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An observation on the decomposition process of gasoline-ingested monkey carcasses in a secondary forest in Malaysia

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Abstract. This study was designed to mimic homicide or suicide cases using gasoline. Six adult long-tailed macaque (*Macaca fascicularis*), weighing between 2.5 to 4.0 kg, were equally divided into control and test groups. The control group was sacrificed by a lethal dose of phenobarbital intracardiac while test group was force fed with two doses of gasoline LD50 (37.7 ml/kg) after sedation with phenobarbital. All carcasses were then placed in a decomposition site to observe the decomposition and invasion process of cadaveric fauna on the carcasses. A total of five decomposition stages were recognized during this study. This study was performed during July 2007. Fresh stage of control and test carcasses occurred between 0 to 15 and 0 to 39 hours of exposure, respectively. The subsequent decomposition stages also exhibited the similar pattern whereby the decomposition process of control carcasses were faster than tested one. The first larvae were found on control carcasses after 9 hours of death while the test group carcasses had only their first blowfly eggs after 15 hours of exposure. Blow flies, *Achoetandrus rufifacies* and *Chrysomya megacephala* were the most dominant invader of both carcasses throughout the decaying process. Diptera collected from control carcasses comprised of scuttle fly, *Megaselia scalaris* and flesh fly, sarcophagid. We concluded that the presence of gasoline and its odor on the carcass had delayed the arrival of insect to the carcasses, thereby slowing down the decomposition process in the carcass by 6 hours.

INTRODUCTION

It is crucial that decomposition process be understood due to its significant impact on forensic investigation. Decomposition process has been studied throughout the world to identify pattern of insect succession from a decaying body. Apart from bacteria and fungus, the insects are the most important processors of the dead remains (Byrd & Castner, 2010). Due to this, the estimation of postmortem interval (PMI) by entomological means is usually based on the rough estimation of larvae age (Gerrard, 2007). To date, this area has been recognized as an important tool in crime-solving investigation.

The rate of decomposition is greatly influenced by organisms which feed on the body in different stages of the decomposition process. In general, the process is divided into five stages: fresh, bloated, active decay, advanced decay and dry remains (Early & Goff, 1986; Gunn, 2006). Different populations of insects that are associated with a corpse may vary

geographically and seasonally. The invasion and the different stages of development of those insects are highly dependent on external factors especially ambient temperature and rainfall (Turchetto & Vanin, 2010).

Circumstances surrounding the death scenario can profoundly affect the time of initial appearance of insects on the corpse. These may affect the estimation of time period of PMI. Currently, majority of the decomposition studies focused on the normal process without considering other important factors such as tissue and chemical composition in flesh (Goff, 1993). Presence of toxins or drugs in the corpse not only delayed the invasion of arthropod, but also affected the development of the insect that swarm to the body (Goff *et al.*, 1997; Carvalho *et al.*, 2001; Wolff *et al.*, 2004; Mahat *et al.*, 2009).

The increase in cases of intoxication-related death justifies the great interest aroused in the studies on decomposition and insect succession. Gasoline is one of the most potential intoxicating agents that are easily accessible to human. These are reflected by several recent reports on intoxication and death cases in humans due to accidental or intentional ingestion of gasoline (Carnevale *et al.*, 1983; Grufferman & Walker, 2005; Nichani *et al.*, 2006). There is also a recent report of gasoline abuse by injection (Fink *et al.*, 2009). It has been reported that gasoline reaches toxic level when 12 ounces are swallowed at a single consumption (Agency of Toxic Substances and Disease Registry, 1995). The acute oral lethal dose for gasoline has also been reported to be 18.8 ml/kg (Beck *et al.*, 1983). Based on the escalating prevalence of death due to poison and the lacking of data on the decomposition of chemical-contained tissue, a study was conducted to observe the effect of gasoline in decomposition process of long-tailed monkeys and the invasion of cadaveric fauna in Malaysia.

