Praca oryginalna ***Original paper***

Succession pattern of invertebrates on an unburied corpse of a cat suffering from cancer: A case study

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Received 05.06.2019 *Accepted 16.10.2019*

Czepiel-Mil K., Stryjecki R., Listos P., Kowalczyk-Pecka D., Wydra K., Sudak J.
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Summary

Forensic entomology frequently assists forensic medicine in legal investigations. It makes it possible to estimate the time of death when a cadaver is recovered at a relatively advanced stage of decomposition. In criminalistics practice, unburied bodies are found the most commonly, and therefore the fauna of these cadavers is the best investigated. The aim of this study was to collect a succession of insects and other invertebrates occurring on an unburied corpse. The experiment was conducted on the carcass of a cat euthanized due to an advanced cancer process. The carcass was colonized by three phyla of animals: Annelidae, Mollusca, and Arthropoda. They belonged to 7 classes and 10 orders. The most diverse were Arthropoda. They were classified into 5 classes: Insecta, Diplopoda, Malacostraca, Entognata, and Arachnida, and into 8 orders: Julida, Isopoda, Collembola, Diptera, Coleoptera, Hemiptera, Araneae, and Acari. The fly species *Calliphora vicina* from the family Calliphoridae is of particular interest among the insects collected because it is one of the fundamental indicator species whose life cycle makes it possible to determine an approximate time of death. During the study it was noted that arthropods occurred in a certain pattern of succession, predictable in forensic entomology. The first group was *Calliphora vicina* (Calliphoridae, Diptera), which laid eggs. The next (second) group consisted of first-instar *C. vicina* larvae and insects feeding on these larvae, such as *Philonthus tenuicornis* (Staphylinidae, Coleoptera). The first stage of succession was the appearance of eggs of *C. vicina*. The second phase was the appearance of adult flies other than Calliphoridae and of accidental species, as well as beetles (e.g. *Philonthus tenuicornis*, Staphylinidae, Coleoptera) feeding on larvae of *C. vicina*. The third phase of succession was the appearance of all larvae stages of *C. vicina* that continued and finished their life cycle.

Keywords: forensic entomology, *Calliphora vicina*, arthropods, unburied corpse, succession pattern

Forensic entomology frequently assists forensic medicine in legal investigations. It makes it possible to estimate the time of death when a cadaver has been recovered at a relatively advanced stage of decomposition. In addition, it may prove useful in establishing the cause of death when other methods employed for this purpose are hard or impossible to apply. The post mortem interval may be determined on the basis of the analysis of the fauna colonizing the cadaver and the analysis of the growth rate of instar larvae of each insect species recovered. Other important factors, such as temperature, relative air humidity, exposure to the sun, lighting, and rain, also need to be taken into account since they affect the development of necrophages and the rate of cadaver decomposition (29). Apart from the abovementioned external, environmental factors, insects interact with one another, and reactions between various substances take place in the dead body. The composition of the cadaver fauna alters in the course of body decomposition (50). Therefore, a relatively quick determination of arthropods present on dead bodies, combined with knowledge of their biology and ecology, allows researchers to establish the time of death more precisely.

From 1899 to 1900, Niezabitowski, an employee of the Department of Forensic Medicine of the Jagiellonian University, conducted studies on human fetuses and bodies of dead animals, such as cats, foxes, rats, and cattle (29). His experiments demonstrated that the fauna of human and animal corpses does not differ from each other. The species composition of arthropods occurring on them is similar, and the stages of colonization follow the same pattern. Thus, entomological research methods may be applied in veterinary medicine to determine the time of death of animals, and the results obtained may be used with regard to human medicine.

In criminalistics practice, unburied bodies are encountered most often, and therefore the fauna of these cadavers is the best investigated (30, 41, 53). There is a need for ongoing studies into growth rates of necrophagous insects under both natural and laboratory conditions. The results of such studies may help determine the time of death more precisely (5, 6, 17, 33).

The aim of this study was to observe the succession of insects and some other invertebrates occurring on an unburied corpse of a cat suffering from cancer in a temperate climate in spring time.

