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Determination of Species of Diptera Feeding on Carcasses and their Evaluation in Forensic Entomology in Kırıkkale Province

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Abstract

Forensic Entomology, which is a branch of science in which insects and other arthropods are used in forensic cases, is a newly developing field in Turkey ,although it has been used effectively in Europe and the United States for nearly 50 years inthe investigation of violent crimes.Studies carried out in this area contribute to the establishment of literature,the development of scientists and the administration of justice.This study makes an analysis of the Diptera species and their seasonal distribution, using rabbit carcasses left on the campus of Kırıkkale University between May 2009 and March 2010. The rabbits, obtained from Ankara Refik Saydam Hıfzıssıhha Serum Production Farm, were killed and left to decompose in the study area, and species of the Diptera order were identified among the insects that infested the carcasses. A species identification was made of the collected Diptera eggs, larvae, pupae and adults, during which it was found that some larvae of the Diptera species that infested the carcasses were higher in number, being ***Chrysomya albiceps, Lucilia sericata*** and ***Calliphora vicina*** of the **Calliphoridae** family. Aside from these, adult specimens were detected also of ***Calliphora vomitoria*** of the **Calliphoridae** family; ***Muscida stabulans, Musca domestica*** and ***Ophyra sp.*** of the **Muscidae** family; ***Sarcophaga crassipalpis***, ***Wohlfahrtia magnifica*** and ***Sarcophaga carnaria*** of the **Sarcophagidae** family; and members of the **Tachinidae Heleomysidae, Anthomyiidae, Piophilidae, Tabanidae, Syriphidae** and **Stratiomyidae** families.

Keywords: Carcass, Diptera, Fauna, Forensic Entomology, Estimation of Post Mortem Interval.

This study was carried out as a part of Master’s program at Kırıkkale University, Faculty of Science Department of Biology.

INTRODUCTION

Forensic Entomology is generally defined as a discipline in which insects and other Arthropods are used in forensic cases.

Arthropods are used in three basic areas within the judicial system [1,2,3]. The first of these, “Urban entomology”, deals specifically with the resolution of problems of human beings caused by termites, cockroaches and some other insects, and problems caused by insects on livestock farms and similar facilities are also categorized under this area [3]. The second area is “Stored product entomology”, relating to insect residues in cereal products, caterpillars inside vegetables, insect larvae found inside sandwiches in restaurants, and the like. The third area is “Medicocriminal entomology”, in which Arthropod evidence is used to solve violent crimes. Medicocriminal entomology generally makes use of all necessary details in order to reveal evidence in a murder case when witnesses are lacking [3].

There are two approaches to the estimation of Post Mortem Interval (PMI); the first one includes the change rate of insect development, depending on temperature. The second is to use data obtained with the help of Arthropod succession, from which it is possible to estimate the post-mortem interval from the colonization patterns of the dead person or animal [3].Through the detection and evaluation of entomologic evidence, it is possible to as certain the time of death, the season of death, whether or not the carcass has been moved from one place to another, the geographical area in which the death occurred, the traumatized areas of the body, whether sexual assault has taken place and whether drugs were used [4].

The aim of this study is to determine the species of Diptera feeding on carcasses, as an area of importance in Forensic Entomology, to determine the seasonal and environmental factors affecting decay and to form a basis for further studies related to the subject in cases of suspicious death.

