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Species richness of scavenger insects on different carcass types

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Abstract. The type and amount of resources available significantly influences the structure and dynamics of food webs. In this study, we analyzed differences in species richness of scavengers based on carcass type in Riyadh, Saudi Arabia. We collected insects from experimental carcasses of three different types, domestic dogs (Canidae, C*anis lupus familiaris*), Hijazi goats (Bovidae, *Capra aegagrus hircus*), and camels (Camelidae, *Camelus dromedarius*). Data collection was conducted during the decay stage in June, 2016. We used mitochondrial cytochrome c oxidase subunit I (mtCOI) barcodes as a marker for the molecular identification of the scavenger insects. The results showed that there were more insects on the camels and goats than the dogs. In total, seven species were found on all carrions. Six species were found on the camels and goats, but only five were found on the dog. *Musca domestica* was the most collected species of flies whereas, *Necrobia rufipes* was the most collected species of beetles. Overall, this study showed that carrion type had an effect on the type and number of insects attracted to the carrions. Thus, one of the significant factors that influence the associated scavenger assemblage is a carcass type.

INTRODUCTION

Three kinds of organisms prevail in any natural or semi-natural habitat i.e., producers, consumers and decomposers; However, the major event involved in proper functioning of ecosystem is the process of decomposition. Decomposers and scavengers play an essential role in environment by eradication of carrion or dead animal flesh. The most noticeable players involved in decomposition process are arthropods and more importantly, carrion-feeding insects (Hanski, 1987). During decomposition, dead bodies go through dramatic physical, biological and chemical changes. Each stage is attracted to a different group of arthropods, primarily insects. Some are attracted directly by the corpse, which is used as food or an oviposition medium, whereas other species are attracted by the large aggregation of other insects they use as food resource (Coe & Curran, 1980). Furthermore, Castner (2001) reported two major groups of insects including flies (Diptera) and beetles (Coleoptera) are predictably attracted to cadavers and provide the majority of information in forensic investigation.

In Saudi Arabia, Mashaly *et al.* (2017) studied the most abundant carrion flies using sheep carcasses in seven cities and recorded that, *Chrysomya albiceps* Wiedmann was the most abundant species, followed by *Musca domestica*. In another study on beetles, Mashaly *et al.* (2018) found that *Dermestes frischii* Kugelann (Coleoptera: Dermestidae) and *Necrobia rufipes* DeGeer (Coleoptera: Cleridae) were the most abundant species in three cities of Saudi Arabia. In Al-Ahsaa city, Shaalan *et al.* (2017) recorded that the maggots of the flies *Calliphora vicina* Robineau-Desvoidy and *Ch. albiceps*

colonized the rabbit carcasses; moreover four beetle species *Hymenorus* sp., *Saprinus chalcites* Illiger, *D. maculatus* De Geer and *Blaps* sp. were presented on the carcasses.

Typically, several studies dealing with patterns of arthropod succession on decomposing bodies have involved a wide variety of animal models from toads and lizards (Cornaby, 1974) to the elephants (Coe, 1978). Results of these studies have been used by forensic entomologists to provide estimations of postmortem intervals based on arthropod development rates, succession patterns, or a combination of both (Goff & Odom, 1987). Other studies have been performed on animal models of various sorts, including sheep (Fuller, 1934), dogs (Boulkenafet *et al.*, 2015), domestic cats (Early & Goff, 1986), fishes, pigeons (Omer, 2014) and most commonly, domestic pig (Tabor *et al.*, 2005).

Besides, a corpse, whether human or animal, is a large food resource for many creatures and supports a large, rapidly changing ecosystem as it decomposes. The body progresses through a recognized sequence of decompositional stages, from fresh to skeletal, over time (Henssge *et al.*, 1995). Once living animal dies and with favorable abiotic conditions this carrion can be colonized by insect species, within minutes after death. Other species arrive and within a few days, the carrion may host a high number of species (ten or hundred) and individuals (thousands). 'Moreover, how is some similar species are able to utilize the same resources within the carrion simultaneously, whereas other species are excluded? Different factors determine who is, and is not, successful; for instance, Matuszewsk *et al.* (2010) described that the temperature affect the rate and speed of decomposition. While, Al-Mesbah *et al.* (2012) recorded that, the decaying rate of rabbit carcasses was depended on the geographical location. Moreover, habitat (Eberhardt & Elliot, 2008) and the physical state of the carcasses (Avila & Goff, 1998) also affected on the decomposition process.