As far as the animal species under investigation is concerned, the study had a preliminary character. It constitutes the first element in a research cycle now underway at the Department of Pathological Anatomy of the Faculty of Veterinary Medicine of the University of Life Sciences in Lublin aimed at introducing research techniques of forensic entomology into legal and veterinary practice.

Material and methods

The experiment was conducted on the carcass of a cat euthanized due to an advanced cancer process. The owner deposited the animal's corpse with the Department of Pathological Anatomy of the Faculty of Veterinary Medicine of the University of Life Sciences in Lublin for didactic purposes. The age of the animal was about 6 years, the race was European, and the weight was 4.8 kg (10.56 lbs.). The cat carcass was placed on the ground surface, within the confines of the Clinics of the Faculty of Veterinary Medicine of the University of Life Sciences in Lublin. The carrion was properly protected from access by people and animals in such a way as to minimize the epizootic threat. The area where the study was conducted was visibly marked.

The study was conducted in spring between March and April (from March 25 to April 28). The material was collected on days 2, 4, 9, 11, 15, 23, and 36 after the death of the cat. The ambient temperature was measured on the days of sample collection between 10 and 11 a.m. Fauna and eggs were gathered with tweezers from the body surface, natural body openings, and the immediate vicinity of the carcass, and they were placed in sterile containers. The material collected was poured with hot water and preserved in 70% ethyl alcohol. The preserved samples were kept in a room with a temperature of 4°C (39.2°F). The insects were identified using Polish studies and keys.

Results and discussion

The carcass was colonized by three phyla of animals: Annelidae, Mollusca, and Arthropoda (Tab. 1). They belonged to 7 classes and 10 orders. The most diverse were Arthropoda. They were classified into 5 classes: Insecta, Diplopoda, Malacostraca, Entognata, and Arachnida, and into 8 orders: Julida, Isopoda, Collembola, Diptera, Coleoptera, Hemiptera, Araneae, and Acari (Tab. 1). The first to occur on the dead body were accidental taxa, such as Stylommatophora (Mollusca), Julida, Isopoda, Collembola, and Araneae (Arthropoda). These taxa were observed mainly in the initial phase of the corpse (Tab. 1). The first proper

Tab. 1. Pattern of succession of invertebrates on the unburied corpse

Days after death	Phylum	Subphylum	Classis/Subclassis	Order/Family/Species
2	Mollusca	Conchifera	Gastropoda/Pulmonata	Stylommatophora
	Arthropoda	Myriapoda	Diplopoda	Julida/Julidae
	Arthropoda	Crustacea	Malacostraca/Eumalacostraca	Isopoda/Porcellionidae/*Porcellio scaber*
4	Arthropoda	Hexapoda	Entognatha/Apterygota	Collembola
9	Arthropoda	Myriapoda	Diplopoda	Julida/Julidae
	Arthropoda	Chelicerata	Arachnida	Araneae
	Arthropoda	Hexapoda	Incecta/Pterygota	*Calliphora vicina* (eggs)
	Arthropoda	Hexapoda	Insecta/Pterygota	Diptera (adults) – other than *Calliphora vicina*
11	Arthropoda	Hexapoda	Insecta/Pterygota	*Calliphora vicina* (adults)
	Arthropoda	Hexapoda	Incecta/Pterygota	*Calliphora vicina* (eggs)
15	Arthropoda	Hexapoda	Incecta/Pterygota	*Calliphora vicina* (adults)
	Arthropoda	Hexapoda	Incecta/Pterygota	*Calliphora vicina* (eggs)
	Arthropoda	Hexapoda	Incecta/Pterygota	*Calliphora vicina* (first-instar larvae)
	Arthropoda	Hexapoda	Insecta/Pterygota	Coleoptera/Staphylinidae/*Philonthus tenuicornis*
	Arthropoda	Hexapoda	Incecta/Pterygota	Hemiptera/Pyrrhocoridae/*Pyrrhocoris apterus*
	Arthropoda	Chelicerata	Arachnida	Acari
	Arthropoda	Myriapoda	Diplopoda	Julida/Julidae
	Annelida	–	Clitellata/Oligochaeta	Haplotaxida/Lumbricidae
23	Arthropoda	Hexapoda	Insecta/Pterygota	*Calliphora vicina* (first-instar larvae)
	Arthropoda	Hexapoda	Insecta/Pterygota	*Calliphora vicina* (second-instar larvae)
	Arthropoda	Hexapoda	Insecta/Pterygota	*Calliphora vicina* (third-instar larvae)
36	Arthropoda	Hexapoda	Insecta/Pterygota	*Calliphora vicina* (third-instar larvae)
	Arthropoda	Hexapoda	Insecta/Pterygota	*Calliphora vicina* (pupae)

