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Case Report

Forensic Entomological Examinations for Animal Welfare Offices under Suboptimal Preservation Conditions

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Abstract: A female dog had allegedly been alive one day before its death. The veterinary office thought about accusing the dog's owner for animal cruelty and commissioned forensic entomological expertise for the calculation of the time of insect colonization on the dog's body. The statement of the dog's owner was proven false by us on the grounds of (a) the advanced state of decomposition and (b) the minimal developmental time of fly larvae found on the dog's body. The darkening and deformation of the fly maggots as well as insufficient temperature data made case work trickier than usual. We worked through the case by creating forensic entomological temperature scenarios. The court used the entomological evidence and issued a penalty order.

Keywords: forensic entomology; insect traces; minimum time since death; neglect; sampling

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1. Introduction

Insects, mostly flies and beetles, can colonize human and animal wounds or bodies both during life and after death. If colonization takes place during life, it is referred to as myiasis. Myiasis may occur in cases of neglect; in such cases, it is sometimes possible to calculate the period of neglect using the development of insects [1–3].

The time of development of insects is temperature-dependent: low temperatures slow down insect development and high temperatures accelerate it. Different insect species develop at different rates at the same temperature [4].

The preference of adult flies to deposit eggs in wounds and body openings applies equally to humans and wild or domestic animals [5,6]. In cases of myiasis, eggs are also laid in the soiled diaper area [1–3]. This applies to soiled cushions in dog baskets and soiled blankets, too.

In neglect cases, the collection and subsequent preservation of fly maggots should be carried out separately according to the place of collection (diaper area, open wounds, or natural body openings) in order to record the possibly different developmental ages of the animals at the different colonization sites of the same body.

In cases of animal cruelty and neglect in wild and domestic animals, the entomological evidence collected from living or deceased animals may also provide investigating authorities with information on the circumstances of death [7–12].

The condition of the preserved insect specimens that reach us do not always allow for easy species determination. The case presented here shows that, despite poorly preserved insect material, the difficulty in calculating a possible development time of fly maggots can be narrowed down by creating various temperature scenarios.

Unlike in high-profile forensic cases, the veterinary office initially left the question open if time since death or time since the beginning of neglect including a possible maggot infestation had to be determined by us. We communicated that in this case, it would be best to operate with colonization time, irrespective of whether this was the colonization time of wounds of the living dog or the colonization of the dead dog.

2. Case Description

In connection with the death of an approximately two-year-old, female French bulldog, a German veterinary authority issued an order to calculate the minimum colonization period of the fly maggots collected from the dead dog.

The dog owner stated that she had left her dog in her uncle's apartment several days before the dog's death and had looked after him there every day during her work breaks. She had allegedly last provided the dog with water and food on 23 July 2022; she claimed that the dog was still alive at that time. When she went to pick up her dog from her uncle the next evening, 24 July 2022, the dog had died.

That same evening, the animal mortician collected the dead dog and froze it in the funeral home at −3 °C, according to his statement. The mortician had noticed "heaps of maggots" on the dead dog's body when he handed it over for examination, i.e., the dog had already been severely decomposed at this point. The veterinary office received the corpse on 25 July 2022; the dog was frozen there at unknown temperatures. On 30 August 2022, the animal's corpse was sent to an Institute for Veterinary Pathology and examined there on 2 September 2022. The fly maggots collected from the dog's body were then frozen at −20 °C until shipment.

The owner's statements were made to officers of the local veterinary authority. Police was not involved since the case was considered to be low-key. The veterinary office then contacted the prosecutors' office. The dog owner did not have to give a sworn statement since it was clear that the case would be handled by the district court and the penalty would be very low because the dog owner had no criminal record and most of such cases are not prosecuted at all in Germany.

3. Veterinary Pathological Examination of the Dog

According to the veterinary pathologist, the dog was already in a high state of "autolysis to putrefaction". There were numerous fly maggots on the dog's body, there were nits (louse eggs) in the fur, and the beginning of skeletonization at the right upper jaw was noted.

The dog had no subcutaneous fat and no structural fat deposits: kidney capsule fat, coronary fat, and intestinal mesentery fat were missing. The stomach was empty; the animal was in a highly reduced nutritional state (Figure 1).

