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Entomofauna of forensic importance on *Canis domesticus* carcasses at Dir Lower, Pakistan

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Abstract

The present study was conducted for the evaluation of forensic insect fauna on *Canis domesticus* carcasses at Dir Lower Pakistan. Two carcasses of *C. domesticus* were evaluated for this purpose in two study periods (28 May to 09 June 2017) and (31 July to 14 August 2017). In first half of the study, 7 species belonging to 5 families and 3 orders were identified including Blow flies (*Chrysomya rufifacies* and *C. megacephala)*, Flesh fly (*Parasarcophaga ruficornis),* House fly (*Musca domestica),* Dune robber fly (*Philonicus albiceps),* Jumper ant (*Myrmecia pilosula)* and *Trox* species. In the second half of the study, 8 species belonging to 7 families and 3 orders were identified. New species in the 2nd study period that were not found in the first half of the study included Dog fly (*Hippobosca longipennis),* Cheese skipper (*Piophila casei)* and Bumble bee (*Bombus lapidarius)*. Decomposition time of the carcasses was the two study periods 13 and 15 days respectively. A taxonomic key was devised for the identified species. Duration of the individual decomposition stages of the two carcasses was correlated with the ambient environmental conditions. This was a preliminary study on forensic insect fauna in the study area and will provide a baseline for future studies in the field of forensic entomology.

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Introduction

Insects colonize carcasses after death and can be used for the calculation of Post Mortem Interval (PMI) and for solving medico-legal issues. Insects are attracted to carcasses just after death in a predictable manner (Payne 1965). Insects play a vital role in the decomposition of carcasses (Prado e Castro *et al.,* 2009) and are thereby classified according to the role played in carcass decomposition. Flies from the family Calliphoridae, order Diptera are the first insects to arrive on carcasses and complete their development (Watson and Carlton 2003). Insects play a key role in determination of postmortem interval through their developmental rates and are used for solving medico-legal issues in criminal investigations (Greenberg 1991; Gruner *et al.,* 2007; Smith 1986; Abajue *et al.,* 2014; Setyaningrum and Dhafer 2014). Insects can be used for terrestrial as well as aquatic crime scene investigations (Vanin 2018).

Entomologists use type of insect species and developmental stages following a crime and different abiotic environmental factors like type of soil, temperature, humidity and plant cover to make decisions about the time since death (Cruise 2018).

The break downing of dead bodies in the exposed environments is often ignored while it can give some important evidences about crime scenes (Moretti *et al.,* 2008). The importance of forensic studies in resolving crimes have been very prominent. The use of forensic evidence does make fighting crimes easier and faster. Mistakes in gaining forensic evidences might be painful to unlock prisoners or to capture the blameless individuals (Fradella *et al.,* 2007).

Arthropods are cold blooded and therefore the growth is greatly dependent on temperature which is a critical factor that greatly influence insect dynamics. Biological activity and metabolic rates in arthropods are limited by temperature. Low temperature decreases metabolic and developmental rate of arthropods and vice versa (Haskell *et al.,* 2008). Forensic entomology is an arena of forensic science through which biological and ecological important forensic aspects of arthropods are discussed (Wells and LaMotte 2001).

Experts of forensic entomology are often asked to check arthropod evidences taken from human carcasses to draw conclusions about how long arthropods exist. The elapsed period is said to be the postmortem interval (Catts and Goff 1992).

Approximately four hundred insect species are discovered at different break-down stages on dead body of pigs (Melted 2016). Biological needs of forensic insects depend on different stages of decomposition.

The present study was aimed to evaluate the diversity of forensically important insect fauna in two study periods with different climatic conditions and to devise a taxonomic key for the identified species found on *Canis domestics* carcasses in District Dir lower, Pakistan.

Materials and methods

Study Area

The present study was conducted at Union council Rabat, District Dir Lower, Khyber Pakhtunkhwa, Pakistan. The study area is located between 34° 51' 58°N 71° 57' 47°E. Elevation of the study area ranges from 1200 m to 2800 m from sea level (Khan *et al.,* 2011).

Materials

Personal protective equipment like overall, gloves, mask and shoe covers were used during the study. To collect evidences from the scene, materials used were: Tool case, protocol sheets (white paper for writing daily information), HB dark pencil, labels, clip board, insect jar, entomological pins, fine and medium forceps, a spoon for collecting larvae, a small brush for collecting eggs, glass vials for collection of sawdust for handling eggs, thermometer, sticking tapes, waxes and insect nets. Chloroform 100 % was used to kill the adult stages of the forensic insects. Ethanol (70–95%) and formalin was used for preservation of the dead specimens. Mobile Camera (5 mega pixels) was used for photography of different stages of the carcasses and insects.