MATERIALS AND METHOD

Following the method of previous Forensic Entomology studies rabbits were used for this study [5,6] after permission for their use was obtained from the Kırıkkale University Experimental Animals Ethics Committee, dated 29.04.2009 and numbered 09.20.160, during meeting number 09/04. The rabbits were killed by a veterinarian through the injection of Ketasol and Xylasol, after which they were left in the study area.The study involved three trials, which were carried out in the spring (28 May–02 July 2010), summer (04 August–04 September 2010) and winter (21 December 2009–29 March 2010) seasons. A total of 12 rabbits were used, four for each trial, and in each trial, one rabbit carcass was taken as the control sample from which no insects were collected, and its process of decay was compared with that of the other three rabbits. The carcasses were placed in a wide open area and were kept in a wire cage to prevent access by dogs, birds, etc., while ensuring the easy access of insects. The area of the study was visited on a daily basis, during which the species of Diptera in the carcasses, the number of each species and the stage of decay were recorded. Diptera samples were collected from the carcasses at noon, when insect activity is high. Adult specimens were collected using an insect trap, while egg, larva and pupa samples were collected using a pens. The adult specimens were killed using death cups filled with ethyl acetate and dried in insect storage boxes after being attached with insect forceps, and stored for identification. The egg and larva samples were put into 70 percent alcohol and were prevented from decomposing, while some larvae were left to develop to permit species identification at the adult stage. The development of larvae was encouraged with 150 gr of beef and cups were used for insect development. The individual specimens that developed into pupa were rehoused in proper cups and underwent species evaluation after developing into adults.

STUDY FINDINGS

Table 1. Members of Diptera order detected in rabbit carcasses, and seasons of detection

Superfamily	Family	Species	Season of study
Oestroidea	Calliphoridae	*L. sericata*	Spring-Summer
		C. albiceps	Spring-Summer
		C. vicina	Spring-Winter
		C. vomitoria	Spring
	Sarcophagidae	*S. carnaria*	Spring
		S.crassipalpis	Spring-Summer
		W. magnifica	Summer
Muscoidea	Muscidae	*M. domestica*	Spring-Summer
		M. stabulans	Spring-Summer
		Ophyra sp.	Summer
	Anthomyiidae		Spring
Sphaeroceroidea	Heleomysidae		Winter
Tephritoidea	Piophilidae		Spring
Oestroidea	Tachinidae		Spring-Summer
Tabanoidea	Tabanidae		Spring-Summer
Syrphoidea	Syriphidae		Summer
Stratiomyoidea	Stratiomyidae		Summer

Temperature and humidity data

Average temperatures were approximately: 16^{0}C in May, 23^{0}C in August and 5^{0}C in December.The peak summer temperature was 36.4^{0}C, while the lowest winter temperature was -10.3^{0}C (Table 2.). This difference in temperatures resulted in significant differences in the Diptera species detected in the carcasses and the process of decay.

Spring Study

No insect activity was observed in the carcasses on the first day. On the third day of the study, *Lucilia sericata* was detected on the carcasses, followed by *Chrysomya albiceps* on the ninth day (Table 4). *Lucilia sericata* larvae were active during the spring study (Table 3), while *Chrysomya albiceps* larvae were also observed after some delay.During the trial, ants, ticks, spiders, hemiptera and bees were also detected in the carcass. As the larvae disappeared, the insects that dined on them also disappeared.

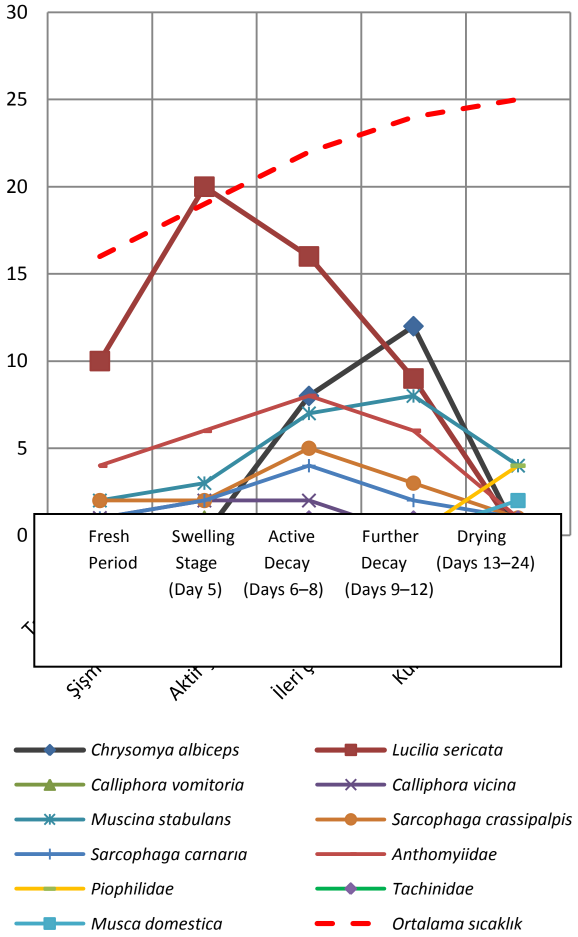
Table 2. Climate values between May 2009 and March 2010