MATERIALS AND METHODS

Experiment was performed on a decomposition site located within the campus of Universiti Kebangsaan Malaysia, Bangi, Selangor (02º 50'N to 02º 54'N, 101º 47'E to 101º 50'E), during July 2007. A grass land and a woody area with direct sunlight were selected as study sites. A total of six adult long-tailed monkeys (*Macaca fascicularis*) weighing between 2.5 to 4.0 kg were used in the study. The monkeys were divided equally into two groups, control and test group. Each monkey in the control group was sacrificed by a lethal dose of phenobarbital (LD_{50} 0.4 mg/kg) via intracardiac route. The test group was first sedated with phenobarbital (dose of 0.2 mg/kg) before they were force fed with double of the lethal dose of gasoline (37.7 ml/kg). The experiment started after the carcasses were introduced to the site at 4 pm and it was considered as day 0. All carcasses were placed on the ground 10 m apart from each other.

The carcasses were then covered individually with protective metal cage (100cm x 100cm x 100cm) made with wire mesh which is 2.0 x 2.0cm in grid-shape. The decomposition process was monitored and recorded twice daily, first at 7 am and then at 4 pm. Climatological data especially temperature, humidity and precipitation was recorded along the experiment using the university weather station. Carcass body temperature, soil temperature and carcasses conditions were also noted using thermometer during each visit.

All insect specimens were collected from around and under the carcasses. Adult dipteran was collected using a hand net. Other adult and immature insect specimens were collected using sampling bottle, forceps and spoon. Sample of soil under and around the body was also taken during the advanced stages of decomposition. All adult specimens were preserved in 70%

ethanol. Some immature specimens (e.g., fly larvae) were reared to adult for identification, while the remaining larvae were fixed in boiling water and then preserved in 70% ethanol.

Taxonomic identification for the adults fly was carried out using the following keys; (Borror *et al.*, 1976; Smith, 1986; Kurahashi *et al.*, 1997). Larvae were identified using keys of Omar (2002) and Thyssen (2010).

RESULTS

There was no variation in daily minimum and maximum temperatures obtained throughout the study (Fig. 1). The mean minimum temperature of the experiment was 23.63°C (±0.56) while the mean maximum temperature recorded was 33.13°C (±0.94). The presence of rain was recorded on day 0, 2, 3, 4 and 5 with the mean of rainfall recorded was 13.29 mm. Ambient temperature was generally higher in the afternoon, except for the third day when it rained heavily (mean ± SD= 28.19 ± 2.11°C). The relative humidity was higher in the morning, except for days with rainfall in the afternoon (mean ± SD = 92.35 ± 2.55%).

A total of five stages of decomposition were observed in this study. Body temperatures of the monkey was mostly recorded from natural orifices such as eyes, mouth and anus. During advanced stages of decomposition, the body temperature was recorded between torn tissues. We observed that the rate of decomposition for control group was much faster in comparison with test group. The arthropods collected on carcasses of both groups are shown in Table 1.

Fresh stage

Fresh stage (day 0) was defined as the stage with no decomposition odor or swelling. Fresh stage started immediately after death. When the carcass was introduced at the site, several adult blow flies (Calliphoridae), namely *Hypopygiopsis fummipenis* (Walker, 1856), *Chrysomya megacephala* (Fabricius, 1794) and *Achoetandrus rufifacies* (Macquart, 1843) and scuttle fly (Phoridae), *Megaselia scalaris* (Loew, 1866) swarmed at the control carcasses. However, no arthropod was found attracted to gasoline-ingested carcasses. This might be due to the strong odor of gasoline that emitted from it.

A sharp decrease in body temperature occurred in both carcass' groups after 9 hours of exposure. The first instar of *M. scalaris* and *C. megacephala* were found and collected from the various natural orifices of control carcasses after 9 hours. Other adult insects collected from the control carcass were fleshfly (Sarcophagidae: sarcophagid) and ants (Hymenoptera: *Componotus gigas* [Latreille, 1802], *Componatus* sp., *Solenopsis* sp.).

