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Frequency of forensic insects on dog and rabbit carcasses in different habitats: use of developmental data of *Chrysomya albiceps* in determining the postmortem interval

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Abstract
The current study was carried out at the Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Nasr City, Cairo, Egypt by using the dog, *Canis lupus familiaris* and the rabbit, *Lepus cuniculus* carcasses as human models. Eight dog carcasses and eight rabbit carcasses were employed in four seasons. The relative abundance of insect species that collected in dog carcasses placed outdoor during the study period was arranged as follows: *Chrysomya albiceps*, followed by *Musca domestica*, *Dermestes maculatus*, *Piophila casei*, *Hister* sp., *Monomorium pharoensis*, *Musca sorbens*, *Necrobia rufipes*, *Sarcophaga carnaria*, *Nasonia vetripennis*, *Calliphora* sp., *Wohlfartia magnifica*, *Megaselia scalaris*, *Chrysomya megacephala*, *Lucilia* sp., *Vespa orientalis*, *Creophilous maxillosous*, *Stomoxys calcitrans*, *Dolichovespula* sp., *Cataglyphis bicolor*, and *Phormia regina*, while the number of occurrence of insect species collected in dog carcasses placed indoor, were arranged as follows: *Nasonia vetripennis*, followed by *Chrysomya albiceps*, *Dermestes maculatus*, *Musca sorbens*, *Megaselia scalaris*, *Necrobia rufipes*, *Monomorium pharoensis*, *Hister* sp., *Musca domestica*, *Sarcophaga carnaria*, *Calliphora* sp., *Creophilous maxillosous*, and *Wohlfartia magnifica*. On the other hand, the abundance of insect species collected in rabbit carcasses placed outdoor during the study period was arranged as follows: *Chrysomya albiceps*, followed by *Musca domestica*, *Piophila casei*, *Hister* sp., *Dermestes maculatus*, *Monomorium pharoensis*, *Nasonia vetripennis*, *Musca sorbens*, *Sarcophaga carinaria*, *Megaselia scalaris*, *Wohlfartia magnifica*, *Lucilia sericata*, *Calliphora* sp., *Vespa orientalis*, *Creophilous maxillosous*, *Cataglyphis* bicolor, *Chrysomya megacephala*, *Necrobia rufipes*, and *Dolichovespula* sp.**,** while the frequency of insect species collected from rabbit carcasses placed indoor, were arranged as follows: *Nasonia vetripennis*, followed by *Chrysomya albiceps*, *Dermestes maculatus*, *Megaselia scalaris*, *Musca sorbens*, *Musca domestica*, *Monomorium pharoensis*, *Hister* sp., *Sarcophaga carnaria*, *Calliphora* sp., and *Cataglyphis bicolor*. In order to estimate the postmortem interval (PMI), the life table of *Chrysomya albiceps* as the first and predominant fly arrive the carcass was studied on dog and rabbit carcasses placed outdoors and indoors.

Keywords: Frequency, forensic insects, *Chrysomya albiceps*, *Canis lupus familiaris*, *Lepus cuniculus*, carcass, postmortem interval (PMI)

1. Introduction
Forensic entomology deals primarily with insects and other arthropods which infest human remains. Insects lay eggs on or in human remains, as well as utilize the corpse for food or habitat. Insect development and successional patterns can be an indication of the postmortem interval (PMI) when the time of death is unknown.

Pathologists can estimate the time of death based on several biological parameters: lividity, rigor mortis, postmortem cooling, changes in the chemical constituents of the body, autolysis of tissue, and decomposition due to bacterial activity in the body. However, these parameters are not reliable beyond about 72 hours after death [1]. The entomological method of determining PMI was found to be statistically more reliable and superior when compared to other pathological methods, particularly during later stages of decay [2].

There are two methods to estimate the PMI; first using the developmental stages of flies found on the corpse as they first lay eggs on the body [3]. A second method uses the succession patterns of carrion-arthropods, the type, and composition of fauna change in predictable pattern as decomposition progresses through different stages [4].

The present study aimed to estimate the postmortem interval (PMI) by studying the developmental stages of the first flies arrive to the carrion and to provide entomological data that can be employed in forensic cases in Egypt.

2. Materials and Methods
2.1 Study site
The study site was located at the Department of Zoology and Entomology, Faculty of Science, Al-Azhar University, Nasr city, Cairo, Egypt. Nasr city is considered semi-arid urban region. It has four distinct seasons; winter, spring, summer, and autumn. According to the meteorological station, summer is hot and dry, winter is cool and rainy, spring and autumn are mild in temperatures and rainfall, the experiments were carried out in four different seasons between December 2013 and December 2014. Each experiment was continued until the entire carcass was consumed. Sites for carcass placement were chosen in a botanical garden (outdoor habitat) of the animal house and in the laboratory (indoor habitat).

