

Determination of Calliphoridae (Insecta: Diptera) Succession on Pig Carcasses in Central Anatolia, Turkey

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Abstract – Insects function as a biological clock in determining the time elapsed after death which is also called minimum postmortem interval (PMImin). Particularly, Calliphoridae family members are the first colonizers. Although the succession behaviors of calliphorids are a potentially useful and accurate tool for determining the time of death, the accuracy of this method has been severely affected by geographical and regional differences in the succession patterns. Therefore, the present study purposes to determine the seasonal activity and succession patterns of Calliphoridae on pig carcasses in Central Anatolia of Turkey and providing the reference data for accurate PMImin determination in forensic cases. The study was conducted in Eskişehir, Sulu Karaağaç Village located in Central Anatolia, Turkey. The study was carried out by collecting samples from twelve pig carcasses left on the field for each month of the year 2011-2012. During the study, *Calliphora vicina*, *Calliphora vomitoria*, *Chrysomya albiceps*, *Lucilia illustris*, *Lucilia sericata*, *Pollenia labialis*, and *Pollenia rudis* which belonging to the Calliphoridae family, were collected. Additionally, their activity on the pig carcasses was observed. *Pollenia rudis* is firstly recorded from Turkey with this study. This study provides to determine certain PMImin in Turkey and also reference data are provided to solve crimes occurring in different seasons.

Keywords – Calliphoridae, forensic entomology, postmortem interval, seasonal activity, succession

1. Introduction

Forensic entomology is an extensive discipline that uses a combination of entomology and judiciary (Hall R. D., 2001). Investigating the insects and other arthropods on a corpse provides significant evidence to solve cases as estimating the time of death. Forensic entomology also represents medico-criminal entomology, has been used entomological information for the determination of minimum post-mortem intervals (PMImin) (Hall, 2001; Byrd & Castner, 2001).

PMImin is the detection of the time passing after death by using body temperature, livor and rigor mortis analyses, liveliness, and paleness of nails and skin (Byrd & Castner, 2001; Smith, 1986; Catts & Goff, 1992; Nelson, 2000). There is evidence that entomological data plays a crucial role in determining the PMImin from the early to advanced decay stage (Smith, 1986; Nuorteva, 1977; Goff et al., 1986; Greenberg, 1991). The entomological method reliability which is used during the determination of the PMImin, were evaluated by using comparative analyses about the autopsy report, entomological and second-degree evidence on 16 death cases (Kashyap & Pillay, 1989). According to these analyses, the entomological method is more decisive than pathological methods as livor mortis, rigor mortis, algor mortis, and decomposition, which are based on changes in 72 hours after death (Byrd & Castner, 2001).

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Almost 400 insect species could be found at different decomposition stages of corpses (Payne, 1965). Identification of the fly's succession in different areas, the definition of different phases of decomposition and the insect species (eggs, larval stages, pupae, and adults) on carrion, and the information about their development at different environmental conditions can provide proof for the determination of PMImin, the season of death, whether the carcass moved from crime scene to another place, trauma location on the body, sexual molestation, and effects of drugs (Smith, 1986; Greenberg, 1991; Introna, Suman, & Smialek, 1991; Haskell et al., 1997).