Fresh stage for control carcass ended 15 hours after exposure when the bloated stage began thereafter. On the other hand, the carcasses in the test group had only their first blowfly eggs at 15 hours after exposure. Body temperature of test carcasses was lower than control carcasses and the prior carcasses had no visible larvae activity on the body.

Several adult blow flies (*H. fummipenis*, *A. rufifacies*, *C. megacephala*), picture-winged fly (Otitidae/ Ulidiidae), long-legged fly (Dolichopodidae) and Hymenoptera (*Componotus* spp. and *C. gigas*) were collected from the test group at this stage. The fresh stage for test group ended 39 hours after exposure.

Bloated stage

A bloated abdomen was observed on the control carcass after 15 hours of exposure, compared to the gasoline-ingested carcasses which started the bloating stage on 39 hours after exposure. The distention of abdomen was likely due to accumulated gases produced by bacteria within the intestine. The duration of the bloated stage for control carcasses occurred between 15 to 24 hours after exposure, while gasoline-ingested carcasses experienced bloating between 39 to 48 hours.

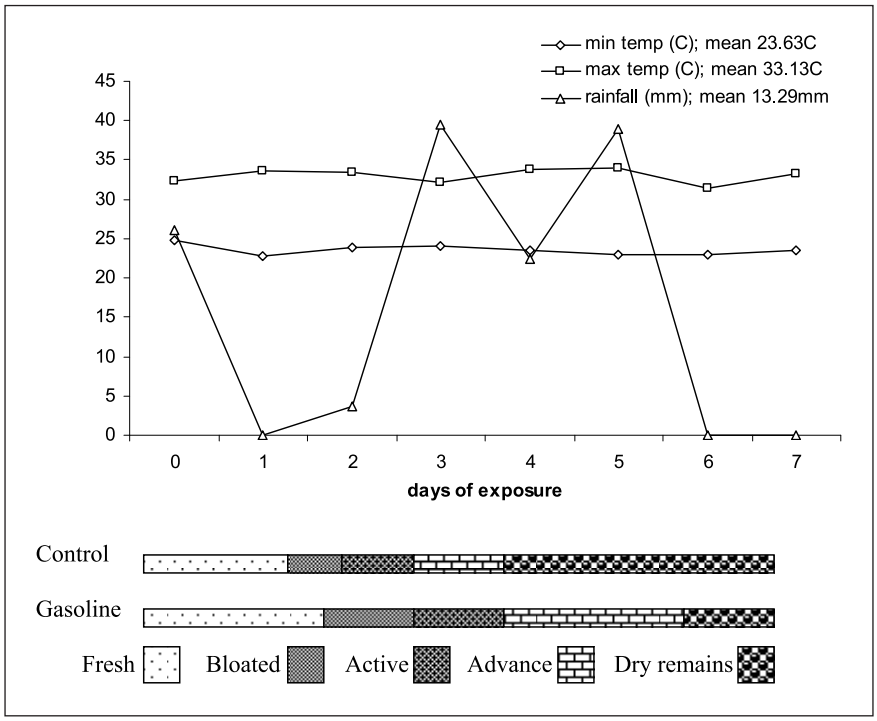

Figure 1. Daily minimum/ maximum temperatures, precipitation and the decomposition stages of the long-tailed macaque (*Macaca fascicularis*) carcasses between control and treated- gasoline carcass during July, 2007. The range of mean ambient temperature during the experiment period was 23.63ºC to 33.13ºC