The succession of insects associated with carrions decomposition is not easily predictable without greater understanding of the species-specific influence of relevant biotic and abiotic factors (Campobasso *et al.*, 2001). In this study, we investigated the differences in species richness of scavenger insects on different carcass types.

MATERIALS AND METHODS

Insect collection

The adult insect specimens used in this study were collected from naturally dead animals. Whereas, animal carcasses were disposed by farmers in a place next to animal farms in Ath Thumamah district, Riyadh. Three types of animals were studies, domestic dogs (Canidae, C*anis lupus familiaris*) about 25±2 KG, Hijazi goats about (Bovidae, *Capra aegagrus hircus*) 40±1.5 Kg, and camels (Camelidae, *Camelus dromedarius*) about 600±15 KG (Fig. 1), during June, 2016.

Insects were collected according to Al-Mesbah *et al.* (2012) by the use of two plastic dishes (traps), which placed by each of the carrions. The dishes filled with water (50 ml) containing 0.5g salts and 0.5g washing powder. Each carcass was examined hourly, for 10 minutes, from 9 am to 2 pm during the decaying stage, where the carcasses were recognized by the breaking up of body skin

Figure 1. Decomposing animals: A, Dog; B, Gaot; C, Camel.

and released of fluid from the body, especially from the abdomen. Each animals were examined for 7 days. The dishes containing insects were removed, and the insects were preserved in 95% ethanol. They were then transferred to the Entomology lab - College of Sciences, King Saud University, Riyadh - in vials labelled with the date and time of collection. Collections were done from three animals of each species. All samples were sorted into groups based on their morphology, determined using a Stereo Microscope SMZ18 (Nikon- Japan) connected to a digital camera. Insect sorting was occurred by specialized taxonomic keys: Pont (1991) and Watson & Dallwitz (2003) for identifying the flies and Catts & Haskell (1990) and Navarrete-Heredia *et al.* (2002) for identifying the beetles. After examination, each group was labelled and then re-preserved in 95% ethanol and maintained at 4°C for molecular identification at a later stage.

Insect identification

Random specimen from each species were selected for molecular analysis and identified to the lowest possible taxonomic level using molecular techniques in order to conduct comparative studies of scavenger insects with respect to host species. Whole genomic DNA extractions were carried out using the QIAGEN DNeasy® Blood & Tissue Kit (Catalogue # 69504) as per the manufacturer's instructions.

To amplify a section of the mitochondrial DNA (mtDNA) cytochrome oxidase subunit I gene (COI) from each specimen, polymerase chain reaction (PCR) was used. The primer utilized was Cyto 1F; Forward sequence (5'-GGTCAA CAAATCCATAAAGATATTGG-3') and reverse sequence (Cyto 1R) (5'-TAAACTTCAGGGTGACCAAAAAATCA-3') (Folmer *et al.*, 1994).

The PCR was performed in a 20 µl reaction volume, containing 2.0 µl of DNA template, 0.6 µl of each primer, 4.0 µl of Solis BioDyne 5 × FIREPol® Master Mix [Reagents, FIREPol® DNA polymerase, 5 × Reaction Buffer B (0.4 M Tris-HCl, 0.1 M $(NH_4)_2SO_4$, 0.1% w/v Tween-20), 12.5 mM MgCl2 (1× PCR solution – 2.5 mM $MgCl_2$), 2 mM of dNTPs (1× PCR solution – 200 µM dATP, 200 µM dCTP, 200 µM dGTP, and 200 µM dTTP), and 12.8 µl of nuclease-free water to make up to 20 µl.

Reactions were performed in an Applied Biosystems® Veriti® Thermal Cycler and the thermal cycling profile used was, an initial denaturation step at 95°C for 5 min; followed by 35 cycles each consisting of three steps, denaturation at 95°C for 15 s, annealing at 51°C for 15 s, and extension at 72°C for 4 min; and then a final extension at 72°C for 7 min. Standard agarose gel electrophoresis was conducted to verify amplification using 1.5% agarose gel to separate the PCR products.

The sequencing of PCR products was carried out using the Big Dye terminator V3.1 kit (Applera, Foster City, CA, USA) with the same primers used in PCR. However, the results were analyzed using an ABI 3700 DNA Analyzer (Applied Biosystem, Foster City, CA, USA).

The sequence chromatograms that were obtained above were edited using Sequencher® v4.0.5 software (Gene Codes Corp., USA). For molecular identification based on the partial sequence of the *mtCoI* gene, each sequence was identified using the Basic Local Alignment Search Tool (BLAST) of the National Center for Biotechnology Information (NCBI), and a reference for each species was chosen. Sequences were trimmed up to approximately 700 bp and were aligned with their respective reference species existing in GenBank using BioEdit Sequence Alignment Editor version 7.2.5 (Hall, 1999). The interaction between the two studied factors, animal and insect species was studied by comparing treatment means using an L.S.D Bayesian test.