Many necrophagous insects can be found on the corpse but Calliphoridae (blowflies) family members are primary colonizers which provide an investigation of PMImin. Calliphoridae is very large group and members of this family can be found worldwide. They have antennae that can detect putrefaction and gases released during the decaying of the corpses (Ashworth & Wall, 1994). Calliphoridae can be used in forensic cases to determine the accurate PMImin, the cause of death, the geographical environment in which the death occurred, the season, the time of dismemberment if the body was dismembered, the location of the corpse, the presence of toxic substances or pharmaceutical drugs, cases of abuse, sexual abuse, the time of trauma on the corpse, and whether the corpse was transported (Catts & Goff, 1992; Benecke & Lessig, 2001; Campobasso & Introna, 2001). Development data of blowflies provide the most precise estimations of PMImin. Besides, succession analyses of arthropods on the carrion are the second method to estimate the PMImin. However, the succession patterns of flies on forensic cases are not typical and insect colonization is affected by several factors, including carcass size, habitat, seasonality, climate, temperature, altitude, humidity, chemicals and drugs and geographic region (Hall & Brandt, 2006; Wells & LaMotte, 2010; Mullany et al., 2014). Evaluating the effect of these factors is important for correctly assessing legal cases. In Turkey, few studies were conducted for determining the succession parameters of forensically important species especially Calliphoridae. (Özdemir & Sert, 2009); surveyed an investigation to determine Coleoptera fauna on Carcasses in Ankara province. Şabanoglu & Sert, 2010; determined the seasonal distribution of Calliphoridae in Ankara province. Seasonality of insect succession on decomposing dog carcasses in Samsun province was evaluated by (Kökdeniz & Polat, 2014). Differences in succession of Coleoptera species attracted to pig carcasses in rural and urban habitats in Eskişehir was investigated (Altunsoy, Turan, Firat, & Sert, 2017). This study seeks to obtain data which will help to address these factors. Recently, a considerable literature has grown up around the theme of determining faunal density and succession patterns of insects on the corpses. Succession parameters of insects associated with the corpses in a particular geographic area is essential tool to determine of PMImin. However, the forensically important species vary according to geographical regions and these studies provide reference data to solve crimes. The specific aim of this study was to determine the seasonal activity and succession patterns of the Calliphoridae species during five decomposing stages of the pig carcasses, in Eskişehir province, Turkey. The present research explores *Pollenia rudis* Fabricius, 1794 (Diptera: Calliphoridae) as a forensically important fly that colonizes on the corpses in Turkey.

2. Materials and Methods

The study was conducted in Eskişehir, Sulu Karağaç Village (39°55'27''N 30°29'18''E, 1233 m) located in Central Anatolia, Turkey between 2011 and 2012. The study area is surrounded by a forest composed of *Quercus* spp. Linnaeus (Fagales: Fagaceae) and *Pinus* spp. Linnaeus (Pinales: Pinaceae). Four female and eight male pig carcasses were used and placed inside separate metal cages to isolate them from wild animals. Each cage placed the study area as one pig per month. They were partitioned into four groups as spring, summer, autumn, and winter to facilitate working on pig carcasses (Table 1).

Table 1

The placing dates and duration of the decomposition stages of pig carcasses in each experimental period. (*: The pig carcasses never completed this stages)

Season	Pig Number	Date	Fresh	Bloated	Active	Postdecay	Skeletal
Spring	5	4 March	8 days	8-9 days	9-11 days	*	*
	6	2 April	5-6 days	3-4 days	15-20 days	*	*
	7	1.May	3 days	5-6 days	14-15 days	5-6 days	*
Summer	8	1 June	1-2 days	4 days	10 days	15-16 days	*
	9	3 July	1 day	4-5 days	5-6 days	6-7 days	10 days
	10	1 August	1 day	3-4 days	8 days	7-8 days	10-11 days
Autumn	11	6 September	1 day	8 days	10-11 days	8-9 days	3-4 days
	12	5 October	1-2 days	8-9 days	13-14 days	3-4 days	*
Winter	1	4 November	1-4 days	18-20 days	4-6 days	*	*
	2	4 December	5-7 days	20-23 days	*	*	*
	3	3 January	8-9 days	22-24 days	*	*	*
	4	5 February	25-26 days	4-5 days	*	*	*

The pigs were supplied from Başkent University Generation and Research Center and Çukurova University Medical Science Experimental Research and Application Center. Ethical approval for the study was provided by Anadolu University Experimental Animals Local Ethics Committee (Date and no. 2012/01). Pigs were sacrificed by intravenous injection of 300 mg pentobarbital sodium under veterinary control. During the study, a total of twelve pigs were sacrificed as one pig per month.

A "Forensic Entomology Daily Data Sheet" (F.E.D.D.S.) was prepared for the environmental variables such as relative humidity, ambient temperature, and climatic conditions, Calliphoridae species and their density, physical changes, and decaying stages of the carcasses were recorded during each sampling on the F.E.D.D.S. Pig carcasses were assessed three times daily; in the morning, afternoon, and sunset. Due to the fly activity was more enhanced, specimens were collected in the morning. Min and Max temperature parameters were recorded during the study (Table 2). Besides, corresponding photographs were taken. Adult specimens were collected with a sweep net and the forceps were used for larvae and pupae. Adult specimens were killed with ethyl acetate jars and preserved in 70% ethanol. Calliphoridae specimens were identified using identification keys as described (Greenberg & Kunich, 2002; Szpila, 2010; Whitworth, 2006; Rognes, 1987). All specimens were preserved in the Zoological Museum of Technical University.

