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Early View

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Insect-Mediated Decomposition of Buried and Unburied Pig Remains for Postmortem Interval Determination in Different Seasons under Temperate Slovenian Conditions

Key words

postmortem;
entomology;
insect;
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Abstract: Forensic entomology is an increasingly important tool in veterinary forensic investigations, particularly in cases of suspected animal abuse, which is classified as a criminal offence under Article 341 of the Slovenian Criminal Code. Reliable estimation of the postmortem interval (PMI) is essential in such cases; however, region-specific forensic entomology data for Slovenia have been lacking. This study investigated the effects of season and burial on insect colonisation, larval development, and decomposition under natural conditions in a temperate Slovenian environment. Hind limbs from 40 pigs (*Sus scrofa domestica*), were studied across four seasons (autumn 2022 to summer 2023). In each season, paired limbs were either left exposed on the ground surface or buried at a depth of 50 cm. No insect activity was observed on unburied limbs in autumn or winter, most likely due to low ambient temperatures and precipitation. In spring, limited colonisation by flies of the families *Calliphoridae* and *Sarcophagidae* occurred on unburied limbs after the 5th day, with minimal larval development. In summer, rapid and extensive insect colonisation of unburied limbs was observed from the 2nd day, peaking on the 5th day and resulting in near-complete soft tissue loss by the 8th day. Buried limbs showed no insect presence throughout the study, with only occasional beetles of the genus *Creophilus* and *Saprinus* detected in summer. These findings demonstrate that season and burial strongly influence insect activity and decomposition rates in Slovenia. The results provide the first locally derived detailed forensic entomology reference data applicable to veterinary forensic practice and support more accurate PMI estimation in criminal investigations involving animal abuse, based on the entomological findings at the crime scene.

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Introduction

Following death, a carcass begins to decompose, providing a food source for scavengers and a variety of insects. These insects colonise the remains at different times, often within seconds of the animal's death. In some cases, colonisation may even occur while the animal is still alive if the animal is in agony (1). The application of knowledge of the life cycle of insects and native fauna is used in forensic entomology to determine the age of collected specimens and therefore the time of colonisation (TOC), which is often associated with the time elapsed since the death of an individual, i.e. the postmortem interval (PMI), an essential yet frequently challenging parameter to estimate in both human and veterinary forensic investigations (2). The application of forensic entomology extends beyond the estimation of the postmortem interval (PMI); it can also provide evidence that a carcass was moved from its original deposition site, contribute to the assessment of the circumstances and cause of death, and support the association of suspects with the scene of death (2).

The forensically important insect species most frequently involved in the decomposition of a carcass include blowflies (order *Diptera*, family *Calliphoridae*), flesh flies (order *Diptera*, family

Sarcophagidae) and houseflies (order *Diptera*, family *Muscidae*), but also carrion beetles (order *Coleoptera*, family *Silphidae*) and skin beetles (order *Coleoptera*, family *Dermestidae*) (1, 3, 4). Blowflies and houseflies are the first to arrive on a carcass, followed by flesh flies. The adult flies enter a dead animal and lay their eggs or larvae at the natural orifices (nostrils, mouth, ears, anus and later the genitalia), eyes and wounds (5). The resulting larvae feed on the body and remains and pass through three stages or instars before they stop feeding and are ready to pupate (6). The larvae hatch from the blowfly eggs within 6 to 40 hours, their development depends primarily on the temperature humidity and precipitation of the environment and can take 3 to 10 days (5). The pupal stage lasts 6 to 18 days (5). Immediately after hatching, the adult fly can mate and lay eggs, but often leaves the body (7). The developing larvae feed on the soft tissues of the carcass, destroying the skin and mucous membranes, which facilitates access for many other insect species and microbiota that accelerate the decomposition of the carcass. During their development, they excrete volatile organic compounds that attract other insect species, especially ants, wasps and various beetles (8), which usually appear on the carcass in the later stages of decomposition when it becomes bloated (5).