Statistical analysis

Significant differences in the species diversity (as quantified by the number of insect species) between the three different animals were evaluated using the Kruskal-Wallis test (Minitab 2017). F-tests were used to determine the effects of interaction between different variances.

RESULTS

The ambient temperature was high throughout the study period (25 to 27-VI-2016), with a daily average (± standard error) of 41.3 ± 2.4°C. However, the average relative humidity was low (13.7 ± 2.5%).

We collected 2327 insects from the three carrion types in the decaying stage of decomposition. From the camel and goat, 1038 and 927 insects were collected, respectively. This was significantly higher than the number collected from the dog (362). Totally, seven insect species were collected belong to flies and beetles. There were six species found on each of the camel and goat, but only five species were found on the dog (Table 1). By applying molecular techniques to identify the collected insects, sequences were identified using the BLAST tool. Based on the results of molecular identification, the selected sequence from each identified species was deposited in the gene bank with the accession numbers shown in Table 2.

Results showed that collected insects belonged to two orders, Diptera and Coleoptera. Three families of Diptera were collected Muscidae, Calliphoridae, and Nemestrinidae, including three species *Musca domestica* Linnaeus 1758, *Chrysomya albiceps* Wiedemann 1819, and *Trichophthalma* sp. Moreover, three families of Coleoptera were collected Dermestidae, Cleridae, and Histeridae, including four species, *Dermestes frischii* Kugelann 1792, *Necrobia rufipes* Fabricius 1781, *Histeridae* sp. Gyllenhal 1808, and *Saprinus semistriatus* Erichson 1834. The fly species, *Trichophthalma* sp., was found only on camel carcasses and the beetle *S. semistriatus* was

Table 1. Number of carcasses (n) monitored and mean number of recorded insects per carcass type and total richness (total number of scavenger species)

Carcass type	n	Mean number of insects ± SD	Total richness
Camel	3	1038.1±19.1 a	6
Dog	3	362.2±17.61 c	5
Goat	3	927.4±21.08 b	6
Total	9	2327	7

At vertical level: Means that do not share a small letter are significantly different.

Table 2. Individuals included in the study with animal and accession number

Order	Family	Species	Animal			Accession No.	Accession No. of reference
			Dog	Goat	Camel		
Diptera	Muscidae	*Musca domestica* Linnaeus, 1758	✓	✓	✓	MG574956	KR262647.1
	Calliphoridae	*Chrysomya albiceps* Wiedemann, 1819	✓	✓	✓	MG574953	KJ193726.1
	Nemestrinidae	*Trichophthalma* sp.			✓	MG574959	DQ631994.1
Coleoptera	Dermestidae	*Dermestes frischii* Kugelann, 1792	✓	✓	✓	MG574954	KM578824.1
	Cleridae	*Necrobia rufipes* Fabricius, 1781	✓	✓	✓	MG574957	KF956176.1
	Histeridae	*Histeridae* sp. Gyllenhal, 1808	✓	✓	✓	MG574955	KF956293.1
		Saprinus semistriatus Erichson, 1834		✓		MG574958	KM439324.1

found only on goat carcasses. The other insect species were found on the all three animal carcasses.

Results in Figure 2 illustrated that, the number of beetles significantly greater than the number of flies on the three carcass types. Moreover, flies and beetles were most common on goat and camel carcasses, while dog carcasses were attracted the lowest number of insects. In addition, goat carcass attracted the highest number of flies. However, the greater numbers of beetles were found on camel carcass. Also, *M. domestica* was the most abundant species found in the carcasses and the most abundant fly species on individual carcasses (Fig. 3). Besides, *M. domestica* and *Ch. albiceps* were most abundant species found on camel and goat carcasses, respectively. *Trichophthalma* sp., was represented only on camel carcasses with a fewer number (Fig. 3). Figure 4 illustrates that *N. rufipes* was the most abundant species of beetle found on the different carcasses, followed by *D. frischii*. In addition, *N. rufipes* and *D. frischii* were most abundant on the camel and goat carcasses, respectively. *Histeridae* sp. was represented on the three animals with slightly similar numbers On the other hand, *S. semistriatus* was found in a fewer number

