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The Abundance of Forensic Insects on Dog and Rabbit Carcasses in Different Habitats and Developmental Stages of *Chrysomya albiceps* as a Forensic Indicator

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ABSTRACT

Eight dog carcasses and eight rabbit carcasses were employed in four seasons. The abundance of insect species collected in dog carcasses placed outdoor during the study period was 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), *Wohlfahrtia 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), while the frequency of insect species collected from 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 *Wohlfahrtia magnifica* (2). 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* (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), *Wohlfahrtia 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), while the frequency of insect species collected from rabbit carcasses placed indoor, 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), and *Cataglyphis bicolor*. In order to estimate the postmortem interval (PMI), the life table of *Chrysomya albiceps* as the first and predominant fly reaches the carcass was studied on dog and rabbit carcasses placed outdoors and indoors. This study aimed to characterize the insect species present on cadavers and to estimate the postmortem interval (PMI) by studying the developmental stages of the first fly arrive at the carrion. These data should contribute to the creation of a database of forensic insects in Egypt.

INTRODUCTION

Entomological evidence on cadavers has been demonstrated to help in elucidating homicides, ranging from the determination of the post-mortem interval (PMI) to indications of the local of the crime (Catts and Goff, 1992). Information on abiotic factors combined with the time taken by the larva to reach a developmental stage may provide a reliable estimation of the time elapsed between insect colonization and the discovery of the corpse (Byrd and Castner, 2001).

The most valuable use of forensic insects associated with the corpse is the estimation of the postmortem interval or the time that elapsed since death (Hall, 2001). 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 (Kashyap and Pillai, 1989).

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 (Catts and Haskell, 1990). A second method uses the succession patterns of carrion-arthropods, the type and composition of fauna change in a predictable pattern as decomposition progress through different stages (Schoenly et al., 1995).

This study aimed to characterize the insect species present on cadavers and to estimate the postmortem interval (PMI) by studying the developmental stages of the first flies arrive at the carrion. These data should contribute to the creation of a database of forensic insects in Egypt.

MATERIALS AND METHODS

Study Site:

The study site was located in Al-Azhar University, Nasr city, Cairo, Egypt. Nasr city is considered a 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, and spring and autumn are mild in temperatures and rainfall. The experiments were carried out during the four seasons from December 2013 to December 2014. Each experiment was continued until the entire carcass was consumed. The sites for carcass placement were chosen in a botanical garden (outdoor) of the animal house and in the laboratory (indoor) at the Department of Zoology and Entomology, Faculty of Science, Al-Azhar University.

Experimental Design:

For each experiment 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 the 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. The sand was placed under each cage to facilitate the collection of larvae, leaving carcasses to pupate.

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 collected adult insect 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 (Greenberg, 1971; Mosallam, 1980; Shaumar et al., 1989; Whitworth, 2006; Carvalho and Mello-Patiu, 2008), and by taxonomists in Cairo University and Agriculture Research Center, 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.

Carcass Decomposition:

Carcasses were examined twice daily; in the morning and afternoon in order to determine the duration of each decomposition stage. Images of carcasses throughout decomposition stages were captured using a digital camera.

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.

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.

RESULTS

Frequency of Insect Species Collected in Carcasses:

A) Dog Carcass:

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

According to the number of occurrences (frequency) of the different adult insect 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), *Wohlfahrtia 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 occurrences 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 *Wohlfahrtia 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
		Chrysomya 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
		Wohlfahrtia 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:

Data are given in Table (2) indicate the abundance of adult insect species collected in rabbit carcasses placed indoor and outdoor during the four seasons from December 2013 to December 2014.

As shown from Table (2) the number of occurrences of different insect species (adults) 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), *Wohlfahrtia 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 has 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
		Wohlfahrtia 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

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 at the carcass and lays eggs within hours after death. Hence, the life table of *Chrysomya albiceps* as the first blow fly arrives at the carcass was studied.

