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A Checklist of Forensic Important Flies (Insecta: Diptera) Associated with Indoor Rat Carrion in Iran

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Abstract
The study of Diptera provide useful complementary data to estimate the post-mortem interval in forensic cases. For the use of insects in criminal investigations, study of insect fauna in each region is essential. This is the first report studying the flies associated with indoor carrion of two carcasses of rats (*Rattus norvegicus*), in Iran. Rat carcasses were exposed in an old room (6 m^2) of a house for 6 weeks in the Fars Province, Iran. In this period during; 3 families, 6 genera and 9 species were collected viz. Muscidae: *Musca domestica* and *Ophyra* sp,; Calliphoridae: *Calliphora vicina, Chrysomya albiceps*, *Lucilia sericata*, *Calliphora vomitoria* and *Lucilia caesar*; Sarcophagidae: *Sarcophaga haemorrhoidalis* and *Sarcophaga* sp. *Chrysomya albiceps* and *Musca domestica* were collected in all stages of decomposition. The first flies egg mass seen in fresh stage of decomposition belonged to *Calliphora vicina* species. Information from this study can be helpful in forensic entomology.

Keywords: Diptera; Indoor Forensic entomology; Iran

1. Introduction
Forensic entomology is the application of the study of arthropods to legal study. Issues of the distribution and biology of insects can help many types of forensic investigation, the most important application is in the estimation of the postmortem interval (PMI) [1]. The study of Diptera provide useful complementary data to estimate the post-mortem interval in forensic cases. Diptera infest corpses in various places such as the savana, forest, inside the car, within the water and everywhere else [2]. Insects colonize the corpse in a predictable regularity; as Calliphoridae family are found in the early stages of body decomposition [3]. Therefore, they are useful in the estimation of the minimum postmortem interval (PMI_{min}). Insect colonization and period of colonization depend on many factors such as environmental conditions and conditions of the corpse (position, sunshine or shade, clothes, indoor or outdoor) [4, 5]. Therefore, collection and identification of insects under such circumstances could be important to estimate the time of death. For the use of insects in legal medicine, sufficient data should be collected in each geographical region, such as time arrival of insects to the corpse, insect succession on cadavers and biology of insects [1].
Studies on insects associated to carrion are well documented in different regions. Ahmad Identified insects on indoor and outdoor monkey carrions in Malaysia [6]. Determined Seasonal patterns of arthropods colonizing pig carrion in Argentina [7], Velásquez identified arthropods associated with rat carrion in Venezuela [8].
The first succession study in Iran conducted by Tüzün who made a preliminary study of insect species of forensic importance in Urmia in 2010 [9]. However, such study had never been conducted indoor. Hence, this is the first documented report of entomological evidence from the carrion in a closed environment.
The application of entomology to forensic science, in spite of its great potential, neglected in Iran. Eventually, we hope to advance Forensic entomology in Iran.

2. Materials and Methods
2.1. Study site
The study was carried out in an area of 6 m^2, in an old room of a house in Shiraz city, southern Iran. Shiraz is the capital of Fars Province, located in the southern part of the country.

There are three distinct climatic regions in this province. This province has moderate temperature in winter and very hot weather in summer. This study was conducted in the metropolitan area of the city (29.62° N, 52.53° E) with an annual mean rainfall of 200 mm and an average temperature of 24°C. The averages of minimum and maximum temperatures and relative humidity in the old room of the house were 20.8, 30.3 °C and 58% respectively (Figure 1).

2.2. Study animal and insect collection

The study was carried out using two laboratory bred rats *(Rattus norvegicus* Berkenhout) weighing 341 g as a model for human decomposition. Two carcasses were killed by contusion and placed in separate cages and hanged from the ceiling by a rope and were separated. They were separated from each other with a length of two meters. The old room had a window that was completely opened to allow entrance of the flies and a door which remained closed. Observations and collections of flies were made daily during October and November 2014.

Adult flies and immature were collected with an insect net and forceps. Larvae were collected and divided into two groups; some immature individuals were killed in hot water and stored in 70% alcohol, while others were transferred to the laboratory of entomology for rearing. The live larvae were reared on chicken meat in a thermostatic room. The temperature of the rearing room was 24 ± 1°C and measured daily. The adult flies were killed with ethyl acetate and then pinned with entomological pins for identification. Valid taxonomic keys were used for the identification of different species [10, 11, 12, 13].

3. Results

Decomposition time for two rats lasted 42 days, from October 2 to November 14, 2014. In this period of time, 2254 adults and immature flies were collected on the two carcasses, distributed in 3 families, 6 genera, and 9 species. They were identified by family and species, whenever possible, the most abundant family was Calliphoridae with 1703 (75%) individuals, followed by Muscidae 383 (17%), and Sarcophagidae with 168 (8%). In this study, we also recorded the day of adults capture (Table 1).

Five decaying stages were identified by the morphological changes of the carrions: Fresh, Bloated, Decay, Advanced decay and Remains. In the fresh stage (Day 0-2) minimum and maximum room temperature were 23.3°C and 27.6 °C respectively. During this stage *Musca domestica* adults was the first fly species that were present on the carcasses. We also captured *Chrysomya albiceps, Ophyra* sp and *Sarcophaga* sp. In this stage, between these *Musca domestica* was dominant. The first maggots were seen in this stage which belonged to *Sarcophaga* sp species.