Table 2

Monthly moisture, min and max temperature, and monthly average temperature parameters.

Months	Monthly Moisture Average (%)	Monthly Max.Air Temp. Average (°C)	Monthly Min.Air Temp. Average (°C)	Average Temp. (°C)
March	73	8,7	-2,7	5,4
April	58,6	20,5	5	10,7
May	68,8	21,7	9	15,5
June	52,2	29,3	12,3	19,2
July	50,1	32,3	16,1	21,9
August	50	29,5	12,9	21,8
September	53	28,3	10	17,8
October	67,2	22,4	7,7	12,4
November	70,5	9,2	-4,4	7
December	80,6	6,6	-1,9	2,3
January	91,1	0	-6,1	0,1
February	88,6	-0,9	-7,8	1,8

3. Results and Discussion

Five stages of decomposition were identified during present research: fresh, bloated, decay, postdecay, and skeletal according to the description of decomposition stages as provided by Goff, 1993. (Figure 1) and totally seven species belong to the Calliphoridae family were identified: *Calliphora vicina*, Robineau-Desvoidy, 1830 (Diptera: Calliphoridae), *Calliphora vomitoria* Linnaeus, 1758 (Diptera: Calliphoridae), *Chrysomya albiceps* Wiedemann, 1819 (Diptera: Calliphoridae), *Lucilia illustris* Meigen, 1826 (Diptera: Calliphoridae), *Lucilia sericata* Meigen, 1826 (Diptera: Calliphoridae), *Pollenia labialis* Robineau-Desvoidy, 1863 (Diptera: Calliphoridae), and *Pollenia rudis* Fabricius, 1794 (Diptera: Calliphoridae) and seasonal succession of these species were briefly discussed.



Figure 1. Decomposition stages of pig carcasses. a) fresh stage b) bloated stage c) active stage d) postdecay stage e) skeletal stage

There were seven species of Calliphoridae collected from the carcasses. Adults in the families Calliphoridae were the primary colonizers of the carcasses, arriving shortly after exposure of the carcasses and colonised in 2-3 minutes. Min and Max temperature parameters when the species seen first and last time, were recorded (Table 3). It was determined that the breeding activity of Calliphoridae decreases and time for laying increases on carcasses in a day when the temperature was lower than 20°C. In addition, hatching delays more than normal. The breeding behavior of the Calliphoridae species affected by cloudiness and rain. If the weather was rainy, Calliphoridae species do not lay on carcasses but their activity proceeded around the carcass.

The presenting of evidence from carcasses which examined four different periods. The autumn study was conducted from Sep. 6th to Dec. 4th (Table 1). Seven species were collected from carcasses in this period; *C. vicina*, *C. vomitoria*, *Chr. albiceps*, *L. illustris*, *L. sericata*, *P. labialis*, and *P. rudis*. The winter study was completed from Dec. 4th to Mar. 4th (Table 1). Only *C. vicina* was collected from the carcasses. The spring study was conducted from March. 4th to June 1st (Table 1). Six species were collected; *C. vicina*, *C. vomitoria*, *Chr. albiceps*, *L. illustris*, *L. sericata*, and *P. rudis*. The summer study was conducted from June 1st to Sep. 6th (Table 1). Five species were collected; *C. vicina*, *C. vomitoria*, *Chr. albiceps*, *L. illustris*, and *L. sericata*. *C. vicina* was the most common species on the carcasses at Eskişehir during the year. *C. vicina* showed major activity until 13 January and its activity was not observed from 13 January to 10 March. Interestingly, *C. vicina* has observed the rest of the months on the carcasses, and oviposition density is weaker than *C. vomitoria* and *L. sericata* (Figure 2 and Figure 3).

The activity of *L. sericata* was observed from Apr. 5th to Oct. 17th on the carcasses. This species was the most common in April than other species and maintain density until 18 May. The activity of *L. sericata* decreases during the high density of laying. When the larval activity of *Chr. albiceps* (known as predators of *L. sericata*)

increases on the carcasses, the adult activity of *L. sericata* decreases (from last week of May to June) (Figure 2 and Figure 3).