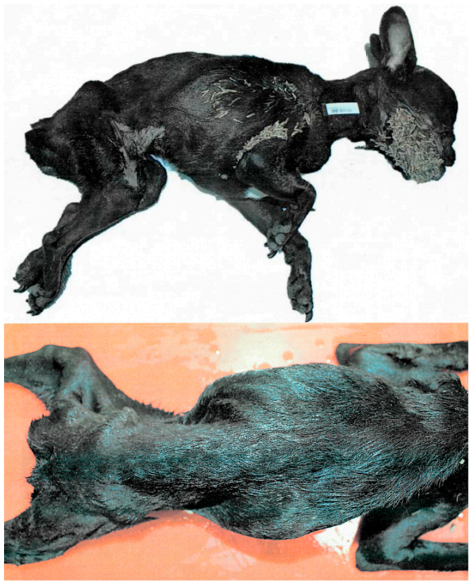

Figure 1. Condition of the emaciated dog's body on delivery to the mortician; the dog had allegedly been alive and well the day before its death. (Note: the insufficient image quality of Figures 1 and 2 is due to the fact that the veterinary pathologist did not allow the use of original images, so copies from the report of the veterinary office had to be used.).

Figure 2. Eyes and brain of the dog missing due to the feeding activity of fly maggots and advanced decomposition. (Photo quality: see remark in Figure 1.)

4. Forensic Entomological Examinations

4.1. Material and Methods

The sample from the Institute of Veterinary Pathology included 146 individual fly maggots and eight clusters of several maggots that were connected (as if glued) together.

The previously frozen fly maggots were sent to us by the veterinarian in 96% ethanol and reached us on 16 January 2023. The thawed maggots were predominantly brown to black in color (Figure 3), the tissue was rubbery, and the animals were predominantly deformed. The discoloration and deformations made it difficult to determine the fly species, as certain body characteristics must be visible to do so. The length of a stretched maggot is used to determine age and cannot be measured correctly if the animals are bent and contorted. The proper and immediate storage of the fly maggots at the mortician's office could have prevented the discoloration and tissue deformation [13].

Species determination was performed based on morphological features using stereomicroscopes (Leica Mz 12.5, Leica S9E, Wetzlar, Germany) and a light microscope (Leica DM LM, Wetzlar, Germany) with identification keys from Szpila [14,15].

One third of the maggots were therefore placed in an 8% potassium hydroxide solution (KOH) at room temperature. The tissue of the maggots was so firm and tough that it only became soft enough to micro-dissect after about a week in the softening solution. The

bleaching effect of the potassium hydroxide made body features relevant to determination largely visible again (Figure 4).

Figure 3. Discoloration and deformation of the fly maggots to be examined.

Figure 4. Dark discoloration of the flesh fly maggot (**top**) and clear tissue after treatment with KOH (**bottom**).

The length of 20 blow fly maggots was measured, the stage of development determined, and their species identified (see Section 4.2). After examination, the maggots—with some body parts removed during the examination (anal plate, head capsule, and mouth parts)—were each transferred to a reaction tube (1.5 mL).

The remaining maggots left in the potassium hydroxide solution were placed in a container of methylated spirits for further storage. The sample also contained a maggot of a flesh fly species: this maggot was also placed in 8% KOH solution for one week at room temperature and then examined microscopically.

4.2. Results of the Species Identification

All 20 blow fly maggots examined belonged to the species *Lucilia sericata* (Meigen, 1826); the average length of the animals was 1.3 cm. All maggots had reached the third and thus last larval stage of development. It was not possible to determine whether the animals had already emptied their intestinal contents in preparation for the subsequent

pupation phase (so-called postfeeders) or whether they still possessed them at the time of preservation due to the dark tissue discoloration.

The single, 2 cm long flesh fly larva belonged to the species *Sarcophaga argyrostoma* (Robineau-Desvoidy, 1830) in the third (and last) larval stage of development.

4.3. Results of the Calculations of a Possible Egg-Laying Time

4.3.1. Temperature Data

Fly maggots develop depending on the surrounding temperature. The temperatures at which the animals developed until the body was found are therefore required.

Normally, the following steps are necessary to recalculate the temperatures in the best case [16]:

- Comparison of hourly temperatures over a certain period of time (e.g., three days or longer) between the location and a nearby weather station;
- Calculation of temperature deviations between these locations;
- Calculation of a correction factor;
- Recalculation of the temperatures for the location where a corpse was found for the time before it was found = time in which the insects developed on the body. The temperature data from a weather station for this period and a previously calculated correction factor are used for this purpose.

In our case, the German Weather Service (DWD) provided daily average, maximum, and minimum temperature values for the period from the beginning of June to the end of July, but no hourly temperature readings. However, a privately operated weather station, which was located 6.8 km away from the dog owner's uncle's home, transmitted hourly air temperature values for the period from the beginning of June 2022 to the end of July 2022.