	Maximum temperature (^{0}C)	Min temperature	Average temperature	Average relative humidity
May 2009	30	4.9	16	60.7
June	36.6	10.5	22.2	47.1
July	35.9	14.1	24	47.4
August	36.4	12.1	23.1	37.5
September	33.1	4.6	18.8	49
October	29.4	6	16.8	47.9
November	20.1	-3.7	6.9	81.6
December	13.3	-4	5.1	88.3
January 2010	16.7	-10.3	3.1	83.8
February	17.9	-5	6.8	74.2
March	22.9	-4.4	8.4	59.2
April	24.5	0.1	11.8	56.7

Table 3. Duration of egg, larva and pupa stages of ***L. sericata*** according to the study in May

Species	Start date	First egg laying date	Larva stage	Pupa stage
Lucilia sericata	28.05.2009	30.05.2009	7 days	10 days

Table 4. Comparison of number of adult insects detected in the carcass and time of detection



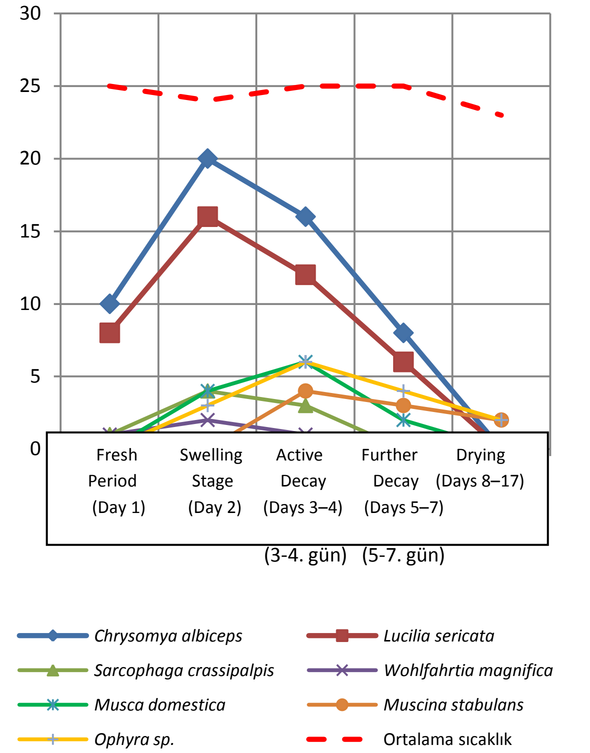
Summer Study

By the fourth hour after the carcasses were left in the study area, *Chrysomya albiceps* and a few *Lucilia sericata* could be detected on the carcasses (Table 6), but by the fifth day, insects were no longer detectable. It was observed that bees began eating the insects, insect larvae and insects that had just completed their pupa stage from the first day of the trial. Insects that managed to avoid the high temperatures and bees laid eggs in the lower part of the carcasses. Furthermore, after eating the lower parts of the carcasses, they remained there and turned into pupae.During the summer study, *Chrysomya albiceps* larvae were active (Table 5).