Species	November				December				January				February				March				April				May				June				July				August				September				October							
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4				
<i>Calliphora vicina</i>	█	█			█	█	█	█	█	█											█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█					█	█										
<i>Calliphra vomitoria</i>	█	█	█																		█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█					█	█	█	█	█	█	█	█				
<i>Chyrisomya albiceps</i>																									█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█				
<i>Lucilia illustris</i>																									█								█	█	█	█	█	█	█	█												
<i>Lulilia sericata</i>																					█	█	█	█	█	█	█	█	█	█	█	█	█	█	█	█					█	█	█	█	█	█	█	█				
<i>Pollenia labialis</i>																																																				
<i>Pollenia rudis</i>																	█	█	█	█																																

Figure 2. Insect succession patterns on the pig carcasses from November to October. (White color indicates absence of species and each color represents different species)

Chr. albiceps was the most common species during the summer period. The activity of *Chr. albiceps* began in May and the activity increased on carcasses when the average temperature was 29.5°C in June. Effect of relative humidity reduced the time of the maturation of larvae for almost two days. Because of this, the activity of the *L. sericata* larvae decreased and none of the pupae were observed on the carcass or around the carcass (Figure 2 and Figure 3). Although *P. rudis* was the most common species in autumn, *Chr. albiceps* was still on the carcass during autumn.

The activity of the *C. vomitoria* was observed every study period except winter. Besides, the activity is minimized in the summer because this species prefers cool weather and cannot see on the carcass in fully sunny days. So, the activity of this species was observed until last week of June, and rarely in July and August, on days that are not completely sunny (Figure 2 and Figure 3).

L. illustris was observed on the carcasses in May and the activity of the *L. illustris* from May to September was lower than other species. Also, *P. rudis* and *P. labialis* was observed on the pig carcasses at the same time and these species have not been reported in the previous studies. The activity of the *P. rudis* has been observed in every examination period except the winter (Figure 2 and Figure 3).

Pig	Species	November	December	January	February	March	April	May	June	July	August	September	October
1	<i>C. vicina</i>	■	■										
	<i>C. vomitoria</i>	■	■										
2	<i>C. vicina</i>		■	■	■								
	<i>C. vicina</i>			■	■								
4	None												
5	<i>C. vicina</i>					■	■						
	<i>P. rudis</i>						■						
6	<i>C. vicina</i>						■	■	■				
	<i>C. vomitoria</i>						■	■	■				
	<i>L. sericata</i>						■	■	■				
	<i>P. labialis</i>						■	■	■				
7	<i>P. rudis</i>						■	■	■				
	<i>C. vicina</i>						■	■	■				
	<i>C. vomitoria</i>						■	■	■				
	<i>Chr. albiceps</i>						■	■	■				
8	<i>L. illustris</i>							■	■				
	<i>L. sericata</i>							■	■				
	<i>C. vicina</i>							■	■				
	<i>C. vomitoria</i>							■	■				
9	<i>Chr. albiceps</i>							■	■				
	<i>L. illustris</i>							■	■				
	<i>L. sericata</i>							■	■				
	<i>C. vicina</i>							■	■				
10	<i>C. vicina</i>									■	■		
	<i>C. vomitoria</i>									■	■		
	<i>Chr. albiceps</i>									■	■		
	<i>L. illustris</i>									■	■		
11	<i>L. sericata</i>											■	■
	<i>L. illustris</i>											■	■
	<i>P. labialis</i>											■	■
	<i>P. rudis</i>											■	■
12	<i>C. vicina</i>												■
	<i>C. vomitoria</i>												■
	<i>Chr. albiceps</i>												■

Figure 3. Monthly distribution of the species on each carrion during the study period. (White color indicates absence of species and each color represents different species)

It was determined that the presence of the *Vespula germanica* Fabricius, 1793 (Hymenoptera: Vespidae) activity around the carcasses prevents the laying of Calliphoridae during the egg-laying period, from May to August. The population of the Calliphoridae decreased about 30% attacking and killing by *V. germanica*. Also, there was significant predation by the Staphylinidae and Histeridae species feeding on the larvae of the Calliphoridae, especially summer period. Together these results provide important insights for evaluating entomological data, found on the corpses to determine the PMImin.