4.3.2. Calculating the Development Time of Fly Maggots

The steps listed above for calculating the development times of the fly species could not be carried out in this case due to the lack of data and information. The development time of the fly maggots could still be approximated by creating various "temperature scenarios" based on the hourly temperature data from the private weather station and developmental data from the literature.

4.3.3. Development Data for *Lucilia sericata* and *Sarcophaga argyrostoma*

The calculation of the time of oviposition of *Lucilia sericata* was carried out using the developmental data of Wang et al. 2020 [17]. Table 1 shows the results of the calculation of the development time of the maggots of *Lucilia sericata* under the influence of different temperatures.

Table 1. Calculation of possible oviposition times of *Lucilia sericata* according to Wang et al. (2020) [17].

[°C]	Developmental Stage	Possible Oviposition *
Weather station **	L2 > L3 ***	21 July 2022; in the morning
Weather station	L3 > postfeeder ****	18 July 2022; in the evening
15	L2 > L3	16 July 2022; at night
15	L3 > postfeeder	10 July 2022; in the evening
24	L2 > L3	22 July 2022; at night
24	L3 > postfeeder	19 July 2022; in the evening
30	L2 > L3	22 July 2022; in the evening
30	L3 > postfeeder	21 July 2022; in the morning

* 5 a.m.–12 a.m. = morning; 12 p.m.–5 p.m. = afternoon; 5 p.m.–9 p.m. = evening; 9 p.m.–5 a.m. = night; ** fluctuating temperatures during the day; *** transition from the second to the third larval stage; **** transition from the third larval stage to the so-called postfeeder stage (empty intestine before pupation).

Due to the dark coloration of the fly maggots, it could not be determined whether intestinal contents were present at the time of collection. Fly maggots empty their intestinal contents before pupation (postfeeding larvae; so-called postfeeders): maggots without intestinal contents are therefore older than those with intestinal contents. A possible influence of the KOH solution on any remaining intestinal contents (which were no longer visible after the KOH treatment because the tissue was brightened too much) could not be ruled out due to the long soaking time.

Data from Wang et al. [17] for the developmental transition from the second to the third developmental stage did not fully fit because the maggots were clearly in the third larval stage; any transitional stage would have been recognizable by morphological characteristics, e.g., half-shed skin and breathing spiracles. The maggots examined corresponded best with the data for transition from the third to the postfeeder developmental stage. As postfeeders, the maggots migrate from the dead body to pupate, so later developmental stages are not expected on a corpse.

Females of flesh flies, to which *Sarcophaga argyrostoma* belongs, lay live young larvae on decaying tissue in the first stage of development [18]. Since flesh fly larvae of this species do not randomly feed on a living organism but are attracted to decomposed tissue, we calculated the development time as a possible colonization time for this maggot based on the temperature and development data of Grassberger and Reiter (2002) [19] (Table 2).

Table 2. Calculation of possible times of deposition of young larvae of *Sarcophaga argyrostoma* according to Grassberger and Reiter (2002) [19].

[°C]	Developmental Stage	Possible Deposition of Larvae *
20	L2 > L3	20 July 2022
20	postfeeder	17 July 2022
25	L2 > L3	21 July 2022
25	postfeeder	19/20 July 2022
30	L2 > L3	22 July 2022
30	postfeeder	20 July 2022

* 5 a.m.–12 a.m. = morning; 12 p.m.–5 p.m. = afternoon; 5 p.m.–9 p.m. = evening; 9 p.m.–5 a.m. = night.

4.4. Answering the Client's Questions

Our calculations were based on both the fluctuating daily temperatures of the weather station 6.8 km away from the uncle's home and constant temperatures, e.g., 15 °C for a possible colonization in the basement room, 24 °C for an indoor room in summer (during the day), and 30 °C daytime temperature for the outdoor colonization in that summer.

Further influences on egg laying and larval development such as rain [20–23], night, and dark conditions [23–29] were disregarded since we were told that the case took place inside or close to the apartment.

Since the maggots of both fly species had reached the third and last stage of development as larvae (before pupation), the larvae could not have developed within one day (e.g., from 23 to 24 July 2022) from oviposition.

We do not know whether the dog was still alive at the time the eggs were laid. It is possible that the dog was neglected and that its wounds were colonized by fly maggots during its lifetime. *Lucilia sericata* and *Sarcophaga argyrostoma* are fly species that may colonize living yet neglected bodies [9].

Since we also did not know whether the fly maggots sent in for examination were the oldest maggots that had developed on the dog, the calculated time periods were the minimum development time of the larvae.

The extensive maggot infestation of the oral cavity, the loss of substance on the muzzle due to autolysis, and the strong odor of decomposition before the body was frozen also spoke against the statement that the dog had been healthy and alive on 23 July 2022.