The rate of larval development depends primarily on ambient temperatures (9), with lower ambient temperatures leading to slower insect growth and higher ambient temperatures leading to faster growth. For each species, there is an upper and lower threshold above or below which the insect stops growing (6, 10), and each developmental stage has its own temperature requirement, so that each species has its own defined number of accumulated degree-days or accumulated degree-hours to complete its development (11). Once the temperature-dependent development of the larvae is known, it can be compared with the temperatures at the site of death, and the PMI can be estimated (11). Developmental thresholds for forensically important flies are generally between 6 and 10 °C (12, 13), with larval development slowing considerably at low temperatures and coming to a complete halt below 4 °C (14). Additionally, decreasing relative humidity has been shown to slow larval development by prolonging the time to eclosion, although humidity requirements vary among fly species (15).

The number and species of insects depends on the local vegetation and the season (16, 17), their number and activity are primarily influenced by environmental conditions such as temperature, precipitation, humidity and wind, as well as by the microbiota of the carcass, which attracts insects with metabolic by-products (gases, alcohols, etc.) produced during the decomposition process. The composition of these products changes over time, and attracts different insect species at different times during decomposition (7, 18).

Burial is an important factor influencing the colonisation of carcasses by insects. Although the decomposition process of buried and unburied remains is fundamentally similar, the rate of decomposition varies considerably. The depth of burial and the properties of the soil are decisive factors for the rate of decomposition (19, 20, 21). The soil surrounding the buried carcasses forms an insulating layer that protects the carcass from weather phenomena such as rain, snow and direct sunlight as well as from insects and scavengers (22, 23). Non-biological factors such as temperature, oxygen content and humidity are directly related to burial depth, and therefore indirectly to biological factors such as the quantity and composition of insects and microbiota (24, 25).

The aim of this study was to assess the effects of temperature and burial under natural environmental conditions on the rate of insect colonisation and decomposition of buried and unburied pig (*Sus scrofa domestica*) hind limbs in a temperate region of Slovenia, with the objective of establishing PMI estimates for the first time in this specific environmental context. The results will provide invaluable support for estimating the PMI in forensic investigations.

Material and methods

This work was carried out at the Institute of Pathology, Forensic Veterinary Medicine, Wildlife, Bees and Aquaculture at the Veterinary Faculty, University of Ljubljana, with experimental work conducted in a shady forest area in central Slovenia in autumn from 15 to 24 November 2022, in winter from 20 to 29 January 2023, in spring from 17 to 26 April 2023 and in summer from 11 to 18 July 2023. Before we started the four series of experiments, we made sure that the outside temperatures were appropriate for each season.

Fourthy pigs of the breed Landrace weighing between 20 and 35 kg were used in a study that primarily focused on the influence of time, temperature and burial on cartilage decomposition (26), while also assessing the decomposition of the intact limb as a whole (Permit for the use of category 2 animal by-products with permit number U34453-38/2021/5 was granted by the Administration of the Republic of Slovenia for Food Safety, Veterinary and Plant Protection on 21 May 2021). The pigs were euthanised due to incurable, chronic diseases that significantly impaired their quality of life and were euthanised in accordance with Article 26 of the Animal Protection Act (27) by the intravenous administration of sodium pentobarbital (Richter Pharma AG, Wels, Austria) 40 mg/kg body weight, having previously been deeply anaesthetised by the intravenous administration of ketamine (Vetoquinol UK Limited, Towcester, UK) 20 mg/kg body weight. Ten pigs were euthanised at the same time in each season, and the hind limbs were removed at the hip joint and labelled, the left limb with the letter P (unburied) and the right limb with the letter G (buried).

In a shady forest area in central Slovenia, limbs labelled G and a thermometer probe (TFA Dostmann GmbH & Co. KG, Reicholzheim, Germany) were buried at a depth of 50 cm in sandy loam with a pH of 4.1, while limbs labelled P were left exposed on the surface above the buried limbs. A cage was placed over the graves and covered with a net with 2 x 5 cm openings to prevent access by large scavengers.