2.2 Experimental design
Two dogs (*Canis lupus familiaris*), weighing approximately 3 kg each, and two rabbits (*Lepus cuniculus*), weighing approximately 1.300 kg each were used. One dog and one rabbit carcasses were placed in the laboratory (indoor) and

other two carcasses were placed in a botanical garden (outdoor) of the animal house.

The dogs and rabbits were taken alive to the study site and killed with a blow on the head. Care was taken to prevent external bleeding that might alter the attractiveness of the carcasses to flies or provide alternate sites for oviposition or larviposition. After death, animals of outdoor experiments were immediately placed into mesh cages to prevent scavenging by large vertebrates and left exposed to natural conditions. The animal carcasses were separated by approximately 4 meters indoor and 10 meters outdoor. Sand was placed under each cage to facilitate the collection of larvae, leaving carcasses to pupate.

2.3 Collection, sampling and identification

Adult insects were collected on a daily basis until apparent insect activity had ceased. Insect collection was carried out twice daily, one in the morning from 8 to 9 am and the other collection was in the afternoon before sunset, from 4 to 5 pm. The numbers of adult insect collected were counted and representative samples were preserved in 70% ethanol and taken to the laboratory for identification. Adult Diptera and Hymenoptera were collected using a hand net, while adult Coleoptera was collected using hand picking forceps and vial glasses.

Identification and taxonomic determinations were made using current keys [5-9], and by taxonomists in Cairo University and Ministry of Agriculture, Dokki, Giza, Egypt. All insects were identified at least to the family level. All efforts were made to identify Diptera and Coleoptera to the species level as they were considered of forensic importance.

2.4 Carcass decomposition

Carcasses were examined twice daily; in the morning and afternoon in order to determine the duration of each decomposition stage.

2.5 Climatic conditions

The ambient conditions of temperature and relative humidity in outdoor habitat were obtained monthly from the meteorological station of Kobri El-Kobba in Cairo, Egypt. Temperatures and relative humidity indoor were daily measured using max./min. thermometer and hygrometer.

2.6 Insect succession tables

Insect succession tables were developed by combining data from sweeping nets and hand collections. The different insect species that collected from each carcass were distributed according to the decomposition stages of carcasses i.e. according to postmortem interval (PMI) giving their numbers.

3. Results

3.1 Frequency of insect species collected in carcasses

a) Dog carcass

Data given in table (1) indicate the number of occurrence (frequency) or abundance of different insect species (Adults) that collected in dog carcasses placed indoor and outdoor during the four seasons from December 2013 to December 2014.

According to the number of occurrence (Frequency) the different adult species collected in dog carcasses placed outdoor were arranged as follows: *Chrysomya albiceps* (1679), followed by *Musca domestica* (1151), *Dermestes maculatus* (417), *Piophila casei* (224), *Hister* sp. (117), *Monomorium pharoensis* (87), *Musca sorbens* (81), *Necrobia rufipes* (69), *Sarcophaga carnaria* (60), *Nasonia vetripennis* (59), *Calliphora* sp. (44), *Wohlfartia magnifica* (42), *Megaselia scalaris* (34), *Chrysomya megacephala* (31), *Lucilia sericata* (29), *Vespa orientalis* (15), *Creophilous maxillosous* (14), *Stomoxys calcitranas* (9), *Dolichovespula* sp. (9), *Cataglyphis bicolor* (6), and *Phormia regina* (3).

On the other hand, the number of occurrence of insect species collected in dog carcasses placed indoor were arranged as follows: *Nasonia vetripennis* (571), followed by *Chrysomya albiceps* (459), *Dermestes maculatus* (304), *Musca sorbens* (199), *Megaselia scalaris* (57), *Necrobia rufipes* (50), *Monomorium pharoensis* (40), *Hister* sp. (32), *Musca domestica (*31), *Sarcophaga carnaria* (21), *Calliphora* sp. (5), *Creophilous maxillosous* (3), and *Wohlfartia magnifica* (2).

Table 1: Entomofauna associated with dog carcass placed outdoor and indoor during four seasons, 2014.

