DIVERSITY OF COPROPHILIC DIPTERA ASSOCIATED WITH BUFFALO DUNG IN SELANGOR, MALAYSIA

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ABSTRACT

Dung flies and blood-sucking flies are known as vectors of pathogens to human and animal worldwide. However, little is known about dipteran biodiversity associated with animal dung (e.g., buffalo, horse, cattle) in Malaysia, especially those are important in public health and zoonosis. Thus, field trips to a buffalo (*Bubalus bubalis* (L.)) farm located in Beranang, Selangor was conducted in March-April 2017. We examined adults and larvae of dipterans associated with 140 buffalo dung pats. A total of 2,775 arthropod specimens from 15 families were collected including Sepsidae, Lauxaniidae, Heleomyzidae, Calliphoridae, Dolichopodidae, Muscidae, Sarcophagidae and others. The scavenger fly (Sepsidae: *Allosepsis* sp.) was the most abundant dipteran followed by lauxaniids and heleomyzids. Eleven species of muscids were collected from the buffalo farm and three of them were haematophagic namely *Musca conducens* Walker, *Musca crassirostris* Stein, and *Stomoxys calcitrans* (Linnaeus). The biodiversity of Diptera associated with buffalo dung was briefly compared with previous animal dung studies in Malaysia.

Keywords: buffalo dung, Diptera, coprophilic, hematophagy, zoonosis, Malaysia

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INTRODUCTION

Arthropods especially insects, play a major role in the ecosystem functions such as nutrient recycling, plant propagation, maintenance of plant community composition and structure and maintenance of animal community structure (Gullan & Cranston, 2009). However, some insects are carriers for pathogens of humans and animals, and the term zoonosis indicates diseases that can be transmitted to human from animals and most of them are vector-borne diseases (Eldridge & Edman, 2004). The major insect vectors are the members of the Order Diptera, for example, mosquitoes, which are the insects responsible for deadly diseases such as malaria and dengue (Service, 2012).

Diptera is one of the largest orders of Insecta (Triplehorn & Johnson, 2005) and it is one of the contributors that may transmit diseases from a source to a recipient as they often come in contact with pathogenic fungi, bacteria, viruses, protozoa and metazoan parasites (Grabovac & Petrić, 2003). Other than playing a role as mechanical and biological vectors, some larvae of Diptera species is of coprophagous, necrophagous or myiasis-producing in human and animal tissues (Hall & Wall, 1995).

Myiasis is defined as the invasion of fly larvae on living host animal. The facultative myiasis-causing flies are generally capable of affecting living hosts and it usually develops in carrion or faeces (James, 1947) while the obligatory myiasis-causing flies involves fly species whose larvae are completely parasitic in nature and they require a living host to complete their life cycle, such examples include *Cochliomyia hominivorax* and *Chrysomya bezziana* (Robbins & Khachemoune, 2010). Necrophagous is the species of which the immature stages of a fly (i.e., maggot) feed on dead tissues and therefore considered as forensic importance (Catts & Goff, 1992). On the other hand, coprophagous species are the flies which ingest animals' faeces (Capinera, 2010). The Diptera families that performed coprophagous include Muscidae, Scathophagidae, Sepsidae, Sphaeroceridae and others (Hanski & Cambefort, 2014) while some members of Calliphoridae, Sarcophagidae, Muscidae, Fanniidae, Phoridae, Piophilidae, Stratiomyiidae, Syrphidae are necrophagous (Greenberg, 1991; Catts & Goff, 1992). Many members of coprophagous dipterans are also considered as pest insects to the livestock industry as they cause many economic and veterinary related problems such as low productivity, injury, infection, and annoyance to the animals (Bowman, 2014).

In Malaysia, medically important bacteria, parasites, and viruses (Rotavirus) have been isolated from house flies (*Musca domestica*) and other cyclorrhaphan flies (Tan *et al*., 1997; Nazni *et al*., 2005). Previous studies on coprophilic Diptera associated with cattle and horse dung has been conducted and species of veterinary important Diptera have been identified. These species include *Stomoxys calcitrans, Musca conduces, Musca crassirostris, Musca inferior*, *Musca ventrosa*, and *Tabanus* spp. (Heo *et al*., 2010, 2015). However, little is known for veterinary important dipterans associated with buffalo dung in Malaysia. Thus, there is a need to document the biodiversity of Diptera associated with buffalo dung, especially the blood-sucking species.