The limbs were examined on the day of euthanasia (day 1), then at 1-day intervals over the next 4 days and on the 8th and 10th day after death, once on site between 5:00 and 5:30 am. During the examination, the limbs were photographed, and the number of insects (eggs, larvae) was assessed semi-quantitatively using a scale developed for the purposes of this study, based to the proportion of limb surface they covered. If the insects covered less than 3 % of the limb, we categorised the number of insects as individual (I), if insects covered 3–25 % of the limb, we categorised the number of insects as low (+), if they covered 25–50% of the limb, we categorised the number of insects as moderate (++), and if they covered more than 50% of the limb, we categorised the number of insects as high (+++). Four larval specimens ~~larvae~~ were numbed by submersion in 70% ethanol for 10 minutes and measured once using a ruler. At least 10 representatives of each species were collected and preserved in 70% ethanol (Kefo, Ljubljana, Slovenia). Insect species and their developmental stages were identified using a Leica M50 stereomicroscope (Zeiss, Stuttgart, Germany) according to the classifications of Szpila (2009) and Byrd&Tomberlin (2019) (28, 29). Prior to sampling, surface temperature and temperature at the depth of the limb were measured; if the limb was inhabited by insects, temperature was also measured adjacent to the limb; temperature and humidity data were also collected from a local government weather station at the distance of 3.4 km. Temperature, humidity and precipitation data for each time period are presented in Table 1. The temperature adjacent to the unburied limbs is shown in Table 2.

Results

Insects were found on unburied limbs in spring and summer, while on the buried limbs individual insects were only found in summer. In winter and autumn, no insects were found on the limbs.

Spring

In spring, individual flies from the family *Calliphoridae* and *Sarcophagidae* along with their eggs were first observed on the 5th day after exposure, with a slight increase in egg numbers noted after the 8th day. On the 8th day, in addition to eggs, a low number of larvae from flies of the family *Calliphoridae* and individual larvae of flies of the family *Sarcophagidae* were observed. The larvae measured 0.3 to 0.5 cm in length. On the 10th day, the number of larvae from flies of the family *Calliphoridae* and *Sarcophagidae* did not increase, on this day in addition to the larvae a few beetles of the genus *Oiceoptoma* spp. were found. The larvae measured 0.5 cm in length. As neither egg nor larval amount increased substantially after the 8th day, or during the subsequent observation period, the recording of limb decomposition was discontinued. The temperature adjacent to the unburied limbs did not differ considerably from the temperature on the surface of the graves. No insects, their larvae or eggs were found on the articular cartilage of the buried limbs in spring.

The number of insects, the temperatures next to the unburied limbs and on the surface of the grave, the average daily temperatures and the presence of precipitation in spring are shown in Table 2.

Summer

In summer the first flies arrived on the limbs within 3 minutes, with a marked increase in their numbers after 20 minutes. The first eggs were detected after 90 minutes, followed by a substantial increase in egg deposition after 10 hours. In addition to flies, individual wasps were observed from 20 minutes onwards.

The first larvae of flies of the family *Calliphoridae* and larvae of the family *Sarcophagidae* were found on unburied limbs in summer on the 2nd day and covered less than 25% of the limb. The larvae measured 0.3 cm in length. The temperature adjacent to the unburied limbs was 0.6 °C higher than the temperature on the surface of the graves.

On the 3rd day the larvae covered 25% of the limb. The larvae measured 0.6 cm in length. The temperature adjacent to the unburied limbs was 0.5 °C higher than the temperature on the surface of the graves.

On the 4th day the number of fly larvae of the family *Calliphoridae* and *Sarcophagidae* increased substantially, the insects covered between 25 and 50% of the limb, the soft parts of the limbs were slightly gnawed. The larvae measured 0.6 to 1.1 cm in length. The temperature adjacent to the unburied limbs was 1.1 °C higher than the temperature on the surface of the graves.