5. Discussion

5.1. Temperatures

Temperature data from the colonization site of the dog were missing. Therefore, the temperatures at which the insects colonized the dog before 24 July 2022 could not be mathematically reconstructed.

If the owner claimed to have visited the dog at about the same time on both days, the maximum PMI hypothesis to test would be approximately 24 h. Assuming the most rapid development rates in the reference papers [17,19], both a 13 mm third larval instar of *L. sericata* and a 20 mm third larval instar of *S. argyrostoma* would be too old for the owner to have told the truth, irrespective of the temperatures of the dog carcass.

We decided to use local weather data as well as the most recent developmental data including ADH information to build and check our temperature scenarios (Table 1). Since only one *Sarcophaga* larva was sent to us and since no recent developmental ADH data were available, we decided to use an older data set for this species that did cover the temperatures we used in our scenarios. This was sufficient because the statement of the owner of the dog was found to be false in all our calculations.

We decided to use the most current data for *Lucilia sericata* that also include ADH values. Since we observe a massive impact of climate change in Europe, we considered the most modern data to be the most reliable in this particular case. For *Sarcophaga argyrostoma*, we had to rely on the older data set because the most recent data sets did not cover the temperature ranges that we needed to include in our "check the scenarios" tests.

5.2. Colonization Site of the Dog

It remained unclear whether the dog was colonized inside the house or outside and if windows were closed or not. "Closed" doors in Germany often provide access points for flies, as the adults can squeeze through old keyholes or gaps between the door and the floor.

5.3. Fly Maggots

It is unknown to which colonization wave the fly maggots collected from the dead dog belonged, and especially, if there were older developmental stages of the flies or other insects in the vicinity of the dog. In a strict court room setting, one could also question if the maggots had been alive on the dog. The color changes and conservation state of the maggots did not allow a reliable length measurement. Our measured lengths were minimum lengths.

Concerning a possible lack of information in the scientific literature relating to the variability of the postfeeding stage, we restricted ourselves to the information contained in the sources that we used [17,19]. In the *Lucilia* larvae as well as the single *Sarcophaga* larva, we saw three slits in the abdominal (posterior) spiracles. Therefore, we decided that any developmental interval beginning from the transition from larval stage 2 to larval stage 3 until the possible postfeeding stage should be considered. Our minimum developmental estimate already excluded the dog owner's statement, so in this particular case the question was answered without further examination of a possible postfeeding stage.

We did not aim for the inclusion of larval length data because on the one hand, we wanted to support the veterinary office even though hardly any budget was available and we thought that a discussion about larval lengths might lead to further unpaid work. On the other hand, our approach using scenarios sufficiently covered the questions that we were asked to answer. Since exact environmental information (the dog's exact place of death, etc.) were unknown, we decided to work on the simplest and safest level so that a possible defense could not use a confusion strategy over numbers. Finally, in our lab, we are hesitant to work in an overprecise manner when larvae arrive in a hardened state. In higher profile cases, we would naturally determine the minimal developmental time from shrunken, hardened maggots, but this case had to be handled under minimalistic conditions, yet with simple and safe conclusions due to the circumstances described above.

5.4. The Dog Owner's Statement

Strictly speaking, it is unknown whether the dog may have died elsewhere and was then transported to the uncle's apartment.

5.5. Conclusions

Despite all limitations, our measurements show that the dog could not have been healthily alive on the evening of 23 July 2022. This entomological exclusion matches the observation that the dog's brain was severely decomposed and largely missing.

The dog was colonized by cadaver flies on the morning of 21 July 2022 at the latest; if the dead or living animal had been in a colder environment than 30 °C outside temperature, then colonization could even have taken place much earlier.

In the trial, the court warned the dog owner and ordered her to pay 1200 Euro to a charitable organization. She was banned from keeping animals for one year. After that, the owner may legally own animals again.

Even though the larvae were in poor condition and not all data were available, the question of the animal welfare office could be answered in a useful, legal way. This allowed the office to go on trial.

We believe that this case might remind veterinarians and veterinary pathologists to preserve and document entomological traces in the best possible way. In more difficult cases, a better preservation of the maggots would have been necessary. Here, the relevant question could be answered sufficiently.

6. Impact Statement

This case report emphasizes the importance of the best possible preservation and documentation of forensic entomological traces and stains, here concerning neglected pets in animal welfare cases. Without such evidence, legal proceedings cannot be carried out objectively and would have to rely on guesswork.

This paper was published in a different form (as an expert witness statement) in a small German language publication [30].

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