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# Forensically important Diptera species associated with Dog carcass (*Canis domesticus* L.) for a case study in District Mardan, Pakistan

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**Abstract**

Forensic Entomology is the scientific methods used by entomologist to calculate the time of an organism death through insect communities as a biological indicator. In the present study, insect fauna identification in dead dog, *Canis domesticus* (L.) has been used as in substitute of dead human body in tropical region Mardan, a tool for forensic entomology were carried out in five different decomposition stages, fresh, bloat, active decay, advanced decay and dry. The collected Diptera species were *Chrysomya rufifacies, Chrysomya megacephala, Parasarcophaga ruficornis, Musca domestica* and *Piophila casei. Chrysomya rufifacies* was first arrived to dog carcass and dominated first stages of decomposition. The average temperature (28.3±1.8-40.4±1.7) was found affected Diptera fauna and rate of decomposition of *C. domesticus* during the observation period for (11 days from 15 to 25 May 2011). This research will be helpful for forensic entomologist for a case study of death to investigate of crimes in District Mardan of Pakistan.

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**Introduction**

Insects acts as biological indicators for the death of an organism or person since time passed. Forensic means the scientific method to help in investigation to solve crimes by scientists, who examine evidence in order to help the law and enforcement agencies in investigation of crimes. Entomology is the study of insects biology (Collins, 2001). This study is based on the principal of ecological faunal succession of these insects observed within dead body. They have ecological succession pattern that represents an important tools in legal investigations (Erzinclioglu, 1983; Catts *et al.,* 1992). The time and arrival of insect colonies can be estimated in the dead body (Greenberg, 1991). These conclusive studies are commonly attempted to suspected criminal, for the purpose of knowing hidden facts for an investigation (Smith, 1986; Catts *et al.,* 1990; Byrd *et al.,* 2001).

Research for this purpose is conducted across the world. It is a very fast growing field of research and study, because the entomologists understand insect's successions pattern for a case their life cycles stages, habitats and identification to perform an investigation. The first observed case was found in 1235 AD in China, where the first study of flies attraction to blood were used to solve a murder investigation (Goff, 2000; Benecke, 2001).

Insect life cycles begin within minutes of death which act as precise clocks (Haskell *et al.,* 1990). Two time dependent processes for the calculation of death time period is involved in these studies. The first is the growth of insect larvae that feed upon the victim. The second is the succession of carrion arthropod species found in the body, which has the potential of providing both a minimum and maximum estimated post mortem interval (PMI) (Greenberg and Kunich, 2002). Those that consume the soft tissues of fresh carcasses i.e. Diptera (Payne, 1965; Putman, 1983). Blow flies *Chrysomya rufifaies (*Macquart, 1842), *Chrysomya megacephala (*Fabricius, 1794), *Chrysomya albiceps (*Wiedmann, 1819*), Calliphora vicina (*Desvoidy, 1830*), Phormia regina (*Meigen, 1826*), (*Diptera*:* Calliphoridae*),* flesh flies, *Parasarcophaga ruficornis* (Meigen, 1826*),* *Parasarcophaga dux (*Thompson, 1869*),* *Parasarcophaga albiceps* (Meigen, 1826*),* *Boerttcherisca peregrina (*Desvoidy, 1830*),* (Diptera: Sarcophagidae*)* and house flies *Musca domestica* (Linnaeus, 1758), *Stomoxys calcitrans* (Linnaeus, 1758), (Diptera: Muscida*)* are tissues decomposers (Fuller, 1934). These flies are considered to be the vultures of the insect world, with the ability to recognize decomposing bodies over vast distances and in any landscape type. They can travel for 20 km in a day, these flies could probably cover greater distances (Greenberg, 1991). In fact, almost every aspect of their biology suggests intense competition for the rapid location and consumption of decomposing bodies (Fuller, 1934; Putman, 1983). Blow flies and flesh flies are observed one of the important forensic indicators during the initial stages of carcass decomposition (Lord, 1990). The larvae of blow flies *C. rufifaies, C. vicinia*, *C. megacephala* and flesh flies *P. ruficornis*, *P. dux, P. albiceps* consume maximum carcass. Blow flies *C. ruficaies, C. vicinia*, *C. megacephala* lay eggs while flesh flies *P. ruficornis, P. albiceps, P. dux,* deposit larvae in natural body orifices. These larvae quickly invade most of the regions of the dead body (Putman, 1983; Payne, 1965; Putman, 1977; Goff, 1993; Tantawi *et al.,* 1996). They complete every stage of their life cycle on the carcasses but mostly in initial stages of decomposition and not in decay (Putman, 1983; Braack, 1981; Early and Goff, 1986). Grassberger and Frank (2004) observed in pig carcasses succession of insects in urban area of Austria for sequence analyzing and composition of the local carrion visiting fauna from May to November 2001. During this study total 42 arthropod species collected from families of Drosophilidae, Muscidae, Calliphoridae, Fannidae, Sarcophagidae, Sepsidae, Piophilidae, Sphaeroceridae, Phoridae, Anthomyiidae (Diptera) Formicidae, Vespidae (Hymenoptera) and Cleridae, Braconidae, Pteromalidae, Silphidae, Stapphylinidae, Histeridae, and Dermestidae (Coleoptera) while orders Isopoda and Acari species were collected during the decomposition of carcasses. In this study the high abundance was of *C. vomitoria* and *C.*