On the 5th day the number of fly larvae of the family *Calliphoridae* reached its peak, the insects covered 80% of the limb, also individual beetles of the genus *Creophilus* and *Saprinus* were observed. The soft tissues of the limbs were moderately eaten away, the bones were partially visible, and white foam could be seen on the ground around the limbs. The larvae measured 0.9 to 1.6 cm in length. The temperature adjacent to the unburied limbs was 2.2 °C higher than the temperature on the surface of the graves.

On the 8th day the number of fly larvae of the family *Calliphoridae* decreased substantially, the insects covered less than 25% of the limbs, and individual beetles of the genus *Creophilus* and *Saprinus* were also observed. The soft tissues of the limbs were mostly eaten away; the bones were almost completely exposed. The larvae measured 0.3 to 1 cm in length. The temperature adjacent to the unburied limbs was 0.2 °C higher than the temperature on the surface of the graves.

Only individual beetles of the genus *Oiceoptoma* were observed on the buried limbs on the 4th, 5th and 8th day; fly larvae were not found on the limbs. The temperature adjacent to the unburied limbs corresponded to the temperature on the surface of the graves. The decomposition of the unburied limbs is shown in Figure 1.

The number of insects, the temperatures next to the unburied limbs and on the surface of the grave, the average daily temperatures and the presence of precipitation in summer are shown in Table 2.

Table 1: Temperatures in the burial area and data on relative humidity, precipitation as well as average, maximum and minimum temperatures from the main meteorological station

Day	Season	T. bur. (°C)	T. sur. (°C)	T. avg. (°C)	T. max. (°C)	T. min. (°C)	Humi d. (%)	Precip. (mm)	Snow (mm)	Rain
1	Autumn	12.5	10.2	11.3	14.1	8.9	75	2.5	0	Yes
2	Autumn	12.4	8.2	10	11.6	9	87	0.5	0	Yes
3	Autumn	12.3	8	10	11.6	8.6	84	3.3	0	Yes
4	Autumn	12.2	8.1	7.7	10.7	6.2	88	4.8	0	Yes
5	Autumn	11.7	4.5	5.2	7.9	4.4	88	9.9	0	Yes
8	Autumn	10.1	2.6	3.9	5	3.1	87	1.4	0	Yes
10	Autumn	8.2	1.8	4	7.6	2.3	88	0	0	No
1	Winter	2.5	–4	0.1	1.7	–0.5	86	7.3	12	No
2	Winter	3	–1.5	0.2	3.7	–5.1	76	0.3	12	No
3	Winter	2.8	–1.1	0.6	1.8	–0.6	83	0	11	No
4	Winter	4.1	–0.5	1.6	3.4	0.4	89	6.7	13	Yes
5	Winter	4.4	–0.5	2	3.5	0.9	89	17.4	10	Yes
8	Winter	5.2	–0.5	1.1	3.9	0.1	71	0	7	No
10	Winter	5.3	–0.8	0.9	2.5	–0.1	77	0	6	No
1	Spring	11.8	11.1	13	17.4	7.1	61	0	0	No
2	Spring	9.9	6.1	12.1	16.9	7.5	71	0	0	No
3	Spring	9.2	8.3	12.7	17.8	9.6	68	0	0	No
4	Spring	9.3	9.3	10.1	13	9.5	74	0.3	0	Yes
5	Spring	9.3	7.3	11.3	18.4	8.1	73	0	0	No
8	Spring	10.2	11.2	11.2	15.1	10.4	83	0.1	0	Yes
10	Spring	9.4	7	11.6	18.2	4.2	62	0	0	No
1	Summer	19.8	26.3	28.1	34	19.5	58	0	0	No
2	Summer	17.7	20.9	25.2	31.5	19.3	67	5	0	Yes
3	Summer	17.5	17.4	18.5	26.5	17.2	84	14.6	0	Yes
4	Summer	18.3	16.7	22.4	28.4	15.7	70	54.8	0	Yes
5	Summer	17.9	16	25.8	32.6	16.8	62	0	0	No
8	Summer	19.8	20.5	23.2	31.6	19.1	69	0	0	No

