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Preliminary study of necrophagous Diptera succession on a dog carrion in Skikda, North-east of Algeria

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Abstract
When an animal dies, it attracts several species of invertebrates and insects, which will feed on its carcass. The first scavenger species that arrive on a carcass belong to the order of Diptera. In this context, we conducted a series of trials in Skikda region with the aim of filling a checklist of the necrophilous species present in this geographical area. Our first trial was performed on a carcass of a dog (22kg), between March and April 2011, in an open field of the School of Agriculture (Skikda) which is used normally as an agricultural area. We collected more than 3126 specimens of Diptera, larvae, pupae and adults. Four families of Diptera, Calliphoridae, Sarcophagidae, Muscidae and Fanniidae were recognized, the most numerous observed family was the Calliphoridae family, represented by the species *Calliphora vicina, Calliphora vomitoria, Lucilia sericata, Lucilia illustris and Phormia Regina.*

Keywords: Necrophagous, Diptera, dog carrion, Skikda, Calliphoridae

1. Introduction
Insects arrive on decomposing corpses in a relatively predictable sequence that is named ecological succession. It's consists of a series of waves of different arthropods which are the primary organisms involved in the major decomposition of the body. They arrive at exposed remains shortly after death, often in less than 10 min, and quickly begin their activities providing valuable scientific information, Flies are the first to invade a corpse [1]. Anomalies in the cast of carrion-frequenting insects (flies, beetles, spiders, ants, etc.) can be used to describe incidents surrounding the death. The major use of insect evidence in forensic science is in the estimation of the post mortem interval (PMI): the time between the death and the discovery of the corpse. The PMI evaluation based on entomological evidences is performed starting from the developmental stage of the species found on a corpse [2].
The necrophagous Diptera, typically, is the first group that arrives to the cadaver, attracted by the odor produced during the early stages of the decomposition [3]. The fauna of necrophagous Diptera has been intensively studied in Australasia, Europe, East and South Asia, and North and South America, with notable effect in the form of a variety of published checklists, keys, and taxonomic monographs [4, 5] However, the fauna of some large geographical regions, like the Afrotropics (except Namibia and the Republic of South Africa) and the Middle East [6] and North Africa (Algeria), remains poorly studied. This situation stops broad application of insects for medico-legal purposes due to a lack of proper tools for species identification of the local fauna. This study addresses our lack of knowledge in this area; it's a part of larger ongoing effort to identify carrion-feeding entomofauna and to start to build a checklist of necrophagous species present in this geographical area.

2. Materials and Methods
This study was carried out in open field at the School of Agriculture, located in Skikda in the north east of Algeria (36°53'N,06°54'E) at the altitude of 1m above sea level. In this region the average annual temperatures fluctuate between (8.8 °C and 29.4 °C) and the annual relative humidity above 76%. The landscape of the school of agriculture, which is used normally as an agricultural area, is characterized by the presence of an orange orchard.
One dog *Canis familiaris* (22 kg) was used as animal model: a stray dog killed with shotgun, immediately after death was placed in a metal cage (120cm x 120cm x 120cm) made with 2cm x 2cm wire meshing. This allowed the access to the carcass by insects while preventing the disturbance by scavenger vertebrate [7].

The carcass was observed for 10 hours following its sacrifice; subsequently the process of decomposition was observed during the daylight from 07 Mars to 22 April, 2011. Samples were taken two times a day (9.00, 14.00) for the first period (2 weeks) and subsequently once a day (at 11.00) until the dry remains stage was reached 45 days after the death.

At each sampling time, adult Diptera were collected with an entomological net. Their immature stages were collected too from the natural cavities (eyes, nose, mouth and anus), the thoracic wound underneath the carcass and in the soil up to some centimeters of depth.

Immature specimens were fixed in 80% alcohol and adults were killed with freezing and mounted with entomological pins.

Environmental temperature and the physical change of the carcass over time (degree of swilling, dischange of liquids and gases, etc.) were recorded during the inspection of the dog's remains.