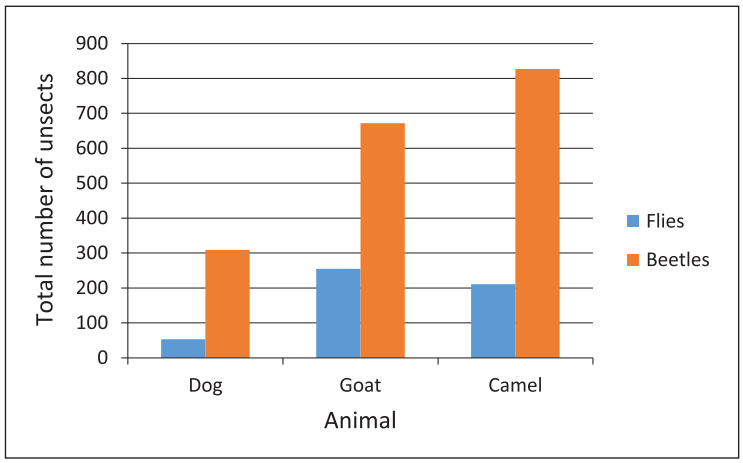

Figure 2. Represents the total number of flies and beetles attracted to the three different animals.

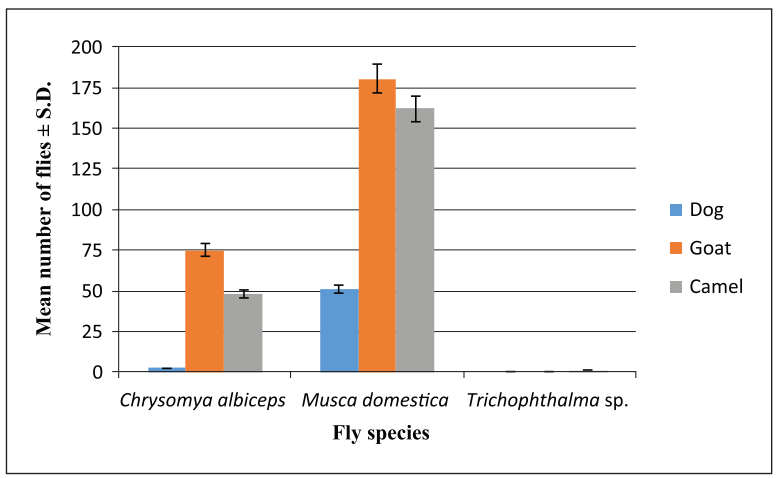

Figure 3. Mean number of each fly species attracted to different animals.

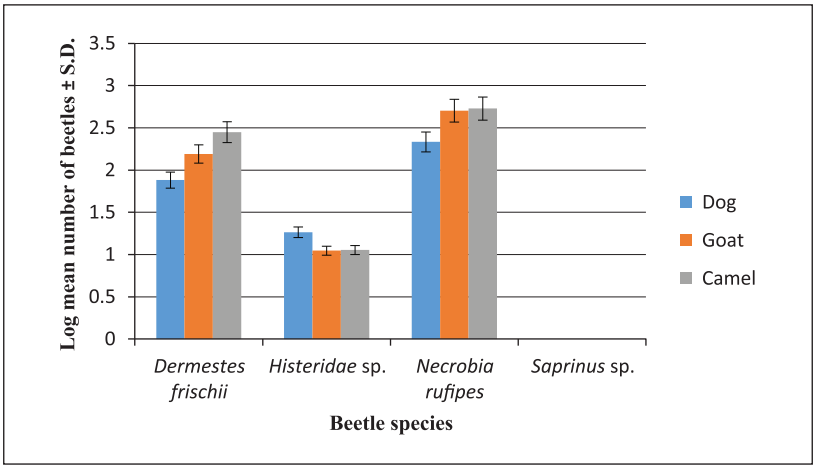

Figure 4. Log mean number of each beetle species attracted to different animals.

Table 3. Analysis of data using Kruskal-Wallis test for adult insects collected from the three different animal carcasses

Comparison	Kruskal-Wallis Test Statistic	d.f.	P-Value	Notes
No. of fly species * animal type	1.13	1	0.032	*
No. of beetle species * animal type	8.23	1	0.001	*

*; Significant (α = 0.05).

on the goat carcasses. In general, results indicated that the number of insects is significantly varied according to carcass type (Table 3).

