Survey of Forensically Important Calliphoridae in Samsun
Samsunda Adli Açıdan Önemli Olan Calliphoridae Hakkında Araştırma

Meltem Kökdener¹, Erdal Polat²
¹Ondokuz Mayıs University, Samsun Health High School, Kurupelit, Samsun
²Istanbul University, Cerrahpasa Medical Faculty, Department of Microbiology and Clinical Microbiology, Istanbul

Abstract
Objective: The objective was to determine the decomposition process and forensically important Calliphoridae (Diptera) succession on dog carcass during one year.

Materials and Methods: This study was conducted from June 2009 to June 2010 in three different area of Samsun province, Turkey. One dog’s carcass (Canis lupus familiaris L.), weighing 15-20 kg, was placed in each area during each season (total 12 carcasses, per year). Ambient daily temperature (maximum and minimum), relative humidity (RH) were recorded and existing keys were used for identification of different species.

Results: Lucillia sericata (Meigen), Chrysomya albiceps (Wiedemann), Calliphora vomitoria (Linnaeus) and Calliphora vicina (Robineau-Desvoidy) species were encountered. It was shown that the seasonal distribution of the collected species was different from each other. Chrysomya albiceps was observed in summer, autumn and spring. In the spring and winter, the dominant fly species were Calliphora vicina. The rate of decomposition of carcass was faster in summer and autumn as compared to spring and winter. Results indicated that ambient temperature is the chief factor determining the seasonal variations in decay rate.

Conclusion: This report also identified some of the Calliphoridae that occur in north of Turkey. The importance of regional faunistic studies of the calliphoridous community, the results of which may be applied to forensic practice in the future.

Keywords: Forensic entomology; Insect succession; Corpse; Postmortem interval; Decomposition.

Özet
Amaç: Çalışmanın amacı köpek karkasındaki çürüme procesini ve adli açıdan önemli Calliphoridae (Diptera) süksesyonlarını belirlemektir.

Gereç ve Yöntem: Bu çalışma Haziran 2009 ve 2010 yılları arasında Türkiye’de Samsun ilinde, üç farklı alanın karşılaştırılması sağlanmıştır. Her mevsimde (1 yılda toplam 12 karkas), her çalışma alanında 15-20 kg ağırlığındaki bir köpek karkası (Canis lupus familiaris L.) konulmuştur. Ortalama günlük sıcaklık (maksimum ve minimum) ve nisbi nem kaydedilmiştir ve farklı türler mevcut anahtarıyla teşhis edilmiştir.

Bulgular: Lucillia sericata (Meigen), Chrysomya albiceps (Wiedemann), Calliphora vomitoria (Linnaeus), and Calliphora vicina (Robineau-Desvoidy) türleri teşhis edilmiştir. Toplanan türlerde mevsimsel dağılımın birbirinden farklı olduğu görülmüştür. Chrysomya albiceps yazın, ilkbahar ve sonbaharda görülmüştür. İlbahar ve kişin Calliphora vicina dominan türdür. Karkasın çürüme hızı kiş ve kişinbaharla karşılaştırılınca çok hızlıdır. Sonuçlar śmın çürüme hızındaki mevsimsel varyasyonların belirlenmesinde çok önemli olduğunu göstermiştir.

Sonuç: Bu rapor Türkiye’nin kuzey bölgelerindeki bazı Calliphoridae türlerini tespit etmiştir. Calliphorid türleri hakkındaki bölgesel faunistik çalışmaların önemli sonuçlarını gelecekteki adli çalışmalarında kullanılabileceğidir.

Anahtar Kelimeler: Adli entomoloji; Böcek süksesyonu; Ceset; Postmortem interval; Çürüme

1. Introduction
After death, insects are attracted to the carcases that they colonize in a relatively predictable sequence (1). Insects play an active role in the decay process (2) and have been classified according to their ecological role in carr...
In addition to estimating the postmortem interval, they can aid in death investigations such as manner of death, place of death, and the presence of drugs or toxins in a corpse (3,8,11-13).

The knowledge of Calliphoridae species is principal for each region because, the species of insect involved in decomposition stages change from region to region.

Studies on successional patterns of arthropods important to forensic entomology and has been carried out in several regions of the world. So far, there were several succession studies done in Turkey. However, such studies are still scarce in many other regions of Turkey. The specific objectives of this study are to identify adults and larvae of the Calliphoridae arriving decomposing dog carcasses. Results of the present study could provide entomological data that can be employed in forensic cases in north of Turkey and other similar biogeoclimatic regions.

2. Materials and Methods

This study was conducted from June 2009 to June 2010 in three different area (urban, coastal and rural) of Samsun province, Turkey. In the study, one dog carcass (Canis lupus familiaris L.), weighing 15-20 kg, was placed in each area during each season (total 12 carcass, per year).