The objectives of the present study were to examine the diversity and abundance of coprophilic flies and other veterinary important arthropods encountered around the dung pats of Murrah milking buffalo, *Bubalus bubalis* in a buffalo farm in Malaysia.

MATERIALS AND METHODS

Study site

The study was conducted in a buffalo farm (mainly for dairy production) which housed about 100 individuals of Murrah breed milking buffalo, *Bubalus bubalis* (L.) at Beranang, Selangor, Malaysia (2°54'11" N 101°52'59" E, 56 m a.m.s.l.). The distant to the study site was approximately 33.94 km south-east from the capital city, Kuala Lumpur. The study site was within the vicinity of an oil palm (*Elaeis guineensis* Jacq.) plantation (Figure 1). There was a freshwater fishing pond located 50 m away from the study site. The canopy of palm trees was able to create a shady environment but allow sufficient sunlight to penetrate to the ground which allows wild vegetation to flourish. There were man-made walking paths between rows of palm trees. The buffalos were reared in a barn; however, the buffalos were released to the oil palm plantation area at certain duration within a day and they were expected to come back to the barn at certain hours.

Figure 1 The surrounding environment of the study site in Beranang, Ulu Langat, Selangor, Malaysia. The study site was within the vicinity of an oil palm plantation

Sample collection

A total of five arranged visits were conducted at the field site over three weeks (from 27 March to 19 April 2017). The time of insect collection was from 11 am to 4 pm (the duration where most of the flies were biologically active). A total of 140 buffalo dung pats were examined externally and internally by observations aided with a pair of forceps. Samples of arthropods observed on and inside the dung pats were collected for further identification and analysis.

Adult flies, beetles as well as other arthropods were observed, recorded and collected from buffalo dung pats. The adult flies were collected using sweep nets or clear plastic bags and the adult insects were killed subsequently in a killing jar containing cotton balls soaked with ethyl acetate (99.8% purity). The insect specimens were brought back and processed at the Parasitology Laboratory, Institute of Medical Molecular Biotechnology (IMMB), Faculty of Medicine, Universiti Teknologi MARA, Sungai Buloh Campus. The adult insect specimens were then pinned, labelled, and dried in an oven at 40ºC for three consecutive days. After that, dried specimens were kept in insect boxes for long term storage.

The Diptera larvae recovered from buffalo dungs were collected using a pair of forceps. Some of the dipteran larvae were placed in vials containing 70% ethanol for preservation. Another subset of dipteran larvae was placed in a clear plastic container (diameter = 12.0 cm; height =

4.3 cm) along with small amounts of buffalo dung which served as a larval food source for rearing process. Several tiny holes were made on the coverlid of the container to allow airflow and respiration. Small amounts of water were added *ad libitum* into the rearing container to maintain dung moisture. The emerged adult flies from pupae reared in the container were then killed using similar methods stated above and proceeded with identification.

Taxonomic identification

The identification of insects to Family level was conducted using the keys of Triplehorn & Johnson (2005) while for Calliphoridae, the identification was performed using Kurahashi *et al*. (1997). Some of the representatives of the families Calliphoridae, Muscidae and Sarcophagidae were sent to the second author for further identification and confirmation. Other Diptera and Coleoptera specimens were identified by taxonomists from related fields.

Data analysis

The data collected was analysed for ecological indices such as Dominance, Species Richness, Simpson's Index, Shannon-Wiener Index, Evenness and Effective Number of Species (ENS). The formula for Dominance (D_i) is:

$$D_i = \frac{n_i}{N} \times 100$$

Where,
n_i - The number of individual collected during the sample collection
N- The total number of specimen collected.

The dominance of the sample collected is classified using Tischler's scale found in Franin *et al*. (2014) and Heo (2015) (Table 1). The total of different species present in the sample is classified as Species richness (S) while the Simpson Index (D) and Shannon-Wiener Index (H') measures the richness and proportion for each species and design on the following formula:

$$D = \sum_{i=1}^{S} P_i^2$$

$$H' = -\sum_{i=0}^{n} P_i(\ln P_i)$$

Where:
S- Species richness
P_i - The proportion of species *i*.