stage initiating the carcass decomposition process was the appearance of numerous eggs of a typical necrophage: *Calliphora vicina*. The second fauna succession stage on the corpse was the appearance of first-instar *Calliphora vicina* larvae and the predator rove beetle *Philonthus tenuicornis* (Staphylinidae, Coleoptera) feeding on these larvae. An additional element was the appearance of taxa not participating in the process of corpse decomposition, such as Haplotaxida (Lumbricidae), Acari, Julida, and *Pyrrhocoris apterus* (Hemiptera). The last (third) phase of entomological succession was the appearance of all larvae stages (I, II and III) of *Calliphora vicina* that continued and finished their life cycle.

The fly species *Calliphora vicina* from the family Calliphoridae is of particular interest among the insects collected because it is one of the fundamental indicator species, whose life cycle makes it possible to determine an approximate time of death.

During the experiment, the ambient temperature varied on particular days, ranging between 10.0° and 21.0°C (50.0°-69.8°F) – Table 2. The average ambient temperature was 14.0°C (57.2°F). Until day 9 after death, the temperature was 10.0°-11.0°C (50.0°-51.8°F). Already at that time the first batches of eggs of *Calliphora vicina* (Calliphoridae, Diptera) were observed. On day 11 after death, the temperature was already 18.0°C (64.4°F), and an increase in the activity of female flies of *Calliphora vicina* laying further eggs was noted. On day 15 after death, further large numbers of eggs and first-instar larvae of *Calliphora vicina* were found on the animal's body. The temperature was then 21.0°C (69.8°F) and it continued for the following week. Twenty-two days after death, the temperature fell to 10.0°C (50.0°F), but all instar larvae of *Calliphora vicina* were already present, with first-instar larvae being the most abundant. Fourteen days elapsed (from day 9 to day 22 after death) from laying the first eggs to the emergence of third-instar larvae of *Calliphora vicina* at a variable temperature of 10.0° to 21.0°C (50.0°-69.8°F). The shortest recorded growth time from eggs to third-instar fly larvae was 8 days, with a recorded day temperature of 21.0°C (69.8°F) – Table 3. The longest recorded growth time from eggs to third-instar fly larvae was 14 days (from day 9 to day 22 after death) – Table 3.

In the temperature range of 10.0°-21.0°C (50.0°-69.8°F), the longest life cycle of *Calliphora vicina* (from the egg to the pupa) took 27 days (from day 9 to day 35 of the experiment), while the shortest one took 21 days (from day 15 to day 35 of the experiment) (Tab. 3).

In many countries all over the world, forensic entomology is widely used in legal investigations (12). The application of entomological methods to estimate the time of death may be useful particularly in cases of advanced decomposition, in which advanced postmortem changes prevent the use of traditional methods.

Tab. 2. Developmental instars of *Calliphora vicina* recovered during the experiment

Days after death	Instar	Temperature
9	Eggs	10°C
11	Eggs	18°C
15	Eggs First-instar larvae	21°C
23	First-instar larvae Second-instar larvae Third-instar larvae	10°C
36	Third-instar larvae Pupae	20°C

Tab. 3. Period of development of *Calliphora vicina* at selected stages of life cycle

Stage	Period of development in days	Days after death
Egg to pupae	27	9 to 35
Egg to pupae	21	15 to 35
Egg to third-instar larvae	14	9 to 22
Egg to third-instar larvae	8	15 to 22
Third-instar larvae to pupae	13	23 to 35

Therefore, it is essential to examine thoroughly necrophagous fauna and the pattern of succession of insects in particular geographical regions and under particular climatic conditions (11, 31, 40, 43, 46, 47, 51).