Legend: T. bur. – temperature in the graves; T. sur. – temperature on the surface of the graves; T. avg. – average daily temperature; T. max. – highest daily temperature; T. min. – lowest daily temperature; humid. – humidity; precip. – precipitation

Table 2: Semiquantitative assessment of the number of insects, the temperature measured adjacent to the hind limbs, the surface temperature and the average daily temperature

Day	Season	Number of insects on/in buried limbs	Number of insects on/in unburied limbs	Temperatures next to unburied limbs (°C)	Surf. temp. (°C)	Avg. temp. (°C)	Rain
1	Spring	/	/	11.1	11.1	13.0	No
2	Spring	/	/	6.1	6.1	12.1	No
3	Spring	/	/	8.3	8.3	12.7	No
4	Spring	/	/	9.3	9.3	10.1	Yes
5	Spring	/	/I	7.3	7.3	11.3	No
8	Spring	/	+	11.3	11.2	11.2	Yes
10	Spring	/	+	7.1	7	11.6	No
1	Summer	/	/	26.3	26.3	28.1	No
2	Summer	/	+	21.5	20.9	25.2	Yes
3	Summer	/	+	17.9	17.4	18.5	Yes
4	Summer	I	++	17.8	16.7	22.4	Yes
5	Summer	I	+++	18.2	16	25.8	No
8	Summer	I	+	20.7	20.5	23.2	No

Legend: / = no insects, I = individual (< 3%) insects, + = low (< 3–25%) number of insects, ++ = moderate (25–50%) number of insects, +++ = high (> 50%) number of insects; surf. temp. – surface temperature; avg. temp. – average daily temperature

Discussion

In recent years, numerous forensic investigations to determine the cause of death in suspected cases of animal abuse have been conducted within pre-trial criminal proceedings in Slovenia, where animal abuse is classified as a criminal offence under Article 341 of the Slovenian Criminal Code. Over the past five years, the number of such cases has increased considerably, with investigators, police officers, and veterinary inspectors increasingly requesting estimates of the PMI. To address this, we currently rely on approximate values reported in the literature; however, it is well recognised that postmortem changes are strongly influenced by local environmental conditions. Consequently, we decided to undertake a study aimed at improving the understanding of postmortem changes under local conditions.

In our study, the season and burial had a substantial influence on the number of insects and their larvae. In autumn and winter, no insects or their larvae were observed on/in the buried and unburied limbs. We believe that the low surface temperature played a crucial role in the absence of insects on the unburied limbs in autumn and winter, as in autumn the average surface temperatures ranged from 4 to 11.3 °C and in winter the average surface temperatures ranged from 0.1 to 2 °C, with the lowest temperature being -5.1 °C. Studies have shown that developmental thresholds for forensically important fly species vary from species to species and generally range between 6 and 10 °C (12, 13), with larval development slowing significantly at low temperatures and low relative humidity and coming to a complete halt below 4 °C (14). In addition, most fly species stop laying eggs at temperatures of 10 °C or below (29), further confirming our hypothesis. Although average temperatures were slightly above 10 °C in the first three days of autumn, we did not see any flies during the entire sampling period, which could be a consequence of the rain in the first eight days of the experiment, as it has been found that rain significantly reduces insect activity and thus affects oviposition (30).