DISCUSSION

Arthropod community associated with carrion is primarily shaped by spatial and temporal constraints. Some arthropod taxa seem to be found nearly everywhere, whereas other carrion arthropod taxa are found only in certain situations; yet, all are in fact guided by these constraints. Temporal constraints include seasonal, successional, and circadian components, whereas spatial constraints include geographical and ecological components (Merritt and Jong, 2016). Moreover, scavenging patterns may depend on characteristics of the carcass, the consumer, and/or extrinsic variables (Moleón *et al.*, 2014). These factors include sun and shaded sites (Sharanowski *et al.*, 2008), clothing of carcass (Mashaly *et al.*, 2019), burning of the carcasses (Vanin *et al.*, 2013) and other factors. Woodward *et al.* (2005) found that carcass size is not closely associated with the direction of inter-specific interactions. However, study by Moleón *et al.* (2015) showed that carcass size is directly related to insect richness.

In this study, the effects of carcass type on the attraction of insect have been studied. It was evident that, one of the key factors affecting the structure and functioning of scavenger species richness, is the carcass type, as the larger number of insects was observed on camels and goats than dogs. Simmons *et al.* (2010) explained that the greater body mass for the carcasses presented a good source for insects to consume and the greater volume of insects the carcass can accommodate. Denno and Cothran (1975) found differences in the species and proportion of necrophagous flies on different types of carrion, including rats, rabbits, and sheep. In our study, we found

differences in the species represented on the studied animals, with some flies found only on camels and some beetles found only on goats. Carrion usually attracts a rather predictable assemblage of arthropods, and several studies have shown that different number of taxa can be acquired simply by visiting different animal carcasses. As examples, Goff *et al.* (1986) reported different taxa from several kinds of carrion in the Hawaiian Islands.

The most conspicuous arthropod taxa of almost any carrion community on land include flies and beetles. Flies are the stereotypical arthropods associated with carrion, both in the adult and in the larval life stages. In fact, in the absence of vertebrate scavengers, flies generally represent the largest amount of arthropod biomass supported in a carcass and can consume over half of the original carcass mass (De Jong and Hoback 2006); however, the total number of families regularly associated with carrion is relatively small. Calliphoridae, Sarcophagidae, and Muscidae are the usual fly families that would be considered normal carrion fauna. In the current study, Muscidae, Calliphoridae, and Nemestrinidae families of were collected from Diptera.

The Calliphoridae is a moderately diverse family, with approximately 1100 species, the majority of which are likely to be found on carrion (Rognes, 1997). These are the most typical members of the carrion fly community throughout the world (Whitworth, 2010). The Muscidae contains about 4500 species (de Carvalho *et al.*, 2005), and between them only a few genera, including *Hydrotaea*, *Musca* and *Muscina* are found on carrion (Skidmore, 1985). Muscids can be among the dominant flies in the carrion fauna (Shi *et al.*, 2009), often arriving a bit later than the calliphorids and sarcophagids, but still before the carrion completely dries out. Flies recorded in this study resembled the species recorded by Mashaly *et al.* (2017) on sheep carcasses in different cities of Saudi Arabia except for *Trichophthalma* sp., which was not found in their study.

Three families of Coleoptera; Dermestidae, Cleridae, and Histeridae were collected during our study. Mashaly *et al.* (2018) recorded the same beetle species which recorded in the current study on sheep carcasses in Riyadh. Histeridae is a large family of about 4300 species. They are efficient predators of calliphorid and other cyclorrhaphan Diptera larvae (Nuorteva, 1970). Dermestidae is a moderately large family with about 600 species, of which likely half can be encountered on carrion. These beetles often arrive later in succession and are commonly characteristic fauna of those later stages (Anderson and VanLaerhoven, 1996), but can arrive even in early stages (De Jong and Hoback, 2006). The majority of the forensically relevant species of dermestids are from the genus Dermestes, where they are important in the decomposition process of humans and other animals (Smith, 1986). Two cosmopolitan species of *Necrobia* are commonly found on carrion and are distinct members of the carrion community. Unlike most clerids, which are generally regarded as predators in both the larval and adult forms (Payne and King, 1970), the *Necrobia* spp. appear to be both necrophagous and predaceous (Anderson and VanLaerhoven, 1996).

We collected the insect sample during the decay stage of decomposition of the all studied animals. Goff (2009) indicated that, in the post-bloating stage, not only large feeding masses of fly maggots, but also predatory members of the Staphylinidae and Histeridae can be observed in their role as predators of fly maggots and, at the end of this stage, most of the maggots have left their food substrate for pupation. This time point is preferred by adult dermestid beetles, because they feed on the remaining cadaver skin and ligamentous tissue (Braack, 1987).

As such, different species of flies and beetles are frequently associated with three different types of carcasses and offer a wealth of potential information to forensic investigators where relevant biological data is available. Unfortunately, the most beneficial aspect of insects evidence

associated with decomposing animals, which could be the indication of minPMI, is hampered by a paucity of such data.

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