*albiceps* species. Shean *et al.* (1993) observed two pig carcasses decomposition carcasses in close proximity to each other, exposed and shaded, in a continuous woodland and different rates of decay were recorded. The exposed pig decomposition was much faster than the shaded pig, which reached a stable minimal weight two weeks before the shaded carcass. Occurrence of blow fly larvae, and ambient air temperatures were compared. Maggot variable development appeared to be a major factor in the overall rate of decomposition which was affected primarily by different temperature patterns at the two sites.

Objective of present study to determine the usefulness and applicability of Forensic Entomology and investigate insect fauna in tropical region for a case study.

## Materials and Methods

*Methodology*

In materials plastic jars, forceps, gloves, mask, insects net, digital camera and a wire gaze cage to cover the dead dog *Canis domesticus* (L.) were used. The dog was killed by hitting on the head with sharp knife. The dog, *canis domesticus* was kept in open ground of Government Degree College, Takht Bhai, Mardan. Adults and larvae and pupae were put in labeled bottles of 70% Formalin.

*Observation*

Trails for observations and collections of insects, larvae, pupae and adults were done daily in morning, afternoon and evening. This ensured that all type of insects were sampled as flies were active in later morning and beetles in evening time while larval growth was more rapid in noon. The wire gaze cage was removed aside at every sampling time and dog was not disturbed during this study. Trails were exercised to the period of total fleshy tissues of dog dead body exhausted (15-25 May, 2011 upto 11 days).

### Data collection

Daily visits were done up to 11 days, three times in morning, noon and evening per day. Adults were collected through insects net while larvae and pupae were collected through forceps from the carcass into the plastic jars for preservation and jars were labeled. Gloves and mask were used to secure the self body. Each stage of decomposition of the carcass, pictures of insects, due to the expanding insect's masses, larvae and pupae were taken through digital camera (5 mg pixel, Sony, Tokyo, Japan). A wire gaze cage (x 36″ × y 54 × z 32″) was used to keep the carcass. A 5 kg stone was also used to safe the dead dog from other living scavengers. Each day insects were put into separate jars. Forensic insect's identification methods of insects were used (White *et al.,* 1940; Dodge, 1953; Seago, 1953; Furman and Catts, 1982; Wells *et al.,* 1999).

## Results

*Observation*

During the present study the decomposition of *C. domesticus* was observed in five stages, fresh, bloated, active, advanced and dry.