Data are 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 period for the first larval appearance on dog and rabbit carcasses placed indoor or outdoor i.e. the egg incubation period was recorded in winter season. This incubation period was (10 ± 1 and 8 ± 1 day) on dog and rabbit carcasses, respectively was recorded in winter season. While, the shortest one (1 ± 0.5 and 1 ± 0.5 days) on dog and rabbit carcasses, respectively was recorded in summer season. The longest pupal duration (7 ± 1 and 6 ± 0.5 days) on dog and rabbit carcasses, respectively was recorded in autumn season. While, the shortest one (3 ± 0.5 and 2 ± 0.5 days) was recorded in summer season.

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

Season / Duration	dog / rabbit indoor							
	Mean		Mean		Mean		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 50

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

Season / Duration	dog / rabbit outdoor							
	Mean		Mean		Mean		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

DISCUSSION

Frequency of Insect Species Collected in Carcasses:

Adults of the blow fly *Chrysomya albiceps* were collected in greater numbers from dog or rabbit carcasses placed outdoor (exposed to direct sunlight). These results are similar to those reported by Rodriguez and Bass (1983), working on human cadavers in Tennessee, and Carvalho and Linhares (2001) working on pig carcass. The abundance or high frequency of *Chrysomya albiceps* may reflect the high dispersal ability and arrival at carcasses shortly following death (Carvalho and Linhares, 2001). 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 (Carvalho and Linhares, 2001). 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 Coleptera increased on the carcasses in both habitats.

Estimation of Postmortem Interval (PMI):

Developmental data for primary blow flies provide the most accurate means of estimating the PMI using arthropod information (Greenberg, 1991).

As they are the first colonizer that arrives and lay eggs in the carcass, therefore the time of death is assumed to close to 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 (29^{o}C), 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 29^{o}C, while varied from 10 ± 1 to 8 ± 1 with a mean of days at 15^{o}C 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 by Grassberger and Reiter, (2001 & 2002) on *Lucilia sericata* and *Protophormia terraenove*, 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 by Joy et al., (2006) for the carrion fly, Nabity et al., (2006) for *Phormia regina*, Villet et al., (2006) for the fresh fly, *Sarcophaga tibialis* and Younis (2013) for *Sarcophaga carnaria* and *Wohlfahrtia magnifica*. However, Niederegger et al., (2010) showed that faster development under fluctuating temperatures for *S. argyrostoma* and *Lucilia illustris*, but slower development for *Calliphora vicina* and *C. vomitoria*.

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ARABIC SUMMARY

وفرة الحشرات الجنائية على جثث الكلاب والأرانب في بيئات مختلفة ومراحل تطور *Chrysomya albiceps* كمؤشر جنائي

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تم استخدام ثمانية جثث كلاب وثمانية جثث أرانب في أربعة مواسم. تم ترتيب وفرة أنواع الحشرات التي تم جمعها من جثث الكلاب الموضوعة في الهواء الطلق خلال فترة الدراسة على النحو التالي: *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), *Wohlfahrtia 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).

بينما تم ترتيب تواتر أنواع الحشرات التي تم جمعها من جثث الكلاب الموضوعة في الأماكن المغلقة، على النحو التالي: *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 *Wohlfahrtia magnifica* (2).

من ناحية أخرى، تم ترتيب وفرة أنواع الحشرات التي تم جمعها من جثث الأرانب الموضوعة في الهواء الطلق خلال فترة الدراسة على النحو التالي: *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), *Wohlfahrtia 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).

في حين تم ترتيب تواتر أنواع الحشرات التي تم جمعها من جثث الأرانب الموضوعة داخل الأماكن المغلقة، على النحو التالي: *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).

تم دراسة دورة حياة *Chrysomya albiceps* من أجل تقدير فترة مابعد الوفاة (PMI)، كأول حشرة سائدة تصل إلى جثث الكلاب والأرانب الموضوعة في الهواء الطلق والمكان المغلق. تهدف هذه الدراسة إلى توصيف أنواع الحشرات الموجودة على الجثث وتقدير فترة مابعد الوفاة (PMI) من خلال دراسة مراحل التطور للذبابة التي وصلت أولاً إلى الجثة. يجب أن تسهم هذه البيانات في إنشاء قاعدة بيانات للحشرات الجنائية في مصر.