In the bloated stage (day 3 to 5) adults of *Calliphora vicina*, *Chrysomya albiceps, Lucilia sericata, Lucilia caesar, Musca domestica*, *Ophyra* sp, *Sarcophaga haemorrhoidalis* and *Sarcophaga* sp were collected. During this period the abdomen of the rat was bloated and room temperature ranged between 23°C and 26 °C. The first flies egg mass seen in this stage belonged to *Calliphora vicina* species, while the adults of this were not collected in the previous stage. Most of the adults were collected during this period (figure 2).

The decay stage lasted for 2 days (day 6 to 7), in this stage the room temperature was varied between 25°C and 30°C. The largest number of larvae collected at this stage. Adults *Sarcophaga* sp, *Musca domestica*, *Calliphora vomitoria*, *Lucilia sericata*, *Chrysomya albiceps and Calliphora vicina* at this stage were collected and between this species *Chrysomya albiceps and Calliphora vicina* were dominant.

In the Advanced stage (day 8 to 13) body weight lost heavily and a combination of bone, skin and internal organ can be seen. Minimum room temperature and maximum room temperature in this stage were 21°C and 25°C respectively. Species that were collected at this stage include; *Chrysomya albiceps*, *Lucilia sericata*, *Musca domestica* and *Sarcophaga sp*. The first pupae were seen in this stage and belonging to the *Chrysomya albiceps*. The remains stage lasted for 29 days (day 14 to 42), in this stage the carcass was reduced to hairs, skin and bones and the minimum and maximum temperature was 20.8°C and 28.6 °C respectively. At this stage the number of adults and larvae of flies decreased and adults *Chrysomya albiceps and Musca domestica* were observed.

Table 1: Adult Diptera species collected on different days of decomposition.

Species	Family	Days of capture (Adult)
Calliphora vicina	Calliphoridae	3,5,6,7
Chrysomya albiceps	Calliphoridae	2 to 9, and 12 to 18
Lucilia sericata	Calliphoridae	1,2,4,5,6,7,9
Calliphora vomitoria	Calliphoridae	6,7
Lucilia caesar	Calliphoridae	4,5
Musca domestica	Muscidae	1 to 8 and 11 to 42
Ophyra sp	Muscidae	2,3,5
Sarcophaga haemorrhoidalis	Sarcophagidae	3,4,5
Sarcophaga sp	Sarcophagidae	5,6

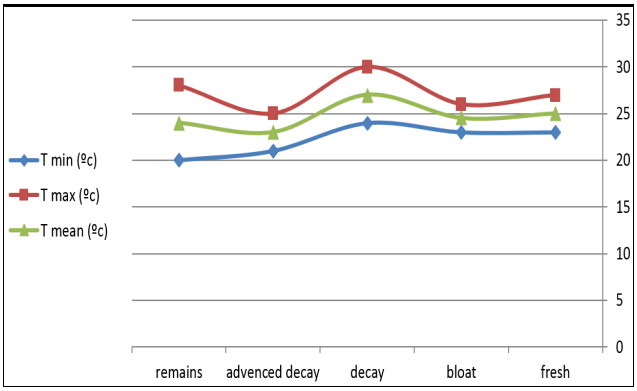

Fig 1: Curve of variation of temperature related with the stage of decomposition in a indoor carcasses in the Fars province, Iran

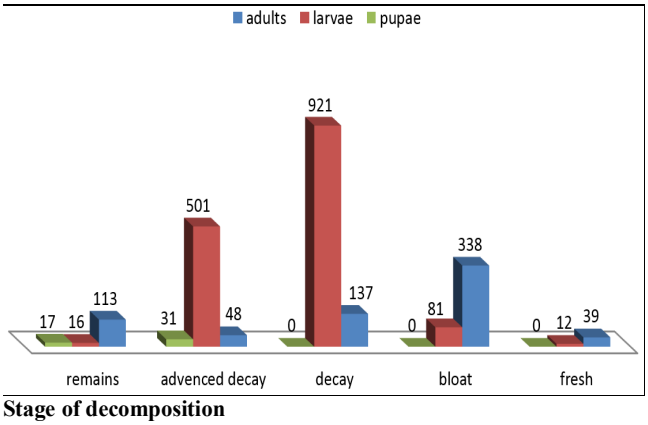

Fig 2: Number of adult, larva and pupa of flies associated with indoor carcasses in the Fars province, Iran.

4. Discussion

In this study Calliphoridae family was more abundant compared to other families as was observed by Pastrana [14], and Goff [15]. In our study, *Musca domestica* adult was the first fly species that was present on the carcass and in the first 2 days had great abundance. This species was available until the end of the decomposition process, but with very low abundance, as was observed by Tuzan [9] and different from the observations in other studies [14, 6]. Wolff observed the arrival of Diptera 30 minutes after placing the carrion, while in the present study it was observed arrival of Diptera 23 minutes after putting the rat carcasses [16]. In our study the first fly larva observed in the fresh stage, similar finding were reported by Pastrana [14]. *Chrysomya albiceps* was the only member of the Calliphoridae family found in all the stages. *Musca domestica* and *Lucilia sericata* were the most frequent species in fresh and bloat stages respectively.

In our study *Chrysomya albiceps* was the most frequent species. Rosa [17], also found this species to be the most abundant among the breeding Diptera in pig carcass in Brazil.

Other studies on carcasses at different seasons can be helpful in the future for criminal investigation in Iran. Study of life cycle of the Diptera species in each geographical region is essential, because populations of the same species can be different physiologically depending on their geographical origin, as a result, the growth rate may be different in various regions [18]. Therefore, further studies on Diptera species are needed to use these species in forensic investigation.

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6. References

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