**Table 1.** The insect species classification into order, family, genus and species of dog, *Canis domesticus* (L.) carcass observed during five different decomposition stages noted during the present research in May 2011 in Takht Bhai, Mardan.

| Order | Family | Genus/Species |
|---|---|---|
| Diptera | Calliphoridae | Chrysomya rufifacies |
| | | Chrysomya megacephala |
| | Sarcophagidae | Parasarcophaga ruficornis |
| | Muscidae | Musca domestica |
| | Piophilidae | Piophila casei |

*Fresh stage*

During the first day after the death up to the evening was declared fresh stage (12 hrs.). The outside appearance of the bodies was similar to those of live *C. domesticus* (Figure, 1a).

*Bloated stage*

This stage started from 1st day evening of death up to third day morning (13-48 hrs.), i.e, (36 hrs.). On second day the body of dog emitted very strong smell

that was highest than the first day and is the characteristic feather of bloat stage of decomposition. *Canis domesticus* abdomen became scratched and blackened (Figure, 1b).

*Active decay stage*

This stage was completed in 3-4 days after death (49-96 hrs.) and the skin of the *C. domesticus* was appeared blacken, emitted less smell and 80-90% of the body was decomposed. There were traces of flesh present on ground side of the body (Figure, 1c).

**Table 2.** The insect species associated with dog, *Canis domesticus* (L.) carcass observed during five different decomposition stages noted during the present research May 2011 in Takht Bhai, Mardan.

| **Scientific names** | **Stages of Decaying *Canis domesticus*** | | | | |
|---|---|---|---|---|---|
| | Fresh (12 h) | Bloated (36 h) | Active (3-4 days) | Advanced (5-6 days) | Dry (7-11 days) |
| *Chrysomya rufifacies* | P* | P* | P* | A* | A* |
| *Chrysomya megacephala* | P | P | P | A | A |
| *Parasarcophaga ruficornis* | P | P | P | A | A |
| *Musca domestica* | P | P | P | A | A |
| *Piophila casei* | A | P | p | A | A |

P*: presence in this stage; A*: absence from this stage.

*Advanced Decay Stage*

This stage was 5-6 days after death (97-144 hrs.) was characterized by minor deep odor and the removal of the soft internal tissues. The body was decomposed up to 90-99% (Fig. 1d).

*Dry stage*

This stage took five days 7-11 (145-265 hrs.) of decomposition. The smell was very minor or absent. There were minor traces of skin and hairs in first two days of this stage, but after that the bones were totally exposed. At this stage flis were absent (Fig. 1e).

*Diptera species found in the carcass of C. domesticus are given in sequence.*

Blow fly, *Chrysoma rufifacies* (Macquart, 1842). The *C. rufifacies* is belonging to order, Diptera and family, Calliphoridae. The first insect that came to the body within 10 minutes was *C. rufifacies*. It was first seen at eyes, mouth and anal regions of the *C. domisticus* and later on occupied the whole body. Their numbers reached to highest than others species within half an hour. The adults of this fly were also present in bloated but was less in number than fresh stage. The adults flies numbers were reduced in active decay than bloated stage while few numbers were present in advanced decay but absent in decay stage of decomposition (Fig. 2a). Flesh fly, *Parasarcophaga ruficornis* (Meigen, 1826). *Parasarcophaga ruficornis* is belonging to order, Diptera and family, Sarcophagidae. This fly was second after *C. rufifacies* and less in number and arrived earlier than *M. domisticus*. Its arrival time was 13 minutes after death. This fly was present in large number in fresh stage reduced in bloated stage, while few in number in active decay and absent in later stages of decomposition (Fig. 2c). House fly, *Musca domestica* (Linnaeus, 1758). It was the 3rd fly that arrived in 15 minutes after the dead body. This is also less described forensically important species. The adult fly occupied abdominal region of carcass in fresh stage and reached highest in numbers within 2 hours. This was present less than *C. rufifacies* but second in number after *C. megacephala*. *Musca Domestica* existed in all stages except decay stage and also their numbers were reduced from fresh to advanced decay stage (Figure, 2e). Blow fly, *Chrysomya megacephala* (Fabricius, 1794) This fly is cosmopolitan in forensic

entomology. As compared to other regions it had less number than *C. rufifacies* This fly arrived after 17 minutes of death of *C. domesticus* and was also present in abdomen region of the carcass and at the evening time both *M. domesticus* and *P. ruficornis* were not found except *C. rufifacies* (Fig. 2b). Chese skipper, *Piophila casei* (Linnaeus, 1758). This fly was observed in later morning of first day bloat stage till the end of active decay stages only and were less in number than the other flies due to their habit of feeding on liquefied food. *Piophila casei* was present in in only bloated and active decay stages (Figure, 2d; Table, 1 and 2).