The taxonomic identification of the adults and larvae was carried out using the following keys [8,-11]. When necessary, the larvae were cleared with a cold solution of 5% KOH and permanently mounted in Canada balsam.

3. Results

In total, 3126 individuals of flies (dipterans) were collected (larvae, pupae and adults) belonging to 4 families, 7 genera and 11 species (Table1). Among these one family was predominant; the family of Calliphoridae (blowflies) constituted 94.82% followed by the other families as Muscidae 3.13%, Sarcophagidae (flesh flies) 1.12% and Fanniidae with 0.93%.

Table 1: Succession of Dipterans associated with exposed carcass on Skikda.

Family	Species	Fresh	Bloated	Decay	Dry
		L P A	L P A	L P A	L P A
Calliphoridae	*Calliphora vicina*	x x	x x	x x x	x x
	Calliphora vomitoria	x x	x x	x x x	
	Lucilia sericata	x	x x	x x x	x
	Lucilia illustis	x	x	x x	
	Phormia regina	x	x x	x	
Sarcophagidae	*Sarcophaga sp*	x	x		
	Sacophaga africa	x	x		X
Muscidae	*Musca domestica*		x	x	
	Musca sp		x		
	Muscina sp		x x	x	
Fanniidae	*Fannia sp*		x		

A: adult stage ; L: larval stage ; P: pupal stage

The results show that the most abundant species among the larvae collected is *Calliphora vicina* (Robineau-Desvoidy, 1830) with 71.82%, followed by *Calliphora vomitoria* (Linnaeus, 1758) with rate of 14.67%; while the other species are poorly represented (Fig1).

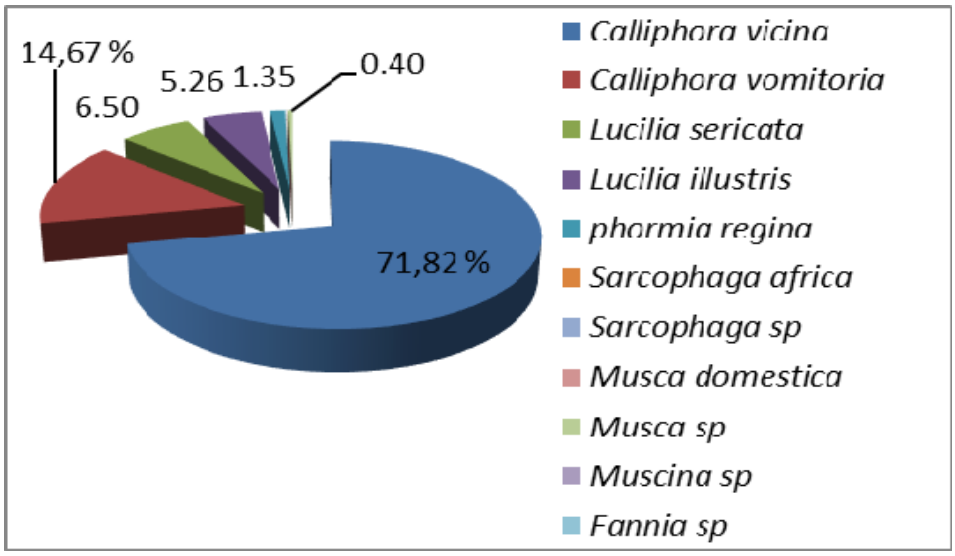

Fig 1: Relative abundance of larvae collected on the corpse during the study period.