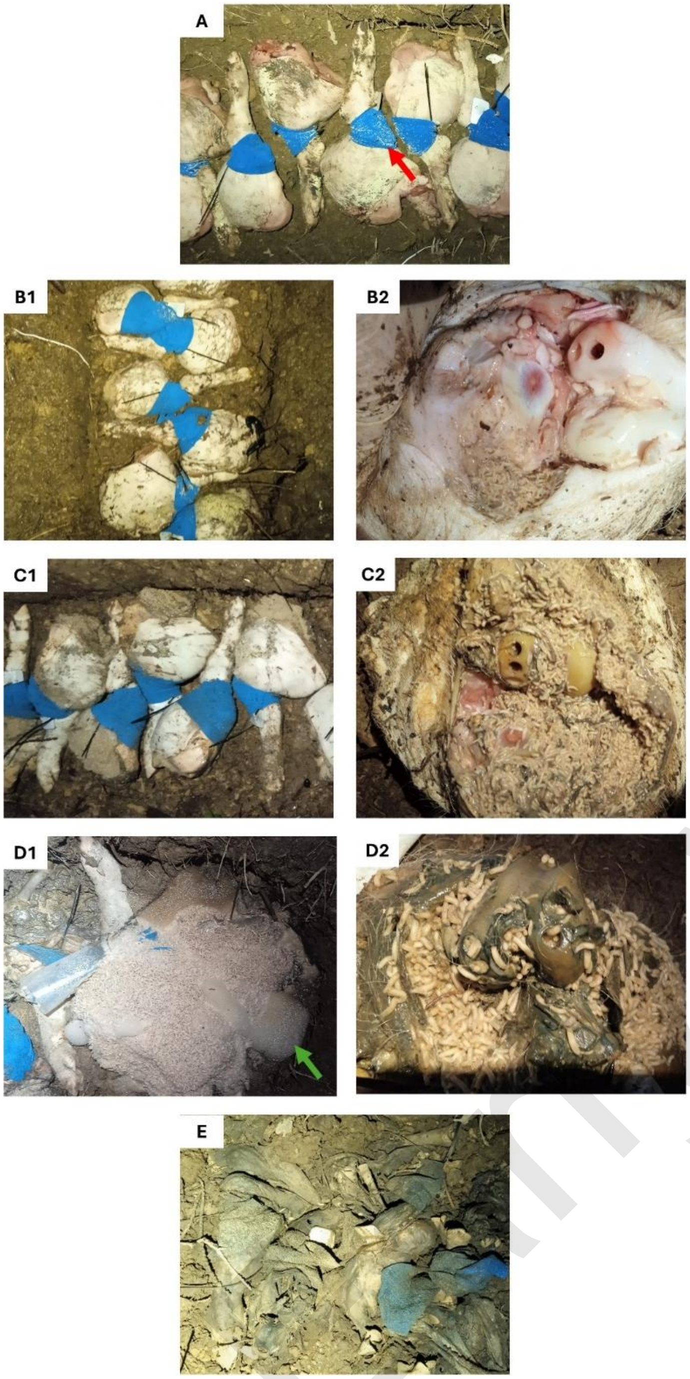

Figure 1: Unburied hind limbs in summer; A – 2nd day of sampling; small number of larvae (red arrow). B1, B2 – 3rd day of sampling; small number of larvae (red arrow). C1, C2 – 4th day of sampling; moderate number of larvae (red arrow). D1, D2 – 5th day of sampling; large number of larvae, foam (green arrow). E – 8th day of sampling; soft tissues are largely eaten away

In spring, we observed small numbers of flies from the family *Calliphoridae* and *Sarcophagidae*, along with their eggs in the unburied limbs only on the 5th day, with their number increasing slightly on the 8th and 10th day, when we first noticed a low number of larvae. The surface temperatures were between 10.1 and 13 °C. Although the average ambient temperatures during the study period were within the range typically associated with *Calliphoridae* activity – between 12 and 30 °C (31, 32, 33) – no insects or larvae were observed during the first four days of the study. We hypothesise that this absence of insect activity may be attributed to persistently low temperatures, which reached as low as 4.2 °C for most of the day, thereby effectively inhibiting insect activity and colonisation. Because of the small number of flies from the family *Calliphoridae* and their larvae on the unburied limbs in spring, we did not measure any temperature increase in the vicinity of the unburied limbs, which is consistent with the results of similar studies that have shown that individual larvae and larvae in the early stages of development have no significant effect on the temperature of the carcass and its immediate surroundings (16, 20, 34). During spring, the observed fly larvae did not exceed 0.5 cm in length.