**Discussion**

In the present study of Forensically Important Diptera Species Associated with Dog carcass (Canis domesticus (L.)) for a case study in District Mardan, Pakistan. *Canis domesticus* carcass was divided into fresh, bloat, active decay, advanced decay and dry stages of decomposition. In present study C. rufifacies arrived first to carcass and dominated while Shi *et al.* (2009) observed in China *C. megacephala* was the dominant specie and arrived to carrion first. Ahmad and Ahmad (2008) reported from Malaysia that *C. megacephala* and blow fly, *Chrysomya nigripes* (Aubertin) (Calliphoridae, Diptera) were first arrived of the carcass and were dominant. Souza *et al.* (2008) reported from Brazil that species Lucilia eximia (Wiedemann, 1819) and Chrysomya albiceps (Wiedemann, 1819) (Calliphoridae, Diptera) were better indicators of post-mortem interval (PMI) and first arrived arrived to carcass and were dominant. This study shows that these species of Diptera have different arrivals in different regions of the world and should be noted before apply for a case study of Post mortem intervals. Present study shows five species of Diptera *C. rufifacies, C. megacephala, P. ruficornis, M. domestica* and *P. a casei* were observed due to fast rate of decomposition While Szymon *et al.* (2008) in Poland reported *Lucilia Caesar* (Linnaeus) (Calliohoridae, Diptera), *P. regina*, *Calliphora vomitoria* (Linnaeus) (Calliohoridae, Diptera), dump fly, *Hydrotaea* sp. (Fibricius) (Mucidae, Diptera), true fly, *Parapiophila vulgaris* (Fallén) (Piophilidae, Diptera), *Stearibia nigriceps* (Meigen) (of family Piophilidae), *Necrodes littoralis* (Linnaeus) (Piophilidae, Diptera). Vitta *et al.* (2008) Repoted from Thailand These species of *Diptera C.rufifacies, C. megacephala, M. domestica, Fannia canicularis, P. ruficornis and Piophila casei*

**Fig. 1.** Decomposition of dog, *Canis domesticus* (L.) body during May 2011 in Takht Bhai, Mardan has been divided into 5 stages. a: fresh 1-12 hrs.; b: bloat 13-36 hrs.; c: active decay 37-84 hrs.; d: advanced decay 85-166 hrs.; e: decay stage 167-286 hrs.

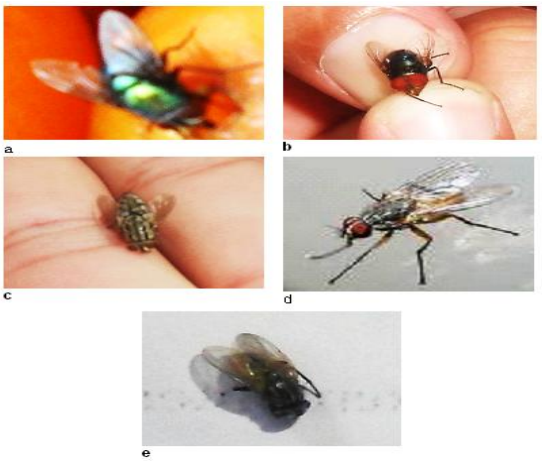

**Fig. 2.** Following insect species associated with dog, *Canis domisticus* (L.) carcass during this study in may 2011 in Takht Bhai, Mardan has been given below: a: Blow fly, *Chrysomya rufifacies* (Macquart); b: Blow fly, *Chrysomya megacephala* (Fabricus); c: Flesh fly, *Parasarcophga ruficornis* (Meigen); d: Chese skipper fly, *Piophila casei* (Linnaeus); e: House fly, *Musca domisticus* (Linnaeus).

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