I. Taflan region: The study area was chosen at Taflan. This study location was a coast of Samsun that is located 40 km from the north of the city. The area is located at 41°26’ N; 36°0.8’ E and 500 m from Black sea. The main road is about 1.5 km away. In its native form, this region is characterized by, broad-leaved herbs, mixed grasses and deciduous shrubs.

II. Atakum region: A rural, farming area in the Atakum region of Samsun was chosen as the research site. This area, is located at 3035 m above sea level and situated 10 km from centre of the city (41°15’N, 36°19’E). The predominant vegetation in this area consists broad-leaved herbs, mixed grasses, and deciduous shrubs.

III. City center: The third study area(urban) chosen at city center(41°16.2’K; 36° 17’D). The study sites in this habitat were located approximately 3 km from the Black sea and were partially shaded by low shrubs.

The animal model used for this study was the dog (canis lupus familiaris l.) that had died of natural causes from animal shelter of municipality. Each dog carcasses (canis lupus familiaris l.) double bagged in 100-gal plastic garbage bags immediately after death and transported to the studies area. Each carcass was placed inside a cage constructed of wire mesh 2.5 cm mesh 2.5 cm wide all sides. Cages were open on the front so that the carcasses were in direct contact with the ground, allowed for full environmental exposure and staked to the ground to prevent disturbance of the carcasses by vertebrate scavengers. The cages is designed to collect a large proportion of insect. No mark of animal or human discomfort was established during the study. The trap is designed to collect a big proportion of arthropods. Carcasses were exposed directly to sunlight during certain parts of the day, whereas at other times they were shaded and placed under a wooden cage for attracting arthropod specimens during season. All dogs were placed on study site in four seasons, three dogs were used in each of the four experiments and left to decompose naturally. Each experiment period lasted until the whole carcass was expended. Environmental data for each season were gathered at the local weather station, in same time weather data were collected during each visit for the duration of the study. Carcass temperature was taken with a thermometer. Carcasses decomposition proceeded at different rates during seasons therefore the sampling protocol was arranged accordingly for each seasons. Sampling was conducted daily in summer and autumn and three times a week in winter and spring. During each sampling, the state of the carcass, physical modification, weather conditions and insect activity were recorded. During collection, samples of insects were collected from on, in and under the carcasses as well as the surrounding soil to provide ensure as many diverse species as possible were gathered.

Methodology of Laboratory

All insects observed were sampled, according to developmental stages of insects. Larvae, pupae and adults were collected from the carcasses. Adult insects specimens associated with carrion were collected from the carcasses using aerial sweeps and net and manual collection the larvae, and pupae samples were sampled using forceps. Adult beetles and crawling insects, were captured by hand or with forceps and pinned for later identification. Samples of larvae were divided in two where half were killed at the site by immersion in near-boiling water and preserved in 70% ethanol and half were taken to the laboratory for rearing. The rearing of larvae was for confirmatory identification objectives. Larvae were placed on a small piece of raw chicken liver (approximately 10 g) and then a 3 cm layer of vermiculite was added to 200 ml clear plastic containers. Pieces of furnished with small holes for air circulation, were used to cover containers. Containers were held at room temperature (i.e., 22–24 C) with a light: dark regime of 12:12 hours. Containers were checked twice daily for the presence of adult blow flies. Some of adult specimens were put in 70% ethanol. The remainder were directly killed in cyanide jars. Ethanol-
killed insects were preserved in plastic specimen containers and cyanide killed insects were pinned and put in the collection for identification and observation. Soil samples were taken, especially for observation of pupae. Pupae were collected then transferred to laboratory to be reared to adulthood. Samples of pupa placed in 200 ml clear plastic containers with sawdust and covered with paper towel secured with rubber bands. All samples were labeled with the date and time of collection and the area of the carcass the samples came from. Taxonomic determination was made by using current keys (14-17).

3. Results
Decomposition Process
The identified samples have been represented in Table 1. In accordance with Catts and Golf (3), five stages of decomposition were examined: fresh, bloated, active decomposition, advanced decomposition, dry.

Decomposition of the carcasses progressed at different rates during the spring, summer, winter and autumn studies. Corpses decomposed at a faster rate in seasons with higher temperatures. Carcass which was placed in summer reached the dry stage in only 23 days, 45 days in spring. In contrast, during winter 86 days were required for carcasses to reach the dry stage and 76 days in autumn because of the evident cooling of the season (Fig.1). Differences in the duration of decomposition stages at each area and between the seasons studied were only observed in the winter. The carcasses which was placed in winter had not reached the dry stage in Atakum region.

In summer, two subfamilies of Calliphoridae family were identified: *Ch. albiceps* arrived to the carcass on the first day in all study site. *L. sericata* arrived on the second day in Atakum region during summer. *L. sericata* wasn’t not observed during spring and winter (Table 1). In summer, *Ch. albiceps* exhibited the longest period of residence in all study area.

During autumn, three subfamilies of Calliphoridae family were identified: The arrival of dipteran species each season was during the early days of decomposition.