Evenness (E) represents the similarity in the abundance of different species. Evenness is calculated using the formula:

$$E = \frac{H'}{\ln S}$$

Where:
H'- Shannon-Wiener Index
ln S- Natural log of species richness

Evenness is expressed on the scale 0 to 1. When the measurement of Evenness approaches to 0, it indicates more variation in the sample collected while if its approaches to 1, it means that the sample collected has a complete evenness.

Biodiversity index is used to describe the amount of species diversity in a given area. It ranges from 0 to 1 where the closer to 1 indicates the higher diversity. The Biodiversity Index is obtained by dividing the number of species in the area (S) with the total number of individuals in the area (N) as follow:

$$Biodiversity\ Index = \frac{S}{N}$$

Effective Number of Species (ENS) is the number of equally abundant species required to produce a meaningful observe value. The unit of ENS is the number of species and thus allows for a direct comparison with the diversity profile (Jost, 2006). The formula for ENS is

$$ENS = EXP\ (H')$$

Table 1 Tischler's scale for species dominance (Tischler, 1949)

Symbol	Description	Range
E	Eudominant	10% - 100%
D	Dominant	5% - 10%
Sd	Subdominant	2% - 5%
R	Recedent	1% - 2%
Sr	Subrecedent	< 1%

RESULTS

From the data collected, a total of 2,775 arthropod specimens were collected from 140 buffalo dungs with the Order Diptera being the dominant Order followed by the Order Coleoptera. Other orders (i.e., Hemiptera, Hymenoptera, Orthoptera, Dermaptera, Scorpiones and Acari) have a total of one occurrence which indicates that it was an incident or adventitious visitor (Table 2).

A total of 2,557 dipteran specimens from 14 families and one unidentified family (due to a damaged specimen) were collected from the buffalo farm. The most abundance Diptera family was Sepsidae (64.96%), followed by Lauxaniidae (12.05%), Heleomyzidae (7.67%), Calliphoridae (6.73%), Dolichopodidae (4.42%), Muscidae (2.78%), Sarcophagidae (0.43%), Platystomatidae (0.31%), Ulidiidae (0.20%), Dryomyzidae (0.16%), Stratiomyidae (0.12%), Syrphidae (0.08%) while Asilidae and Tephritidae both have the abundance of 0.04% each (Figure 2). Tabanidae was collected from the body of the animals but it was not included in the analysis as it was not obtained from the dung of the buffalos. Ecological indices according to Family are presented in Table 3 where both Sepsidae and Lauxaniidae were the eudominant families in this study.

Table 2 Ecological indices by arthropod's Order collected from 140 buffalo dungs at Beranang, Selangor, Malaysia

Arthropod / Insect Order	Abundant	Dominance	Tischler's scale
Diptera	2,557	92.14	Eudominant
Coleoptera	210	7.57	Dominant
Hemiptera	1	0.04	Subrecedent
Hymenoptera	1	0.04	Subrecedent
Orthoptera	1	0.04	Subrecedent
Scorpiones	1	0.04	Subrecedent
Dermaptera	1	0.04	Subrecedent
Mites (Acari: Acariformes)	3	0.11	Subrecedent
Ecological Index		**Value**	
Simpson		0.854783	
Shannon		0.292399	
Richness		8	
Evenness		0.140614	
Biodiversity Index		0.002	
Effective Number of Species		1	