Order	Family	Species	Dog	
			Outdoor	Indoor
Diptera	Calliphoridae	*Chrysomya albiceps*	1679	459
		Chrysom megacephala	31	0
		Lucilia sericata	29	0
		Calliphora sp.	44	5
		Phormia regina	3	0
	Muscidae	*Musca domestica*	1151	31
		Musca sorbens	81	199
		Stomoxys calcitranas	9	0
	Sarcophagidae	*Sarcophaga carnaria*	60	21
		Wohlfartia magnifica	42	2
	Piophilidae	*Piophila casei*	224	0
	Phoridae	*Megaselia scalaris*	34	57
Coleoptera	Dermestidae	*Dermestes maculatus*	417	304
	Histeridae	*Hister* sp.	117	32
	Celeridae	*Necrobia rufipes*	69	50
	Staphylinidae	*Creophilous maxillosous*	14	3
Hymenoptera	Pteromalidae	*Nasonia vetripennis*	59	571
	Vespidae	*Dolichovespula* sp.	9	0
		Vespa orientalis	15	0
	Formicidae	*Cataglyphis bicolor*	6	0
		Monomorium pharoensis	87	40

b) Rabbit carcass

As shown from table (2) the number of occurrence of different insect species (adults) that collected in rabbit carcasses placed outdoor were arranged as follows: *Chrysomya albiceps* (645), followed by *Musca domestica* (511), *Piophila casei* (238), *Hister* sp. (149), *Dermestes maculatus* (123), *Monomorium pharoensis* (123), *Nasonia vetripennis* (107), *Musca sorbens* (45), *Sarcophaga carinaria* (31), *Megaselia scalaris* (27), *Wohlfartia magnifica* (26), *Lucilia sericata* (9), *Calliphora* sp. (9), *Vespa orientalis* (6), *Creophilous maxillosous* (5), *Cataglyphus bicolor* (5), *Chrysomya megacephala* (4), *Necrobia rufipes* (4), and *Dolichovespula* sp. (2).

On the other hand, the frequency of insect species collected from rabbit carcasses placed indoor throughout the four seasons were arranged as follows: *Nasonia vetripennis* (360), followed by *Chrysomya albiceps* (109), *Dermestes maculatus* (78), *Megaselia scalaris* (47), *Musca sorbens* (29), *Musca domestica* (21), *Monomorium pharoensis* (16), *Hister* sp. (8), *Sarcophaga carnaria* (6), *Calliphora* sp. (5), and *Cataglyphis bicolor* (3).

From the aforementioned results, it is appeared that *Chrysomya albiceps* was predominated throughout the four seasons on dogs and/or rabbits during the study period.

Table 2: Entomofauna associated with rabbit carcass placed outdoor and indoor during four seasons, 2014.

Order	Family	Species	Rabbit	
			Outdoor	Indoor
Diptera	Calliphoridae	*Chrysomya albiceps*	645	109
		Chrysomya megacephala	4	0
		Lucilia sp.	9	0
		Calliphora sp.	9	5
	Muscidae	*Musca domestica*	511	21
		Musca sorbens	45	29
	Sarcophagidae	*Sarcophaga carnaria*	31	6
		Wohlfartia magnifica	26	0
	Piophilidae	*Piophila casei*	238	0
	Phoridae	*Megaselia scalaris*	27	47
Coleoptera	Dermestidae	*Dermestes maculatus*	123	78
	Histeridae	*Hister* sp.	149	8
	Celeridae	*Necrobia rufipes*	4	0
	Staphylinidae	*Creophilous maxillosous*	5	0
Hymenoptera	Pteromalidae	*Nasonia vetripennisis*	107	360
	Vespidae	*Dolichovespula* sp.	2	0
		Vespa orientalis	6	0
	Formicidae	*Cataglyphis bicolor*	5	3
		Monomorium pharoensis	123	16

3.2 Effect of seasons on the development of *Chrysomya albiceps* on dog and rabbit carcasses (Estimation of PMI)

Developmental data for primary blow flies provide the most accurate means of estimating the PMI using arthropod information. So it was necessary to estimate the developmental stages of the first insect species that arrive to the carcass and lays eggs within hours after death. Hence, the life table of *Chrysomya albiceps* as the first blow fly arrive to the carcass was studied.

Data given in tables (3 & 4) represent the effects of different seasons on the development of *Chrysomya albiceps* on dog and rabbit carcasses placed indoor or outdoor.

The longest egg incubation period was (10 ± 1 and 8 ± 1 day) on dog and rabbit carcasses placed indoor or outdoor, respectively was recorded in winter. While, the shortest one (1 ± 0.5 and 1± 0.5 day) was recorded in summer. The longest larval duration (7 ± 1 and 4 ± 0.5 day) on dog and rabbit carcasses was recorded in autumn and spring, respectively. While, the shortest one (2 ± 0.5 and 2 ± 0.5 day) was recorded in autumn and summer, respectively. The longest pupal duration (7 ± 1 and 6 ± 0.5 day) on dog and rabbit carcasses, respectively was recorded in autumn. While, the shortest one (3 ± 0.5 and 2 ± 0.5 day) was recorded in summer.