In a temperate climate, the activity of arthropods, mainly insects, depends on the succession of the seasons of the year, and therefore temperature is of enormous importance (28). Insects are ectothermal animals, so they are strongly dependent on ambient thermal conditions. During periods of low temperature, the activity of arthropods, and especially of insects, decreases. Most of them enter a state of diapause (10).

Apart from temperature, an important factor initiating the state of diapause is photoperiodism. Temperature and length of day stimulate the neurohormones of ecdysone-producing glands. Changes in the production of hormones inhibit or initiate development. When the level of ecdysone decreases, diapause is initiated, whereas an increase in the ecdysone level stimulates growth and development. The same is true of flies from the family Calliphoridae. Their activity decreases at low temperatures (27). The development of their larvae is also strongly dependent on ambient temperature. Thanks to temperature receptors, they sense thermal changes; their behavior and physiology change accordingly. For most of the known necrophagous fly species, the temperature threshold below which the development of a given species is inhibited is about 10.0°C (50.0°F) (29). Below this temperature, development does not take place and adult forms are inactive. Therefore, if the temperature is too low, an unburied body may not be colonized by flies until the temperature rises above the threshold value characteristic of a given species of Diptera (39).

Under our climatic conditions, temperature often fluctuates significantly in the spring period. At the time of the experiment, the temperature varied on particular days, ranging from 10.0° to 21.0°C (50.0°-69.8°F). In the first week of the study, with the temperature at 10.0°C (50.0°F), the following invertebrates were caught: Gastropoda, Diplopoda, Malacostraca, Entognatha, Arachnida, and Insecta. Moreover, the first batches of eggs of *Calliphora vicina*, as well as flying adult specimens of this species, were observed although a favourable temperature for them to deposit eggs is more than 12°C (53.6°F) (13). This species occurs mostly from April to October (18). Thanks to its excellent sensory organs, it deposits eggs in natural body openings (29). The development of eggs of Calliphoridae takes one or two days, depending on the temperature, air humidity and the species of the fly. In the case of *Calliphora vicina*, their development at a temperature of 10.0°C (50.0°F) takes 88 hours. During our experiment, temperature fluctuations delayed the appearance of the first-instar larvae of the species. On day 23 of the experiment, all instar larvae were present, but no eggs of the fly were observed. As Marchenko (37) notes, the activity of female flies depositing eggs clearly ceases with the appearance of larvae.

According to Marchenko (37) and Greenberg and Kunich (22), the time of development of *Calliphora vicina* from eggs to pupae is 21.2 days at a temperature of 11.0°C (51.8°F), 17 days at 12.5°C (54.5°F), 15.9 days at 14.0°C (57.2°F), 10 days at 21.0°C (69.8°F), and 8.4 days at 22.0°C (71.6°F). At variable temperatures from 18.0°-20.0°C (64.4°-68.0°F), the time of development varies from 10.5 to 14 days (2). These slight differences in development may be explained by the genetic disparity of the species acquired in distinct zoogeographical regions (21). Higher temperatures in the summer season cause increased insect activity and a greater species diversity than temperatures in the winter season (46). Some species are active from April to November, others from May to October. *Calliphora vicina* larvae are capable of development between 5.0° and 29°C (41.0°-84.2°F) (42). Temperature fluctuations result in a slower growth of flies of this species. They develop faster at steady temperatures.

The present research confirmed an essential impact of temperature on the development of *Calliphora vicina*. The development from eggs to pupae took from 21 to 27 days, depending on the temperature. Generally, a longer developmental cycle of the blowfly was observed in comparison to the results presented above. With an average temperature in the month of April between 10.0° and 21.0°C (50.0°-69.8°F), the longest period of development from eggs to the emergence of pupae was 27 days (from day 9 to day 35 of the experiment). The shortest period was 21 days (from day 15 to day 35 of the experiment). This result was, arguably, influenced not only by temperature fluctuations during the day, but also by a significant fall in temperature at night.