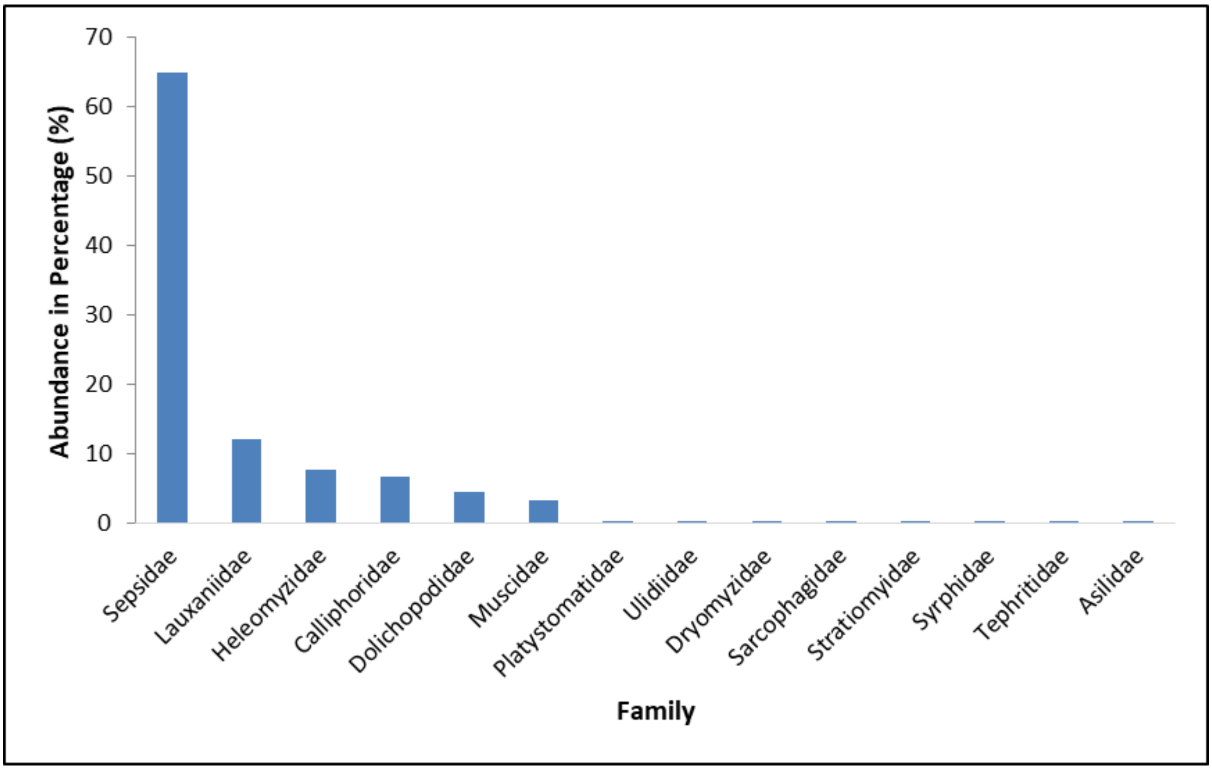

Figure 2 The abundance of Diptera according to family in percentage (%) collected from the buffalo dung in Beranang, Selangor, Malaysia

Table 3 Ecological indices by Dipteral Family collected from 140 buffalo dungs at Beranang, Selangor, Malaysia

Family	**Number**	**Dominance**	**Tischler scale**
Sepsidae	1,661	64.96	Eudominant
Lauxaniidae	308	12.05	Eudominant
Heleomyzidae	196	7.67	Dominant
Calliphoridae	170	6.65	Dominant
Dolichopodidae	113	4.42	Subdominant
Muscidae	82	3.21	Subdominant
Platystomatidae	8	0.31	Subrecedent
Ulidiidae	5	0.20	Subrecedent
Dryomyzidae	4	0.16	Subrecedent
Sarcophagidae	3	0.12	Subrecedent
Stratiomyidae	3	0.12	Subrecedent
Syrphidae	2	0.08	Subrecedent
Tephritidae	1	0.04	Subrecedent
Asilidae	1	0.04	Subrecedent
Ecological index		**Value**	
Simpson		0.449637	
Shannon		1.237482	
Richness		15	
Evenness		0.446327	
Biodiversity Index		0.005866	
Effect Number of Species		3	

As for Diptera species identification, a total of 11 species in Muscidae, five species of Calliphoridae and two species in Sarcophagidae were identified (Table 4). *Neomyia lauta* (Wiedemann) was the most dominant (29.3%) species in Muscidae, followed by *Myospila tarsalis* (Malloch) (18.3%) and others. There were three different blood-sucking muscids collected in this study namely *Musca crossirostris* Stein, *Musca conducens* Walker and *Stomoxys calcitrans* (L.) (all have a similar abundant percentage, 6.10%). Note that *Tabanus* sp. (Diptera: Tabanidae) (a member of another blood-sucking family) was also collected in this study. Furthermore, four different species of *Neomyia* (i.e., *Neomyia lauta* (Wiedemann), *Neomyia coeruleifrons* (Macquart), *Neomyia timorensis* (Robineau-Desvoidy), and *Neomyia indica* (Robineau-Desvoidy)) were recorded at this farm. There were three unidentified *Musca* specimens collected at buffalo dung (currently identified as *Musca* sp. nr. *pattoni*) (Figure 3). Further collection of Diptera fauna at the buffalo farm is needed to confirm its identity.