Table 5. Duration of egg, larva and pupa stages of ***C. albiceps*** during the summer study

Species	Start date	First egg laying date	Larva stage	Pupa stage
C. albiceps	04.08.2009	04.08.2009	5 days	6 days

Table 6. Comparison of the number of adult insects detected on the carcass during the summer study and time of detection



Winter Study

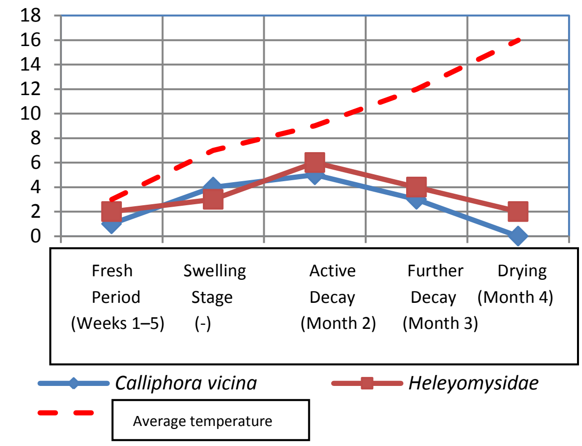
The carcasses were left in the study area on 21 December 2009,when the maximum temperature was 10.4^{0}C. No insect activity was observed on the first day. *Calliphora vicina* was detected on the carcass for the first time on 30 December (Day 10), which was a sunny day, while on 31 December, 20 *Calliphora vicina* larvae measuring 0.3 cm were found around the mouth of the carcasses. By 4 January, the larvae had grown to 1cm, and on 26 January, larvae measuring 1.6 cm were found in the same oral area. On 28 January, snowfall prevented a clear examination of the carcasses. Maximum temperatures increased from 29 January, reaching 13.8^{0}C. On 30 January, a few *Calliphora vicina* eggs were detected in the carcasses, and it was found that insects infested the carcasses at intervals on warm days until 11 March, as if they were infesting a fresh carcass for the first time. On 2 February, larvae measuring 0.5 cm were detected in the lower parts of the carcasses, and these larvae had reached a length of 1.2 cm by 12 February. Larvae activity continued until 23 March.

Many pupae were detected up to 3 meters away from the carcasses, detected from their entry holes into the soil surface. It was observed that *Calliphora vicina* had emerged from the pupae stage since 20 March, and adult *Calliphora vicina* were observed around the carcasses until the second week of April. The winter trial lasted 3.5 months (Table 8), during which, the infestation of *Calliphora vicina* and larva lactivity were very intermittent (Table 7). *Calliphora vicina* larvae were active during the winter trial.

Table 7. Duration of egg, larva and pupa stages of *Calliphora vicina* detected on the carcasses during the winter study, between 30.12.2009 and 30.01.2010

Species	Start date	First egg laying	Larva stage	Pupa stage
Calliphora vicina	21.12.2009	30.12.2009	4 weeks	_
		Second egg laying	Larva stage	Pupa stage
		30.01.2010	7 weeks	2weeks

Table 8. Comparison of the number of adult insects detected on rabbit carcasses during the winter study and the time of detection

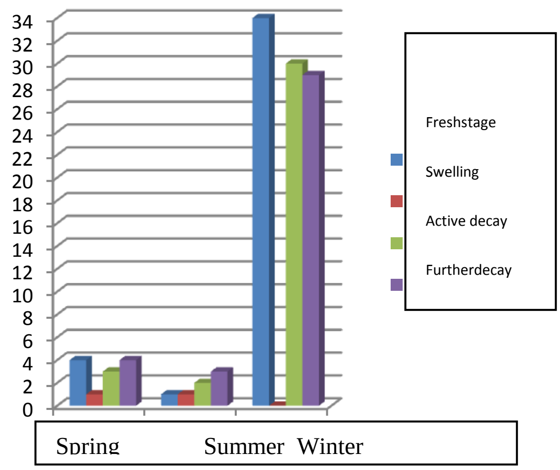


Findings on decaying process

The trials continued until all insect activity on the carcasses came to an end, and thus it was possible to observe all development stages of the insect species. As a result, it was observed that the level of insect infestation of the carcasses depended on the temperature; that is, the number of insect species infesting the carcasses decreased as the temperature decreased, the first egg laying period was extended and the larvae hatched later than normal, and the development stages of larva and pupa were extended. *Chrysomya albiceps* laid eggs only four hours after the carcasses were left in the study area during the summer months when the temperatures were highest; *Lucilia sericata* laid eggs three days after the carcasses were left in the study area during spring months, when the temperature was lower: and *Calliphora vicina* laid eggs nine days after the carcasses were left in the study area during the winter months, when the temperatures were very low. The duration of decay changed according to seasonal conditions.