The present study was designed to determine the succession patterns of Calliphoridae on pig carcasses in Central Anatolia. Because of a certain PMImin estimation process is the most fundamental criteria in forensic entomology, a precise analyzes and assessment of entomological data should be done carefully and intensively. For this purpose; seven Calliphoridae species, *C. vicina*, *C. vomitoria*, *Chr. albiceps*, *L. illustris*, *L. sericata*, *P. labialis*, and *P. rudis* which are evidence for PMImin estimation in criminal cases, were identified in the present study. All of these seven species were observed together in the autumn period of the study which was placed in September and October. It was determined that adults and larvae of *C. vicina*, *C. vomitoria*, *Chr. albiceps*, *L. sericata*, and *L. illustris* can be used for solving any case from spring to autumn. Another forensically important species was *L. Sericata*, which was seen for the first time in March, and keep its existence until

December in this study. It is also reliable to use this species in possible death cases that occur in spring, summer, and autumn. Besides, the seasonal activity and succession of *P. rudis* on carcasses were observed for the first time in Turkey. Although (Šulákova & Barták, 2013), stated that *Pollenia* Robineau-Desvoidy, 1830 is not included in forensically important blowflies, they found the *P. rudis* on the pig carcasses in their study. In the present study, *P. rudis* provides useful evidence to estimate PMI in during spring, summer, and autumn while (Taleb, Tail, & Açıkgöz, 2021) found particularly in Spring and also stated *Pollenia* species should be consisted of in carrion entomofauna. Tabor (2004) determined the *P. rudis* existed on the corpse only bloated stage, other carrion studies confirmed that *Pollenia* is attracted to the dead bodies (Prado E Castro et al., 2012); Benbow et al., 2013).

Literature shows that the tendency to the carrion and distribution of species is different under dissimilar conditions. In our study, the most attracted species to the carrion during autumn were *C. vicina*, *C. vomitoria*, *Chr. albiceps*, *L. illustris*, *L. sericata*, *P. labialis*, and *P. rudis* whereas *Chr. albiceps* and *Hydrotaea ignava* Harris, 1780 (Diptera: Muscidae) were the most in Portugal (Prado E Castro et al., 2012). However, similarly, winter periods represented with *C. vicina* and *C. vomitoria*.

Şabanoğlu & Sert (2010) reported that *C. vomitoria*, *C. vicina*, *L. sericata*, and *Chr. albiceps* in Central Anatolia and explained the succession and the seasonality of these four species. Based on their reports, *L. sericata* was the most common species during the study period while in our study *C. vicina* has the longest duration in Eskişehir. On the other hand, *C. vomitoria* was observed as cool weather species in both studies. *C. vicina* was seen in the subtropical zone in winter, temperate zone in autumn and spring as reported by Greenberg & Povolny (1971). For the present study, *C. vicina* showed major activity until 13 January and its activity was not observed from 13 January to 10 March. *Chr. albiceps* was the most abundant species during summer in Eskişehir. The activity of *Chr. albiceps* began in May and increased when the average temperature was 29.5°C in June. Similarly, Şabanoğlu & Sert (2010) stated that *Chr. albiceps* is the most frequently seen species during summer on the carcasses. Another study surveyed in Brasil, *Chr. albiceps* was determined as the most abundant species for all experimental periods (Carvalho & Linhares, 2001).

4. Conclusion

The most obvious finding from this study is that adult Calliphoridae were the first colonizers on the carcasses. However, the duration time of each decomposition stage depended on the area's particular environmental conditions and also the effect on the activity of the insects associated with the bodies. These findings have significant implications for the understanding of how ecological conditions effect on the forensically important species, especially Calliphoridae, at different zones and different microclimates. The insights gained from this study may be of assistance bio-model for investigations on human cases in this area of Eskişehir, Turkey. Further studies are needed to determine the geographic spreads and succession of different microclimates of forensically important Calliphoridae species in different regions of Turkey.

Author Contributions

Ferhat Altunsoy: Designed the study; identified the insect samples and designed the manuscript.

Cenk Önsoy: Conducted the field studies; collected the insect samples and designed the manuscript.

Conflicts of Interest

The authors declare that they have no conflict of interest.

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