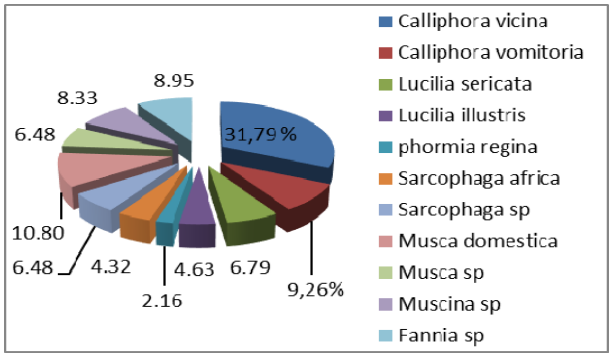

Fig 2: Relative abundance of adults collected on the corpse during the study period.

followed by *Musca domestica* (Linnaeus, 1758) with 10.80% then *C. vomitoria* with 9.28%; the species less represented is *Phormia regina* (Meigen, 1826) with 2.16% (Fig2).

Four different stages of decomposition were observed during the experiment [1].

***Fresh stage (0-3 days):** A sharp decrease in body temperature was observed reaching levels below ambient temperatures (Fig3). Oviposition was observed in natural orifices, around the wound and at the side of carcass touching the ground.

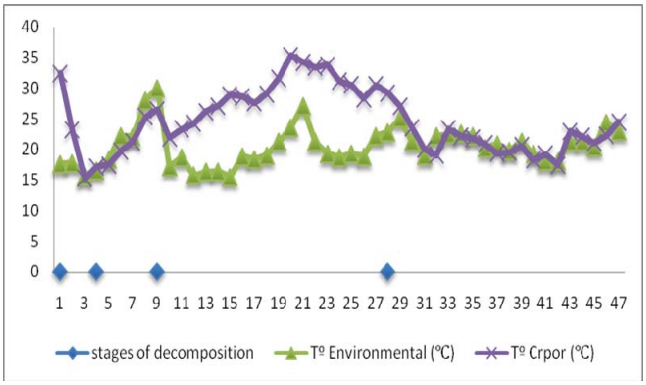

Fig 3: Daily temperature variations related with decomposition Stages

Immediately after death, the first adults of Calliphoridae represented by (*C. vicina* representing 41.30% overall capture of adults in this stage, *Lucilia sericata* (Meigen, 1826) 15.22%, *C. vomitoria* 8.70%, *P. regina* with 4.35%) and Sarcophagidae represented by (*Sarcophaga sp* 19.57% and *Sarcophaga africa* (Wiedemann, 1824) 10.87%) (Table2).

Table 2. Percentage of adult dipterans collected at different stages of decomposition

Family	Speces	Fresh	Bloated	Decay	Dry
Calliphoridae	*Calliphora vicina*	41,30	28,00	26,54	81,25
	Calliphora vomitoria	8,70	9,00	10,49	0,00
	Lucilia sericata	15,22	8,00	4,32	0,00
	Phormia regina	4,35	4,00	4,94	6,25
	Lucilia illustris	0,00	2,00	3,09	0,00
Sarcophagidae	*Sarcophaga africa*	10,87	7,00	0,00	12,50
	Sarcophaga sp	19,57	12,00	0,00	0,00
Muscidae	*Musca domestica*	0,00	0,00	21,60	0,00
	Musca sp	0,00	21,00	0,00	0,00
	Muscina sp	0,00	9,00	11,11	0,00
Fanniidae	*Fannia sp*	0,00	0,00	17,90	0,00

Three days after death, the first instars larvae of *C. vicina* were collected in natural orifices and around the wound.

*Bloated stage (4-8 days): body temperature began increasing during this stage (Fig. 3) as result of insect activity and putrefaction process [12].

A large number of adult species were attracted and collected in the carcass during this stage; *C. vicina, C. vomitoria, L. sericata, Lucilia illustris* (Meigen, 1826), *Ph. regina, S. africa, Sarcophaga sp, Musca sp* and *Muscina sp* of which the most important were *C. vicina* 28% and *Musca sp* 21% (Table2).

Abundant masses of larvae were observed throughout the carcass. First and second instar larvae were found belonging to Calliphoridae: the majority of specimens collected belonging to *C. vicina* with 75.05% and *C. vomitoria* 13.09%. *L. sericata* and *P. Regina* were also collected but with low proportion, 7.57% and 4.29% respectively (Table3).