In summer, we observed flies from the family *Calliphoridae* and their larvae in the unburied limbs from the 2nd to 8th day. Their numbers gradually increased until the 5th and then declined sharply on the 8th day, as by then they had eaten all the soft parts of the unburied limbs. In summer, the number of insects had a substantial impact on the rate of limb decomposition, with larvae consuming most of the soft tissues of all unburied limbs, and leaving only skeletal remains within 8 days. After the 8th day, no flies were observed on the limbs, with only occasional beetles of the genus *Creophilus* present. We also observed a marked increase in insect numbers on the unburied limbs following the end of a 3-day rainy period, supporting the role of rainfall in modulating insect activity. Similar findings were reported by Payne et al. (1965), who observed that carcasses not colonized by insects underwent mummification and remained largely unchanged for several months, whereas carcasses experiencing high insect activity lost up to 90% of their body mass within six days (35). During this period in addition to flies, individual wasps were observed 20 minutes after the limbs were buried or left on the surface, which was contrary to the data in the literature where it was found, that wasps usually appear on the carcass in the later stages of decomposition when it becomes bloated (5). In summer, insects, especially their larvae, had a substantial impact on the temperature near the unburied limbs, as the number of larvae increased and the temperatures near the unburied limbs were up to 2.2 °C higher than the general surface temperature after the 3rd day. This phenomenon was most likely due to the heat released by larval activity and metabolism, which can raise the temperature of a larvae-covered carcass by up to 7 °C above the temperature of the immediate surroundings (16, 20, 34, 36). Consequently, the ambient temperature measured in these cases often does not correspond to the temperature to which the carcass is exposed and can therefore lead to errors when using temperature-based measurement methods to determine the PMI. During summer, the observed larvae measured 0.3 cm on the 2nd day, 0.6 cm on the 3rd day, 0.6 to 1.1 cm on the 4th day, 0.9 to 1.6 cm on the 5th day and 0.3 to 1 cm on the 8th day, showing a gradual increase in size till the 5th day and a slight decrease in the 8th day, possibly reflecting the onset of pre-pupal development. These findings are consistent with Shin et al. (2021), who reported that post-feeding larvae ready to pupate measured

12.12 ± 1.99 mm in length (37). Minor differences in larval size may be attributed to population-specific characteristics of *Calliphoridae* flies in Slovenia and the particular environmental conditions of the study site.

Throughout the study, we did not observe any insects on the buried limbs, apart from the summer, when individual beetles of the genus *Oiceoptoma* were found. The beetles did not cause any visible tissue alterations on the limbs during this period. The absence of insects on the buried limbs was most likely due to the burial depth, which was in our case 50 cm, and subsequently due to the insulating properties of the soil, which protects the carcass from weather phenomena, as well as from insects and scavengers, while preventing major temperature fluctuations and thus providing relatively stable temperatures (22, 23), which in our study were higher in winter and lower in summer compared to surface temperatures. The depth of burial also has a substantial influence on insect colonisation. Gaudry (2010) found that carcasses buried beneath at least 40 cm of soil were not colonized by *Calliphoridae* flies (38). Similarly, Schultz (2007) reported that burying carcasses at a depth of 50 cm largely prevented insect access, with neither larvae nor adult flies or beetles observed at this depth, although a few ants were present. At a depth of 1 m, no insects were observed (39).

In our study, larval size exhibited minimal growth in spring, with no individuals exceeding 0.5 cm. In summer, however, the size of the larvae increased steadily. During this season, the larvae measured 0.3 cm on the 2nd day, 0.6 cm on the 3rd day, 0.6 to 1.1 cm on the 4th day, 0.9 to 1.6 cm on the 5th day and 0.3 to 1 cm on the 8th day.