Study Subjects

Two free ranging dogs 28kg and 25kg each were killed from 10m and 8m distances by a veterinary expert through a shot gun in the two study periods respectively.

Carcasses of the dogs were immediately placed under metallic cages (90cm x 90cm x 100cm) which allowed the access of arthropods to the corpses and prohibited the access of other carnivores. The adult insects were captured with aerial net three times daily and killed with 100% Chloroform and then pinned in insect box. The larvae were collected with the help of a spoon and preserved in 70% ethanol. The first collection was made on 28th May to 6th June 2017 and the second collection was made on 31st July to 9th of August 2017.

Identification of insects

The collected insects were identified with the help of available taxonomic keys (Carvalho and Mello-Patiu 2008; Dodge 1953), internet surfing and consulting with expert entomologists.

Results

In the present study, 550 adult specimens, 175 larvae and 85 pupae were collected from various parts of the dog carcasses and from the near vicinity of the carcasses. The time of decomposition of the dead bodies varied according to weather conditions. Weather data was taken three times daily (Morning, Noon and Evening) in the two study periods. In the first study period, mean daily temperature was 30.13±7.33°C, mean wind speed was 8.47±2.46Km/h while mean humidity was recorded as 26.09±5.85% (Fig. 1).. On the other hand, mean temperature of the second study period was found to be 28.56±5.33°C, mean wind speed was 5.04±2.38 while mean relative humidity was found as 66.12±9.75% (Fig. 1).

In the first study period, a total of 194 adult specimens were collected till the decomposition. Blow fly (*C. rufifacies)* was the most abundant species with 125 specimens followed by House fly *(M. domestica)* with 41 specimens, Flesh fly *(P. ruficornis)* 7 specimens, Skin beetle and Jumper ant *(M. pilosula)* 6 specimens each, Blow fly *(C. megacephala)* 5 specimens and Dune robberfly (*P. albiceps)* with 4 specimens (Fig. 2).

In the second study period, a total of 356 specimens were collected till the decomposition of the carcass. Blow fly (*C. rufifacies)* was the most abundant with a total of 293 specimens followed by House fly *(M. domestica)* with 24 specimens, Blow fly *(C. megacephala)* 15 specimens, Cheese skipper *(P. casei)* 10 specimens, *Trox* species 6 specimens, Flesh fly *(P. ruficornis)* 5 specimens, Dog fly (*H. longipennis)* 2 specimens and Bumble bee (*B. lapidarius)* with only 1 specimen (Fig. 2).

The total time (in days) taken by the two carcasses to reach the dry stage of decomposition was 13 days for the first carcass during May and June 2017 at 30.13°C mean temperature and 26.09% mean humidity. The 2nd carcass in August 2017 took 15 days at 28.56°C mean temperature and 66.12% mean humidity (Table. 4, Fig. 1). The difference in the time interval of the active decay stage and dry decay stage in the two study periods was because of the difference in the climatic conditions in the two study periods.

Table 1. Insect fauna on *Canis domesticus* carcasses collected during the two study periods.

Order	Family	Common Name	Scientific name	Authority: Year
Diptera	Calliphoridae	Blow fly	*Chrysomya rufifacies*	(Meigen 1826)
Diptera	Calliphoridae	Blow fly	*C. megacephala*	(Fabricius 1794)
Diptera	Muscidae	House fly	*Musca domestica*	(Linnaeus 1758)
Diptera	Sarcophagidae	Flesh fly	*Parasarcophaga ruficornis*	(Meigen 1826)
Diptera	Asilidae	Dune robber fly	*Philonicus albiceps*	.....
Diptera	Hippoboscidae	Dog fly	*Hippobosca longipennis*	(Fabricius 1805)
Diptera	Piophiladae	Cheese skipper	*Piophila casei*	(Linnaeus 1758)
Hymenoptera	Formicidae	Jumper ant	*Myrmecia pilosula*	(Smith 1858)
Coleoptera	Trogidae	Skin beetle	*Trox* sp.	(Harold 1872)
Hymenoptera	Apidae	Bumble bee	*Bombus lapidarius*	(Linnaeus 1758)

Table 2. Insects occurrence matrix for the first dog carcass (May/June, 2017).