Table 3: Percentage of larvae collected at different stages of decomposition

Family	Speces	Fresh	Bloated	Decay	Dry
Calliphoridae	*Calliphora vicina*	100	75,05	70,96	0,00
	Calliphora vomitoria	0	13,09	15,10	0,00
	Lucilia sericata	0	7,57	6,30	0,00
	Phormia regina	0	4,29	5,50	0,00
	Lucilia illustris	0	0,00	1,65	0,00
Sarcophagidae	*Sarcophaga africa*	0	0,00	0,00	0,00
	Sarcophaga sp	0	0,00	0,00	0,00
Muscidae	*Musca domestica*	0	0,00	0,00	0,00
	Musca sp	0	0,00	0,00	0,00
	Muscina sp	0	0,00	0,49	0,00
Fanniidae	*Fannia sp*	0	0,00	0,00	0,00

***Decay stage (9-27days)**

Peaks in cadaver temperature reached during this stage with the maximum value of 35℃ (Fig 3). All the larval instars of Callphoridae were present. No individuals of the Sarcophagidae were observed.

Larval activity in the carcass was notably reduced although it continued to be dominated by *C. vicina* with 70.96 %(Table 3), the majority of larval masses were concentrated in the muddy ground under body.

In this stage the number of adults of Calliphoridae decreased considerably (*C. vicina* 26.54), being replaced by small dipterans such as the Fanniidae, *Fannia sp* that represents 17.90% (Table 2).

***Dry stage (28 -47days)**

The last sampling was represented by adults of *C. vicina* 81.25% (Table 2), in this stage about 85% of the initial body weight had been lost. Only remains of skin bones were left.

Several dead larval were found on the body and a small number of larvae were concentrated in the extremities of legs.

In the beginning of this stage some pupae of Calliphoridae were collected at a distance of 1m from the carcass among layers of moss on the ground, these were placed in rearing containers and raised to adults.

4. Discussion

The number of decomposition stages and their types found in the present study were similar to those of previous studies [13-15]. The correspondence of study results from different areas, seasons and habitats supports the model of exposed carcass decomposition with at least 4 stages distinguished.

Due to the initial nature of the study the differences in rate of carrion decomposition are difficult to interpret. It is obvious that decomposition rate depends on many factors [16], of which biotic and abiotic conditions of the habitat, weight of the carcasses or plain chance can explain the observed differences [15].

Insects affect the rate of degradation of a body significantly [17]. Two categories of carrion flies are attracted by dead bodies: insect people in search of food and females in search of a spawning site, the latter are the most part [18]. According to Mann [19], the majority of destruction of soft tissues is due to the insects.

The taxa composition of carrion entomofauna attracted to the rabbit carcasses at Skikda at the family and genus level is similar to what has been reported in previous studies [12-14, 20-22]. In this study, 11 insect species were identified that were directly attracted to the rabbit carcasses throughout the course of the study.

During the first phase of decomposition of the corpse which lasted 3 days, it was noted that the cluster of the eggs was in natural openings and in wounds primarily; the choice of oviposition sites is due to the fragility of eggs and young larvae [23].

The laying also depends on several factors including the temperature of environment and the substrate, are the critical parameters [24].

First attracted and harvested on the corpse belong to the family of Calliphoridae which *C. vicina* is the most frequent with 41.30% and which is present from the first day, These results coincide with those of Wyss's & Cherix's [25] work, which found that *C. vicina* is present from the first day with significant proportions. During the bloated stage; It was noted the significant presence larval mass belongs to several species, mainly Calliphoridae *C. vicina* which represents 75.05% and 13.09% *C. vomitoria*. Fenton *et al.* [26], noted that the presence of other eggs, larvae or adult individuals over the body increases the likelihood of spawning by acting as an attractive signal to gravid females; which explains the presence of different larval stages. In this phase, there is always the presence of a high rate of *C. vicina* adult (28%) and *Musca* sp. with (21%), which is noticed in several works [25, 27]. Adults of *M. domestica* (21.6%) were attracted to the decay stage of the dog carcass. However, no larvae of *M. domestica* were found on the dog carcass during this study. Arnaldos *et al.* [28], reported adults of *M. domestica* as the dominant by during the summer period from the first day of carcass exposure. Voss *et al.* [29], reported that adult *M. domestica* would regularly visit carcasses, although oviposition was rare.