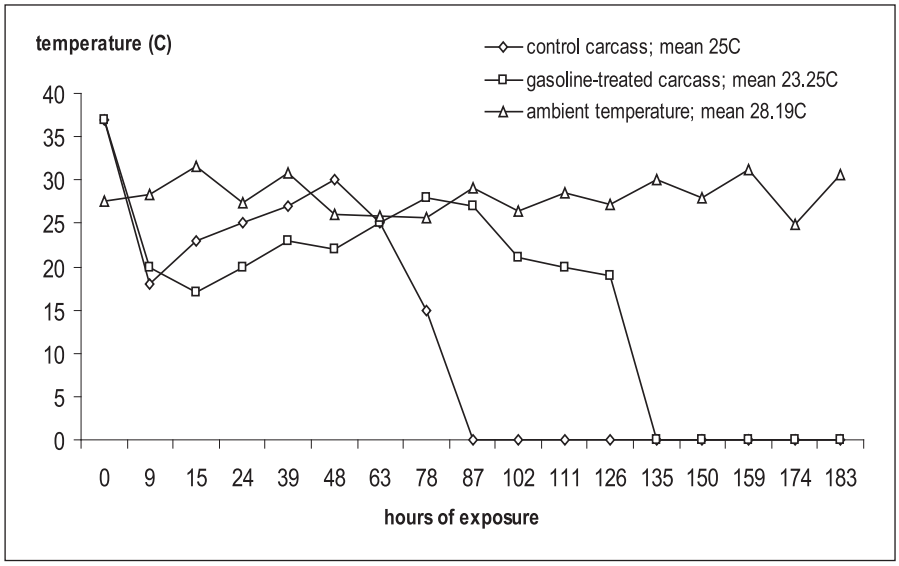

Figure 2. Ambient temperature and body temperature of the long-tailed macaque (*Macaca fascicularis*) carcasses between control and treated-gasoline carcass during July, 2007. Mean of the ambient temperature, control and gasoline-treated carcasses internal temperature were 25ºC, 23.25ºC and 28.19ºC, respectively

Table 1. Entomofauna attracted to various stages of decomposition of the long- tailed macaque (*Macaca fascicularis*) carcasses

Fresh stage	Control 0 – 15 hours	Diptera	Calliphoridae	*Hypopygiosis fummipenis*	Adult
				Chrysomya megacephala	Egg, larvae, adult
				Achoetandrus rufifacies	Egg, larvae, adult
			Phoridae	*Megaselia scalaris*	Egg, larvae, adult
			Sarcophagidae	sarcophagid	Adult
		Hymenoptera	Formicidae	*Componotus* spp.	Adult
				Componotus gigas	Adult
	Gasoline 0 – 39 hours	Diptera	Calliphoridae	*Hypopygiopsis fummipenis*	Adult
				Chrysomya megacephala	Egg, larvae, adult
				Achoetandrus rufifacies	Egg, adult
			Otitidae		Adult
			Dolicopodidae		Adult
		Hymenoptera	Formicidae	*Componotus* spp.	Adult
				Componotus gigas	Adult
Bloated stage	Control 15 – 24 hours	Diptera	Calliphoridae	*Achoetandrus rufifacies*	Egg, larvae, adult
				Chrysomya megacephala	Egg, larvae, adult
		Hymenoptera	Formicidae	*Componotus* spp.	Adult
				Solenopsis spp.	Adult
	Gasoline 39 – 48 hours	Diptera	Calliphoridae	*Achoetandrus rufifacies*	Egg, larvae, adult
				Chrysomya megacephala	Egg, larvae, adult
		Hymenoptera	Formicidae	*Componotus* spp.	Adult
				Solenopsis spp.	Adult
Active decay stage	Control 24 – 48 hours	Diptera	Calliphoridae	*Achoetandrus rufifacies*	Larvae, adult
				Chrysomya megacephala	Larvae, adult
		Hymenoptera	Formicidae	*Componotus* spp.	Adult
				Solenopsis spp.	Adult
	Gasoline 48 – 87 hours	Diptera	Calliphoridae	*Achoetandrus rufifacies*	Larvae, adult
				Chrysomya megacephala	Larvae, adult
		Hymenoptera	Formicidae	*Componotus* spp.	Adult
				Solenopsis spp.	Adult