First Dog Fauna			Day wise occurrence of insect fauna						
Orders	Families	Species	1	2	3	4	5	6	7
Diptera	Calliphoridae	*C. rufifacies*	55	67	5	21	30	34	21
			*A	A	A	*L	L	L	L
Diptera	Calliphoridae	*C. megacephala*	4	1	-	-	-	-	-
			A	A	-	-	-	-	-
Diptera	Muscidae	*M. domestica*	17	22	1	-	-	-	-
			A	A	A	-	-	-	-
Diptera	Sarcophagidae	*P. ruficornis*	3	-	2	-	-	-	-
			A	-	A	-	-	-	-
Hymenoptera	Formicidae	*M. pilosula*	6	-	-	-	-	-	-
			A						
Coleoptera	Trogidae	*Trox species*	-	4	2	-	-	-	-
			-	A	A	-	-	-	-
Diptera	Asilidae	*P. albiceps*	-	1	3	-	-	-	-
			-	A	A	-	-	-	-

*A = adults, *L = larvae.

Table 3. Insects occurrence matrix for the second dog carcass (August, 2017).

Second Dog Fauna			Day wise occurrence of insect fauna							
Order	Family	Species	1	2	3	4	5	6	7	8
Diptera	Calliphoridae	*C. rufifacies*	108	93	82	10	50	34	31	36
			*A	A/L	A/L	A/L	*L	L	L	L
Diptera	Calliphoridae	*C. megacephala*	4	9	2	-	-	-	-	
			A	A	-	-	-	-	-	
Diptera	Muscidae	*M. domestica*	13	4	5	3	-	-	-	
			A	A	A	-	-	-	-	
Diptera	Sarcophagidae	*P. ruficornis*	3	1	1	-	-	-	-	
			A	-	A	-	-	-	-	
Diptera	Piophiladae	*P. casei*	-	-	10	-	-	-	-	
			-	-	-	-	-	-	-	
Coleoptera	Trogidae	*Trox species*	-	-	2	4	-	-	-	
			-	A	A	-	-	-	-	
Hymenoptera	Apidae	*B. lapidaries*	-	-	1	-	-	-	-	
			-	-	A	-	-	-	-	
Diptera	Hippoboscidae	*H. longipennis*	2	-	-	-	-	-	-	
			A	-	-	-	-	-	-	

*A = adults, *L = larvae.

Table 4. Decomposition stages and decomposition time of the two dog carcasses.

Months of 2017	Mean Temp. (°C)	Mean Humidity (%)	Stages of Decomposition (Days)					
			Fresh	Bloated	Active Decay	Advanced Decay	Dry	Total
May/June	30.13	26.09	1	2	3	2	5	13
August	28.56	66.12	1	3	5	3	3	15

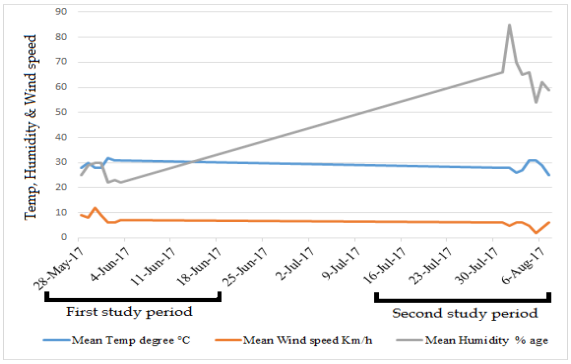

Fig. 1. Daily mean weather data for the two study periods.

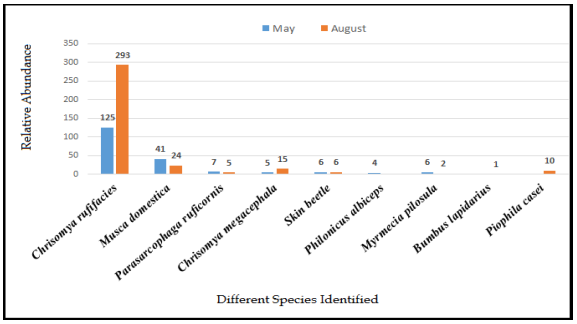

Fig. 2. Relative abundance of aerial collection of each species in May and August.