Decay occurred in five stages,namely: fresh stage, swelling, active decay, further decay and drying. The fresh stage lasted four days during the trial in May 2009, while this stage ended on the day the trial was being carried out during the trial that began in August 2009. In January, when the temperature decreased dramatically, this stage lasted up to five weeks.It was observed that the duration of the swelling stage of rabbit carcasses varied between seasons, as a result of different temperatures. This period was one day in May and August, while in winter, the rate of swelling was very low, and no visible swelling could be observed. The active decay period was three days in May, two days in August and one month in January. Further decay was four days in spring, three days in summer and one month in winter. The drying period of the carcasses began after 10 days in the summer, after 13 days in the spring and after three months in winter (Table 9).

Table 9. Duration of decay stages in spring, summer and winter.



DISCUSSION AND RESULTS

In this study,the insect species that infested the carcasses the most in the study areas during the spring, summer and winter of 2009–2010 were determined. *Lucilia sericata* was the dominant species in the spring, when the average temperature was 16^{0}C; *Chrysomya albiceps* was the dominant species in the summer, when the temperature reached 6.4^{0}C; and *Calliphora vicina* was the dominant species in the winter, when the temperature fell to -10^{0}C (Table 3.2). *Calliphora vicina* and *Lucilia sericata*, which are generally accepted as urban species, [7] were found in high numbers on the carcasses, which matches the fact that the area of study is in transition from a rural area to an urban area.During the spring study,it was found that *Lucilia sericata* started infesting the carcass at an average temperature of 13.7^{0}C on day three, and their eggs hatched on day four. On day 11, no *Lucilia sericata* larvae remained on the carcass, as all had become pupae. On day 20, the adult *Lucilia sericata* were detected around the cage, having left the pupa stage, transitioning from egg to adult in 17 days, which concurs with the finding of Introna and Campobasso (2000), that the development of *Lucilia sericata* is completed in a minimum of 10 days and a maximum of 28 days [8]*Chrysomya albiceps* was found to be the most prevalent species on the rabbit carcasses in the summer months, based on the study that began on 4 August. During the summer study, the carcasses were first infested by *Chrysomya albiceps* four hours after they were left in the study area under a maximum temperature of 36.4^{0}C and an average temperature of 23.1^{0}C. On day two, the eggs hatched, and by day nine, all larvae had turned into pupae, meaning that no larvae were left feeding on the carcasses. From day 13 to day 16, *Chrysomya albiceps* were observed emerging from the pupae. As the maximum temperature reached 36.4^{0}C, all of the carcasses skeletonized in a shorter period of time, and insect activity on the carcasses had ceased completely after 16 days. Marchenko (1978) found that the pupa stage of *Chrysomya albiceps* was 7.2 days at an average temperature of 36^{0}C [9]. It was also found that *Chrysomya albiceps* larvae was a predator of other larvae during summer period.Karapazarlıoğlu (2004) stated that *Lucilia sericata* and *Chrysomya albiceps* were among the most dominant species in a study using swine carcasses in Samsun during the summer period [10]. Şabanoğlu (2007) found in a study carried out in Ankara using swine carcasses that *Lucilia sericata* larvae, when found together with *Chrysomya albiceps* on carcasses, are very few in number [11]. Furthermore, Marchenenko (1978) observed in a study in which *Chrysomya albiceps* larvae were found together with *Musca domestica* and *Protophormia terraenovae* larvae that *Chrysomya albiceps* larvae completed its development by eating other larvae in the event of food in sufficiency, [9] and when the food was sufficient, it destroyed all other larvae before infesting the carcass. *Calliphora vicina* was the dominant species during the winter trial, which began on 21 December. During this trial, the first adult *Calliphora vicina* was detected on the rabbit carcass on day 10, and on day 11, larvae measuring 0.3 cm were found around the oral area of the carcasses, and on day 38, a few larvae measuring 1.6 cm were observed. On day 39, snowfall occurred and no larval activity was detected on the carcasses. Some 43 days after the carcasses were left in the study area, new larvae measuring 0.6 cm were observed, and these had reach a length of 1.2 cm by day 54, and 1.4 cm by day 57. On day 73, dark-colored pupae and post-feeding larvae were observed. It was found that larval activity ended after day 94, by which time all larvae had turned into pupae, and on the same day new adult specimens were observed around the carcasses. As of day 110, new adults of the *Calliphora vicina* species were observed around the carcasses. Introna and Campobasso (2000) reported that *Calliphora vicina* completed its development in a total period of 28–53 days, [8] and Donovan *et al.* (2006) found that the minimum development temperature of *Calliphora vicina* was 1^{0}C [12].This explains why the trial in winter, when the temperature fell to -10.3^{0}C, lasted so long. Larvae in the same location can produce a significant amount of heat, which is generally referred to as larval mass effect [13]. During the winter trial, as the rabbit carcasses were small and temperatures were low, the insects did not infest the carcasses, and thus the larval mass effect was limited. In successive studies, Payne (1965) and Early and Goff (1986) reported the existence of visitor species and their effects on the decay process [14,15].