Advanced decay	Control 48 – 78 hours	Diptera	Calliphoridae	*Achoetandrus rufifacies* *Chrysomya megacephala*	Larvae, pupae, adult Larvae, pupae, adult
		Coleoptera	Staphylinidae		Adult
	Gasoline 87 – 126 hours	Diptera	Calliphoridae	*Achoetandrus rufifacies* *Chrysomya megacephala*	Larvae, pupae, adult Larvae, pupae, adult
			Sarcophagidae	sarcophagid	Adult
Dry remains	Control 78 – 183 hours	Diptera	Calliphoridae	*Achoetandrus rufifacies* *Chrysomya megacephala*	Pupae, adult Pupae, adult
		Coleoptera	Histeridae		Adult
			Staphylinidae		Adult
		Arachnida	Spider		Adult
		Hymenoptera	Wasp		Adult
	Gasoline 126 – 183 hours	Diptera	Calliphoridae	*Achoetandrus rufifacies* *Chrysomya megacephala*	Pupae, adult Pupae, adult
			Dolicopodidae		Adult
		Hymenoptera	Formicidae	*Componotus* spp. *Solenopsis* spp.	Adult Adult

At this stage, masses of larvae were observed from both groups, however, infestation of fly larvae were in different period of exposure. It was suspected that the feeding activity of these larva masses on the carcass body had raised the body temperature and thus, created an ideal environment for rapid larvae growth. However, their body temperatures had not exceeded the ambient temperature throughout the stage.

First and second instar *C. megacephala* were collected from the head part. While the first and second instars *A. rufifacies* were only collected from the anus of the test monkey carcasses. Besides, C*omponotus* sp. and *Solenopsis* sp. (Formicidae) were observed predating on fly larvae found on carcasses in both groups.

Active decay stage

After 24 hours of exposure, a small hole was detected at the abdomen of the control carcasses. The abdomen burst open due to the gas accumulation, and slowly deflated. Larvae of first, second and third instars were found crawling out from the various openings on the body. The skin color of the control carcasses had changed as a result of putrefaction and most of the monkeys' hair were detached from their skin. Active decay stage in control carcass ended 48 hours after exposure. Test carcasses were in active decay stage between 48 to 87 hours after exposure.

Large amount of putrefying liquid or byproduct of decomposition (BOD) was seen around test monkey carcasses but it was not observed over control carcasses. Fly larvae were seen emerging from the abdomen to surrounding area of the carcasses. Close examination of the BOD showed that it consisted of internal tissues and dead insect material. Other products of decomposition were also noted but not identified. The stench from both rotting animal groups was strong. The body temperatures of carcasses in both groups were elevated and this was related to increased larval activity.

At the later stage of active decay, most of the flesh on the head and body were consumed by the larvae. The decaying carcasses from both groups were now dominated by *A. rufifacies* and *C. megacephala* larvae. Other arthropods found were the predators, *Componotus* sp. and *Solenopsis* spp.

Advanced decay stage

Most of the flesh had disappeared with some soft tissues remaining on the feet area. The foul odor had begun to fade. Reduced larval activity on the carcasses was observed and the body temperature declined below the ambient temperature. Similar observations were obtained for both the control and test groups. We also noticed that the majority of fly larvae masses had started to migrate from carcasses to drier places.

The control carcasses underwent advanced decay stage between 48 to 78 hours after exposure. The gasoline-ingested monkey carcasses showed a longer period of advanced decay stage, which occurred between 87 to 126 hours after exposure. These phenomena might be due to the wet surrounding caused by BOD on the carcass. The first beetle (Coleoptera) was found on one of the control carcasses and it was identified as rove beetle (Staphylinidae). At this stage, sarcophagids and *Chrysomya* spp. were the only adult flies seen on the test carcasses.

Dry and remains stage

Dry and remains stages on control carcasses started after 78 hours after exposure while gasoline-ingested monkey carcasses was after 126 hours after exposure. Pupae of *A. rufifacies* and *C. megacephala* were found from the remains of both groups. However, the pupae from test carcasses were collected about 2m apart from the carcasses. BOD from the carcasses had now dried and mixed with the soil at the end of this stage.

