle under fluctuating field temperatures, and with Obafunwa et al. (2025), who identified third-instar laboratory rearing as yielding the greatest accuracy gains in field validation studies.

5.5 Reliability and Limitations of the ADH Method

The ADH method assumes that development is linearly related to temperature above the lower threshold. At 43 °C, this assumption approaches the upper thermal limits of several species, where non-linearity and reduced larval survival may bias estimates. The maggot-mass effect can further raise temperatures within larval aggregations above ambient. Matuszewski *et al.* (2023) demonstrated that PMImin can deviate from the true interval by 2–72 h depending on the method and dataset, underscoring the need for locally validated developmental data. Obafunwa *et al.* (2025) recommend recording soil temperature at multiple depths in buried cases for accurate ADH calculation — a recommendation of particular relevance under Iraqi conditions, where surface and subsurface thermal regimes diverge sharply.

6. CONCLUSION

Third-instar larvae of *Calliphora vicina*, *Calliphora vomitoria*, *Chrysomya megacephala*, and *Chrysomya albiceps*, interpreted through the accumulated degree hours method, provided coherent and discriminating PMImin estimates for dog carcasses under the extreme summer temperatures of Southern Iraq. Surface estimates ranged from 8.143 h to 11.000 h, while buried estimates ranged from 12.727 h to 20.727 h. Burial at 50 cm consistently and substantially increased PMImin — roughly doubling the *C. vicina* estimate at both sites — by delaying insect access and reducing thermal accumulation. Habitat differences between the agricultural area and the cemetery produced modest but ecologically informative variation. *Calliphora vicina* consistently provided the closest approximation to the time of death. A multi-species approach, combined with accurate temperature records and locally validated developmental data, is essential for reliable forensic casework in hot-climate Iraq.

7. Recommendations

(1) Collect from multiple species. Reliance on a single species can introduce errors of up to ~7 h; always recover larvae from every species present.

(2) Record burial status and depth precisely. Even 50 cm of burial markedly delays colonisation and inflates PMImin; forensic reports must specify whether remains were surface-exposed or buried and at what depth.

(3) Document ambient and soil temperature. Measure temperature at multiple depths (5, 10, 20 cm) so that ADH is calculated from conditions actually experienced by larvae, especially in buried cases.

(4) Develop dog-specific developmental datasets. Dog carcasses produce systematically lower PMImin estimates than pig carcasses under the same conditions; dog-specific reference data should be established for Iraqi forensic practice.

(5) Use locally validated developmental data. Temperate-region datasets cannot be transferred uncritically to Southern Iraq; region-specific calibration is essential.

(6) Frame *Calliphora* estimates as lower bounds. Treat *C. vicina* estimates as the closest indicator of the time of death for surface remains; in buried cases, expect Calliphoridae to be delayed and treat their estimates accordingly.

Ethical Approval

This study was approved by the Scientific and Ethical Committee of the College of Science, University of Al-Qadisiyah, Iraq (No. 007). All procedures involving animal carcasses were conducted in accordance with institutional and national ethical guidelines.

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