Table 3: Incubation period and larval and pupal duration of *Chrysomya albiceps* reared on dog /rabbit placed indoor.

Season / Duration	Mean							
	Incubation Period ± S.D		Larval Duration ± S. D		Pupal Duration ± S. D		Temp. (°C)	R. H %
	Dog	Rabbit	Dog	Rabbit	Dog	Rabbit		
Winter	7 ± 1	8 ± 1	-	-	-	-	22	60
Spring	4 ± 0.5	2 ± 0.5	3 ± 0.5	4 ± 0.5	5 ± 0.5	6 ± 0.5	26	54
Summer	1 ± 0.5	1 ± 0.5	3 ± 0.5	2 ± 0.5	3 ± 0.5	4 ± 0.5	29	62
Autumn	2 ± 0.5	2 ± 0.5	2 ± 0.5	3 ± 0.5	5 ± 0.5	6 ± 0.5	24	65

No. of egg tested 50
No. of larvae tested 50
No. of pupae tested 5

Table 4: Incubation period and larval and pupal duration of *Chrysomya albiceps* reared on dog /rabbit placed outdoor.

Season / Duration	Mean							
	Incubation Period ± S.D		Larval Duration ± S. D		Pupal Duration ± S. D		Temp. (°C)	R. H %
	Dog	Rabbit	Dog	Rabbit	Dog	Rabbit		
Winter	10 ± 1	8 ± 1	-	-	-	-	15	57
Spring	3 ± 0.5	3 ± 0.5	5 ± 0.5	2 ± 0.5	4 ± 0.5	3 ± 0.5	23	45
Summer	2.5 ± 0.5	1.5 ± 0.5	4 ± 0.5	2 ± 0.5	3 ± 0.5	2 ± 0.5	29	54
Autumn	3 ± 0.5	2 ± 0.5	7 ± 1	3 ± 0.5	7 ± 1	4 ± 0.5	20	56

No. of egg tested 50
No. of larvae tested 50
No. of pupae tested 50

4. Discussion

4.1 Frequency (abundance) of insect species on dog and rabbit carcasses

Adults of the blow fly *Chrysomya albiceps* were collected in greater number from dog or rabbit carcasses placed outdoor (exposed to direct sunlight). These results are similar to those reported by others [10], working on human cadavers in Tennessee, and others working on pig carcass [11]. The abundance or high frequency of *Chrysomya albiceps* may reflect the high dispersal ability and arrival at carcasses shortly following death [11]. While, *Chrysomya albiceps* is a potential species for estimation of the postmortem interval (PMI) due to its wide distribution, it is of value as an indicator of a particular habitat type, since it does not appear to display habitat specificity [11]. This fact is supported by the present study where *Chrysomya albiceps* was the predominant species on carcasses in both habitats (outdoor and indoor).

It was interesting to note also in the present study that as adult Diptera decreased, the numbers of Coleoptera increased on the carcasses in both habitats.

4.2 Estimation of postmortem interval (PMI)

Developmental data for primary blow flies provide the most accurate means of estimating the PMI using arthropod information [12].

As they are the first colonizer that arrives and lay eggs in the carcass, therefore the time of death is assumed to close to the time of first eggs were laid. The blow fly used in this study to estimate the PMI was *Chrysomya albiceps* as it was a predominant and first arrival fly on the different carcasses used.

The results showed that the shortest larval and pupal durations were recorded at higher temperatures (29ºC), while the longest ones were found to be at lower temperatures. The egg incubation period was varied from 2.5±0.5 to 1.5± 0.5 with a mean of days at 29ºC, while varied from 10±1 to 8±1 with a mean of days at 15ºC for dog and rabbit carcasses placed outdoor, respectively. These results mean that time elapsed since death assumes to be equal to the incubation period and PMI could be estimated from the results of larval and pupal durations. These results agree with those obtained on *Lucilia sericata* and *Protophormia terraenove* [13, 14], where the minimal duration of development from oviposition to adult emergence was inversely related to temperature. Also, the present results are in consistence with those obtained for the carrion fly [15], for *Phormia regina* [16], for the fresh fly, *Sarcophaga tibialis* [17], for *Sarcophaga carnaria* and *Wohlfartia magnifica* [18]. However, some studies showed that faster development under fluctuating temperatures for *S. argyrostoma* and *Lucilia illustris*, but slower development for *Calliphora vicina* and *C. vomitoria* [19].

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