There were fewer numbers of adult blow flies and flesh flies on both carcasses. On control carcasses, another group of

beetle had appeared namely hister beetle (Histeridae), along with rove beetle and other predators such as Hymenoptera (*Componotus* spp. and *Solenopsis* spp.), spider and wasp. No coleopteran was found on gasoline-ingested carcasses. Only a few long-legged fly (Dolicopodidae) and ants (*Solenopsis* spp. and *Componotus* sp.) were found at this stage. It was observed that third instars and pupae were carried away by ants.

DISCUSSION

During the decaying process, we observed all five stages of decomposition: fresh, bloated, active decay, advanced decay and dry remains. Decomposition process of control carcasses was faster in comparison with the gasoline-ingested carcasses. The appearance of first instar larvae were observed at 9 hours after exposure on control carcasses compared to 15 hours exposure on gasoline-ingested monkey carcasses. The increase of body temperature on test carcasses was also delayed compared to control carcasses. From this study, we suspected that the presence of gasoline or gasoline odor on the carcasses had led to the delay of insect invasion, which was attributed to longer duration of decomposition process. It significantly slowed the decomposition rates of carcasses containing gasoline as compared to normal decomposition (control carcass group).

The rate of decomposition process in this study occurred faster as compared to the studies reported from other countries. Previous research had indicated that the stages of decomposition in Brazil were much longer compared to Malaysia (Centeno *et al.*, 2002). The research showed that complete decomposition in Brazil was about 73 days and its duration can vary between seasons. A decomposition study in Colombia showed longer period for decomposition in which it took 83 days (Martinez *et al.*, 2007). Based on these variations, it can be concluded that rate of decomposition is a localized event and very much influenced by variable temperature condition and surrounding fauna. Due to an absence of a definite season in Malaysia, it is very likely that the rate of decomposition is nearly similar throughout the year.

Decomposition rates bear a direct relationship to the successional pattern of carrion frequenting insects. Larvae of blowflies, *A. rufifacies* and *C. megacephala* were the most dominant carcass processor of both groups throughout the decaying process. Blowfly species differ in their abundance from region to region, from habitat to habitat and from season to season. Research have shown that the larvae of the blowfly especially *A. rufifacies* and *C. megacephala* were predominantly recovered from 76.2% human corpses in Malaysia (Lee, 1989), corresponding with that reported from Thailand (Sukontason *et al.* 2007). Both blow flies species have also been reported to be the most common species found in cadavers and carcasses from different ecological habitats in Malaysia (Omar *et al.*, 1994; Hamid *et al.*, 2003; Lee *et al.*, 2004) and Thailand (Sukontason *et al.* 2007).

A number of carcass-frequenting insects had been reported in Malaysia (Lee, 1989; Omar *et al.*, 1994; Hamid *et al.*, 2003; Lee *et al.*, 2004; Heo *et al.*, 2007). In this study, we first reported the adult blowfly *H. fummipenis*, scuttle fly (*Megaselia scalaris*), picture-winged fly (Otitidae) and long-legged fly (Dolicopodidae) collected from monkey carcasses in this country. However, *M. scalaris* is the only fly that has been defined as a fly of forensic importance in many part of the world (Sukontason *et al.* 2001; Campobasso, 2004; Leccese, 2004; Disney, 2008; Kumara *et al.*, 2010).

From our observations, no larvae of *H. fummipenis* were collected from the monkey carcasses in this study; only swarming adults were seen at the control carcass of the fresh stage of decomposition. In this regard, forensic importance of this

fly species is still uncertain. Ants such as *Componotus* spp. and *Solenopsis* spp. were the most dominant predators for *Chrysomya* spp. larvae on carcasses. The presence of blister beetles and rove beetles were only found on the control carcasses as some of these beetles were not attracted to wet surroundings as in gasoline-ingested carcasses.

We conclude that the ingestion of gasoline on monkey carcasses was responsible in delaying the invasion process of necrophagous insects and thus slowing down the decomposition rate of the carcasses up to 6 hours. Future studies of forensic entomotoxicology should be conducted by using different types of poison to obtain a complete data on rate of decomposition and faunal succession.

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