In a study of an unburied mammal corpse, one also needs to allow for the number of larvae, which change the temperature of the dead mammal body while feeding. It should be remembered as well that flies need a few days to find a decomposing body. Taking into account the above factors, the date of death could be established with a relatively high probability. Thus, the knowledge of the biology of *Calliphora vicina* flies and the time of their development under various external conditions is very useful.

Blowflies (Calliphoridae) and Sarcophagidae belong to two families of flies most commonly found on human cadavers (3). In Europe, blowflies from the genus *Calliphora*, and especially the two most common species of bluebottle blowflies, *Calliphora vicina* and *Calliphora vomitoria*, are usually the first to arrive at the corpse, sometimes along with species of the genus *Lucilia* (29).

In southwestern British Columbia, the most commonly collected species during the decomposition process were the family Piophilidae (4). In southern China, on the other hand, the collected larvae were Calliphoridae, mainly *Chrysomya megacephala* and *C. rufifacies*, as well as *Hydrotaea spinigera* of the family Muscidae (52). In southeastern Brazil, the most common were Calliphoridae: *Chrysomya albiceps*, *C. megacephala*, *Hemilucilia semidiaphana*, and Sarcophagidae: *Pattonella intermutans* (15). In southwestern Virginia, the predominant species in spring was *Phormia regina*, in summer *Phaenicia caeruleiviridis*, and in autumn *Calliphora vomitoria* (49).

To date, the literature has not reported on flies collected on a cat suffering from cancer, but only on cancer-free cats (9, 45). The fly species gathered by researchers were *Megaselia scalaris* (Phoridae), *Blaesoxipha plinthopyga*, *Sarcophaga carnaria* (Sarcophagidae), *Phormia regina*, *Lucilia sericata*, *Calliphora vicina* (Calliphoridae), and *Musca domestica* (Muscidae).

In our study, we noted that arthropods occurred in a certain pattern of succession, predictable in forensic entomology. The first stage of succession was the appearance of the eggs of *Calliphora vicina* (Calliphoridae, Diptera). The next (second) phase was the appearance of first-instar *Calliphora vicina* larvae and insects feeding on these larvae (for example, *Philonthus tenuicornis* (Staphylinidae, Coleoptera). It is a common phenomenon that Coleoptera begin to appear during the period when flies deposit their eggs. Coleopterans usually prey on larvae of flies and other insects that occur on dead bodies. Coleopterans inhabit corpses several days after decease, at the stage of the bloated corpse, and remain on them as long as other insects are present (38). *Philonthus tenuicornis*, found in the study, occurs mostly under dead leaves or in leaf molds (48). Another element noted at the second stage of succession was the occurrence of taxa not directly involved in the process of corpse decomposition, such as *Pyrrhocoris apterus* (Hemiptera). These insects overwinter as imagoes to start preying early in spring. They come from adjacent plants and the ground, and feed mainly on fallen seeds and fruit, but can also suck out juices of dead insects using their piercing-sucking mouthpiece (7).

Similar three stages of successive occurrence of insects were reported by Lefebvre and Gaudry (32). Some researchers have also distinguished a fourth, final stage in the succession of insects, in which they observed the end of the growth cycle of insects from the second group, such as Coleoptera. The species composition of arthropods participating in the succession is obviously affected by geographical location (20). This is associated with varying temperature and humidity, which significantly affect the quantitative and qualitative relationships between arthropods and thereby the rate of corpse decomposition. Therefore, a study of an unburied mammal corpse needs to allow for a range of temperatures characteristic of a given geographical region.

The present study was an attempt to examine the fauna infesting a cat corpse in the spring period of the temperate climate of Poland. The experiment was conducted on an animal suffering from cancer. This was a pioneering trial, since such research has hitherto involved only cancer-free cats, as well as other mammals, mainly pig or rabbit carcasses (35, 36, 44, 54). Some of the studies concerned urban, rural, or forest environments and plantations (14, 16, 19, 20, 23-26). Others concerned a selected group of insects, such as necrophagous flies, wasps, and beetles (1, 8, 34). In the future, such experiments may contribute to the development of studies aimed at determining the time of death of animals, which attracts increasing interest in forensic and veterinary medicine. The data presented here confirm a partial repeatability of the occurrence of arthropods and other indicator species on corpses, which is essential to establish the time of death.

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