During the spring and summer trials, it was observed that the bees and ants that infested the rabbit carcasses disturbed the insect species and affected significantly their egg laying. Particularly, during the summer study, it was observed that insects laid their eggs in the lower parts of the carcasses and between the legs rather than in open cavities. It was further noted that *Silphidae* and

Staphylinidae of the *Coleoptera* order, when feeding on the carcasses, ate insect larvae, and that climatic conditions changed the duration of decay of the carcasses. Payne (1965) reported that the decay rate of carcasses is higher in cold weathers, meaning that the decay process is faster in the summer months [14]. Our study concurred with that of Payne, in that the rabbit carcasses that were left on the area dried after nine days in the summer, after 12 days in the spring and after three months in the winter. In the present study, the rabbit carcasses went through five stages of decay, namely the fresh stage, swelling stage, active decay stage, further decay stage and drying stage, as stated in many studies and references. *Chrysomya albiceps*, *Calliphora vicina* and *Lucilia sericata* started infesting the carcass during the fresh stage and their number peaked during the swelling phase, decreasing gradually during active decay.They were rarely observed during further decay and were completely absent during the drying phase. This data is in line with other studies in literature.

As the size and mass of a rabbit carcass is much smaller than a person, there was a delay in the infestation by insects, larval mass effects were low and the decay stages were shorter. Kuusela and Hanski (1982) demonstrated that large carcasses do not attract different insects to small carcasses, although there will be an increase in the number of insects involved in the infestation [16]. It should be considered that the drugs used for the initial killing of the rabbits, being Ketasol and Xylazol, may have affected the delay times.

The rabbit carcasses used in the study weighed around 4 kg, and so were equal to the weight of a new born baby. In this regard, the data obtained may have potential use in cases of baby abuse or murder.

It was observed that Diptera species showed less interest in the carcasses when the temperature was low, and did not infest the carcasses when it rained. After the rain, it was observed that both the decay process and larval activity increased, and Diptera, which had shown less interest, continued its infestation of the carcass again. Anderson’s (2010) comparison of swine carcasses left in sunny and shaded areas revealed that the carcasses in the sunny areas decayed faster than those left in the shade [17]. From our study it can be understood that the warming effect of the sun accelerates decay. Dominant species were identified in both the summer and winter studies, but two types of larvae were identified as dominant during the spring. Depending on this, it may be possible to observe a mixed larval succession during the transitional seasons.

In summary, it has been demonstrated that these three species of the Calliphoridae order can be used effectively to determine the post-mortem interval in a potential case of death that may occur around Kırıkkale University in the Yahşihan district of the Kırıkkale province.

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