Table 1. Distribution of identified species of Calliphoridae during decomposition period.

| Species       | Seasons | Summer | Autumn | Winter | Spring |
|---------------|---------|--------|--------|--------|--------|
|               |         | F      | B      | AC     | AD     | D      | F      | B      | AC     | AD     | D      |
| *Ch. albiceps*|         |        |        |        |        |        |        |        |        |        |        |
| *Ca. vicina*  |         |        |        |        |        |        |        |        |        |        |        |
| *Ca. vomiters*|         |        |        |        |        |        |        |        |        |        |        |
| *L. sericata* |         |        |        |        |        |        |        |        |        |        |        |

(F: Fresh stage, B: Bloat stage, AC: Active decomposition stage, AD: Advanced decomposition stage, D: Dry stage)
of the carcasses in all study area. Of the predominant Diptera taxa, C. albiceps (Diptera: Calliphoridae) consistently colonised all carcasses within the first 24 h in all study area. L. sericata was observed in Taflan region during from bloated stage to end of advanced decomposition stage. Ca. vicina and Ca. vomitoria at the carcasses were delayed in autumn and collected firstly from the end of the bloated stage (Table 1).

During winter, two species from only one subfamilies of Calliphoridae family were identified: Ca. vomitoria and Ca. vicina showed a preference for the lower temperatures. Ca. vicina was observed longer period than Ca. vomitoria. Ca. vomitoria were collect from active decomposition stage to end of advanced decomposition stage. Ca. vicina was observed one of the dominant species in the three study area (Table 1).

During spring, three species from three subfamilies of Calliphoridae family were identified: Calliphoridae was represented by three species in the spring. Ca. vicina was seen longest duration on the carcass, from fresh to dry stage, in this study Other early calliphorid colonisers, Ca. vomitoria appeared from the bloated stage to the middle of the advanced decomposition stage. Ch. albiceps was first collected in the active decomposition stage and present till dry stage.

4. Discussion

From the evidence of our study, all decay stages seen extended from summer to winter and decreased from autumn to summer. Undoubtedly environmental factors such as ambient temperature, seasons, humidity, rainfall, habitat affect arthropod succession and duration of decomposition stage (8). This is consistent with our findings. In this study, the decomposition changes and rate of decomposition observed in all study area were similar.

Carrion insects are impacted by season, with specific peaks in activity, abundance and species richness (8,18).

Calliphorids exhibit definite seasonal preferences in terms of activity and abundance. The Calliphoridae species laid eggs within 1 hour following the placement of the carcass in the study area during summer months but these species laid eggs 5 days following the placement of the carcass in the study area during winter months.

This study showed Ch. albiceps was primary invader on dog carcass. Ch. albiceps is prefer warmer seasons and was disappered when the air temperature is decreased. The species was first observed on May 26 and continued to be found till November 6 (Fig. 2). Tantawi et al. and Wolff et al. recorded Ch. albiceps as secondary breeders on rabbit and pig carrions respectively (18,19).

L. sericata was especially abundant in Atakum region during summer, in Taflan during autumn. During winter and spring, L. sericata was absent.in all study area. Şabanoğlu and Sert (20), Vega and Baz (21), reported that L. sericata existed during summer, spring and autumn. Horenstein et al. (22), identified L. sericata during winter spring and autum.

Due to Ca. vicina and Ca. vomitoria showed pereference for the lower temperatures, these species were the most abundant species during winter and autumn. This finding is parallel to the findings of the study that was carried out by de Prado et al. (23) Ca. vicina was recorded for the longest period during our studies in all study area. This species was present on the dog carcasses from October 1, 2009 until May 10 2010 (Fig. 2). When temperature increased, this species disappeared on the carcasses. The intensity of Ca. vicina are higher than Ca. vomitoria in all study area. When we compare intensity of both species between seasons; the intensity of Ca. vicina and Ca. vomitoria in autumn are lower than winter and spring. This finding is parallel to the findings of the study that was carried out by Tantawi et al. (18), Şabanoğlu and Sert (20), Horestein et al. (22), Arnoldosa et al. (24), Prado et al. (23), Vega and Baz (21).

In conclusion this study obtained data presented the knowledge of the distribution of Calliphoridae species during four season as well as the duration of decay rates in the north of Turkey. These species may be important indicator species of specific stages of decomposition in this region. Besides the faunistic information, Calliphoridae that could recorded in carcasses were used for estimation of PMI. Due to the absence of regional experimental studies, police investigators still do not use entomological samples obtained from a corpse as evidence. This study begin to fill the knowledge gap and highlights the importance of forensic entomology in Turkey. Mean time these data can be used to model future studies. Turkey have great diversity of geoclimatic regions, many addi-
tional studies are necessary to determine the species that are present in different geographic region.

Acknowledgement
We thank Istanbul University Scientific Researches Projects Unit for supporting our study with 3943 numbered project.

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