As with comparable forensic studies conducted under natural environmental conditions, the findings of this study should be interpreted with caution, as the analysis does not fully account for the climatic heterogeneity of Slovenia, which includes four major climate regions: Alpine, Mediterranean, Continental, and Dinaric. The distinct environmental characteristics of these regions may influence insect activity patterns and, consequently, the accuracy of PMI estimations, thus limiting the broader applicability of the results. To address this limitation, future research will include similar studies across the different climatic regions of Slovenia to improve the reliability and precision of PMI estimations derived from field-collected forensic entomological data. Nevertheless, the findings presented here may still provide a useful framework for approximate PMI estimation when used alongside other established forensic methods, thereby contributing to a more robust and accurate assessment of the PMI.

The findings of this study represent a significant contribution to both veterinary and human forensic medicine in Slovenia, as no similar research has previously been conducted in this region. The results have already been successfully applied in forensic assessments of PMI during pre-trial investigations of suspected animal abuse.

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Razgradnja zakopanih in nezakopanih prašičjih ostankov pod vplivom insektov za določanje postmortalnega intervala v različnih letnih časih v razmerah zmernega celinskega podnebja Slovenije

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Izvleček: Forenzična entomologija postaja vse pomembnejše orodje za določanje posmrtnega intervala (PMI) v veterinarskih forenzičnih preiskavah, zlasti v primerih suma mučenja živali, ki je v Republiki Sloveniji opredeljeno kot kaznivo dejanje po 341. členu Kazenskega zakonika. Zanesljiva ocena posmrtnega intervala je v takšnih primerih ključnega pomena. Za čim bolj natančno določanje PMI morajo biti podatki o vplivu žuželk na razpad trupla lokalno specifični, vendar do sedaj v Sloveniji nismo imeli na voljo regijsko pridobljenih forenzično-entomoloških podatkov. Namen raziskave je bil preučiti vpliv letnega časa in zakopa na kolonizacijo žuželk, razvoj njihovih ličink ter razgradnjo tkiv v naravnih razmerah zmernega podnebja v Sloveniji. V raziskavo smo vključili zadnje okončine 40 domačih prašičev (*Sus scrofa domestica*), pri čemer smo polovico okončin pustili na površini tal, drugo polovico pa zakopali v zemljo na globino 50 cm. Kolonizacijo žuželk in razkroj okončin smo spremljali v štirih letnih časih, od jeseni 2022 do poletja 2023. Na nezakopanih okončinah jeseni in pozimi nismo zaznali aktivnosti žuželk, kar je najverjetneje posledica nizkih temperatur in povečane količine padavin. Spomladi smo opazili manjše število muh in ličink iz družin *Calliphoridae* in *Sarcophagidae*, pri čemer je bil razvoj ličink minimalen. Poleti je bila kolonizacija žuželk na nezakopanih okončinah hitra in obsežna; ličinke so bile prisotne že drugi dan po izpostavitvi, vrh njihove aktivnosti pa smo zaznali peti dan. Do konca osmega dne je prišlo do skoraj popolne izgube mehkih tkiv. Na zakopanih okončinah aktivnosti žuželk nismo opazili, z izjemo poletnega obdobja, ko so se pojavili posamezni hrošči iz rodu *Creophilus* in *Saprinus*, ki pa niso imeli bistvenega vpliva na razgradnjo tkiv. Rezultati raziskave poudarjajo pomen lokalnega preučevanja vpliva letnega časa in zakopa na aktivnost žuželk ter posledično na hitrost razgradnje trupla. Izsledki predstavljajo prve lokalne referenčne podatke za forenzično-entomološko presojo PMI v veterinarski forenzični medicini v Sloveniji.

Ključne besede: posmrtno; entomologija; žuželke; Slovenija; forenzična