In decay stage, maggot activity increased tremendously, and maggot mass temperature reached its high (35.3 °C). The most evident consequence of this larval gregariousness is the local elevation of the temperature. This phenomenon, often observed but still poorly understood, seems to be due to the exothermic process of larvae's digestion [26]. The simultaneous presence of a very large number of individuals emitting a very low heat because of their metabolism can lead to extremely high temperatures and local increases: Greenberg [30], recounts an observation of 18 °C higher than the outdoor temperature, while Turner mentions a temperature of 40 °C within a mass of larvae [31]. A study in Hawaii recorded during several days of a temperatures over 50 °C inside a corpse placed pork into wooded area, while the outside temperature was below 30 °C [32].

The environmental conditions of the study area, with temperatures fluctuating between 15.5 °C and 30 °C during the day and 9.3 °C to 14.7 °C at night, influenced the process of decomposition. The decomposition process took 47 days as opposed to the 83 days reported by Martinez *et al.* [1] in Colombia during the wet season where a pig of 10 kg was used. The weight of the two corpses were not equal, the rate of decomposition may have been influenced by the environmental temperature which are lower in our work. Moreover our study was done at 1 m above sea level, whereas the study by Martinez *et al.* [1], was done at 3035 m above sea level.

Many authors are interested in experimental highlighting spatial and temporal variations of populations of Necrophagous Diptera. So, it has been shown that species and orders of succession could vary depending on the geographic area [33, 14], the type of media [34, 35] or even following the seasons and years [18, 28, 36, 37] these data highlight the importance of local studies and the risk of errors related to the use of data from «standard» estates.

In Dry stage about 85% of the initial body weight had been lost. Only remains of skin bones were left, The rapid weight loss of the cadavers was a result of the conversion of carcass biomass into larval biomass and the subsequent exit of insects from the body during pupation [1].

An interesting difference revealed in the present experiment is a small number of Sarcophagidae adults and absence of its larvae. In most previous studies this family was found to be

one of the most important Diptera families in the process of carrion decomposition [15, 38, 39]. However, it seems that this statement should not be generalized, particularly given that the present experiment is not the only one in which Sarcophagidae were of little importance for the decomposition process [13, 15, 20]. This might be due to the fact that several species of the genus *Sarcophaga* are parasites of earthworm [25]. But it seems that even these flies are fewer on a corpse as the Calliphoridae, they can colonize the corpse more can meet them both at the beginning of process of decomposition until much later, and what was found during our experimentation; the presence of the adults at the beginning of the decomposition's process.

5. Conclusion

The major use of insect evidence in forensic science is in estimation of post mortem interval (PMI), Estimates are most commonly determined by examining the developmental stage of particular insect species found on corpse. The reliability of forensic entomological expertise and associated experiments strongly depends on proper species identification of collected material. Necrophagous blowflies (Calliphoridae), the earliest visitors to carrion as adults and the main decomposers of carrion mass as larvae (the usual species forming the first wave the faunal succession to arrive and lay eggs on corpse) play a special role in both casework and research dedicated to medico-legal applications. Those routinely encountered by forensic entomologists are the common blue bottle species (*C. vicina* and *C. vomitoria*) and the green bottle species (*Lucilia sp*). These species had previously been reported in studies of forensic entomology in this region and world, conducted in different climactic zones. These species are important indicator species of specific stages of decomposition in this bio-climatic zone.

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