Identification Key

Identification key for the entomofauna of forensic importance on *C. domesticus* carcasses:

1a Chitinous exoskeleton, jointed legs, wings present or absent-------Phylum Arthropoda

1a (i) Wings transparent or hard with one or two pairs, antennae may be hidden or 1 or 2 pairs segmented with several forms, three pairs of jointed legs----------Class Insecta

1a(ii) Body flattened, one pair of transparent wings, posterior pair of wings modified into haltares, legs modified for jumping, piercing and sucking type of mouth parts, tarsi 4-5 segmented, antennae hidden ---------- Order Diptera

1b One pair of wings, greyish or yellowish abdomen, body length variable----------2

2a(1b) (i) Greyish lines running down the length of the thorax, no hypopleural bristles, wing veins 6 and 7 short and do not move towards each other---------- Family Muscidae

2a(1b)(ii) A greyish fly, about 6-7 mm in length, 4 narrow black stripes along its thorax and a greyish or yellowish stripe along its abdomen ------- *M. domestica*

2b Body with shining metallic coloration, one pair of wings, one pair of haltares -------3

3a(2b) Abdomen and thorax blue, green or bronze----------Family Calliphoridae

3a (2b)(i) Anterior spiracle being white to pale yellow----------*Chrysomya rufifacies*

3a(2b)(ii) Anterior spiracle orange to black-brown----------*C. megacephala*

3b Abdomen and thorax with different coloration, conspicuous dark vittae on gray background----------4

4a(3b)(i) Abdomen and thorax dull gray or brown and conspicuous dark, mesonotum with conspicuous dark vittae on gray background----------Family Sarcophagidae

4a(3b)(ii) Abdomen and thorax dull gray or brown, thorax with 3 stripes ----------*Parasarcophaga ruficornis*

4b Small, black flies with broken wings----------5

5a(4b)(i) Small black flies, costal vein of the wing appears broken at one point-------Family Piophilidae

5a (4b)(ii) Shiny, 2.5-4.5 mm in length, black in color----------*Piophila casei*

5b Wings thickened, cross veins absent and elytra present---------- 6

6a(5b) Front pair of wings thickened hard, elytra shortened, one or more abdominal segments from above (beetles), hind legs not modified for jumping----------Coleoptera

6b Antennae segmented, simple eyes are absent, digging legs---------- 7

7a(6b)(i) Medium size, dull brownish or muddy, rough and hairy elytra---------Family Trogidae

7a(6b)(ii) Dorsal surface of the body rough and brown, elytra hairy, tip of antennae flat, adult legs not broad and modified for digging----------*Trox species*

Discussion

The present study was carried out to evaluate the diversity of forensically important insects on free ranging urban dog *C. domesticus* at District Dir Lower, Pakistan. All the decomposition stages were studied in both of the dog carcasses. Szymon *et al.,* (2009) reported insect fauna in *Sus domesticus* in Poland which conforms in respect of decomposition stages to the present study. They reported 12 species belonging to 7 families and 2 orders. Wangko *et al.,* (2015) also reported five decomposition stages. They reported 11 species belonging to order Diptera and 8 species belonging to order Coleoptera during a period of two weeks. *Chrysomya megacephala, C. rufifacies* and *Hermetia illucens* colonized all corpses. In the present study, a total of 10 species were identified belonging to 9 families and 3 orders. Due to the fast depletion of soft tissues, a more diverse fauna was not seen as compared to other studies worldwide.

Hippobosca longipennis, *B. lapidarius* and *Piophila casei* were only collected during the second study period but were not observed in the first study period. *C. rufifacies and C. megacephala* were found in both of the study periods and are important forensic indicators.

Decomposition of the first dog took 13 days while the second dog decomposed in 15 days. Ahmed and Ahmed (2009) reported that decomposition rate is

affected by the difference in the climatic conditions which disturb fly's activities of food searching, mating and egg laying processes. This ultimately leads to the differences in decomposition rates. The two studies conform in respect of weather conditions in association with decomposition rates.

In the present study, order Diptera was found the leading group for decomposition and has immense importance in death investigations. Coleoptera was found the least observed on the dog carcasses. Succession rate of Hymenoptera in the present study was negligible which shows that Hymenoptera has little or no role in the decomposition of dog carcasses. Hymenoptera therefore may be regarded as accidental visitors. At initial stages of decomposition, different species of flies arrived on the carcasses. The first arrived insect was *C. rufifacies,* reached in 14 minutes after death and was found the most abundant one. The skin beetles were reached the corpses at later stages of decomposition and they were the last contributors in the decomposition process. In the second study period, the dog fly (*H. longipennis)* was found to be present already on the body just after death. *C. rufifacies* was the second fly that reached in 15 minutes after death and was the most abundant one.

Forensic insects on dog carcasses need to be studied thoroughly in all seasons of the year. Detailed taxonomic study for the indoor, outdoor, endemic and exotic insect species of forensic importance needs to be carried out. All geographic areas of Pakistan must be evaluated with reference to forensic studies for future applications in medicolegal issues and crime scene investigations.

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