Table 4 Biodiversity of Dipteral species according to family collected from 140 buffalo dungs at Beranang, Selangor, Malaysia

Family	**Species**
Sepsidae	*Allosepsis* sp.
Calliphoridae	*Chrysomya megacephala* (Fabricius, 1794) *Chrysomya rufifacies* (Macquart, 1843) *Hemipyrellia ligurriens* (Wiedemann, 1830) *Hemipyrellia tagaliana* (Bigot, 1877) *Isomyia viridaurea* (Wiedemann, 1819)
Muscidae	*Brontaea ascendens* (Stein, 1915) *Morellia biseta* van Emden, 1965 *Musca crassirostris* Stein, 1903 *Musca conducens* (Villeneuvi, 1922) *Musca* sp. nr. *pattoni* *Myospila tarsalis* (Malloch, 1935) *Neomyia coeruleifrons* (Macquart, 1851) *Neomyia indica* Robineau-Desvoidy, 1830 *Neomyia lauta* Wiedemann, 1830 *Neomyia timorensis* (Robineau-Desvoidy, 1830) *Stomoxys calcitrans* (Linnaeus, 1758)
Sarcophagidae	*Parasarcophaga misera* (Walker, 1849) *Seniorwhitea princeps* (Wiedemann, 1830)
Lauxaniidae, Heleomyzidae, Dolichopodidae, Platystomatidae, Ulidiidae, Dryomyzidae, Stratiomyidae, Syrphidae, Tephritidae, Asilidae, Tabanidae	Unidentified species

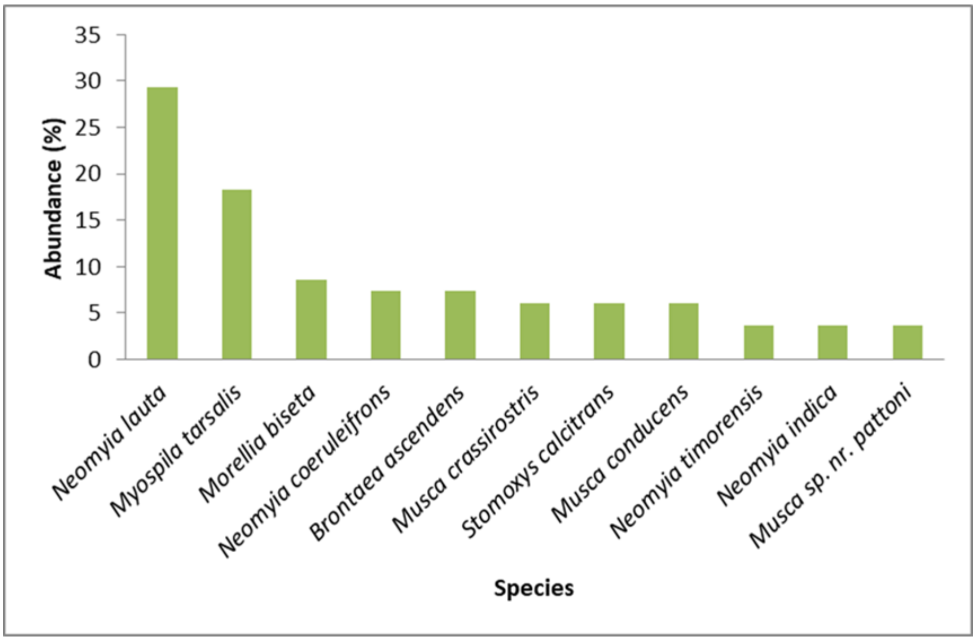

Figure 3 The abundance of Muscidae according to species in percentage (%) collected from the buffalo dung in Beranang, Selangor, Malaysia

Five species of Calliphoridae were identified namely *Chrysomya megacephala* (Fabricius) (75.9%), followed by *Chrysomya rufifacies* (Macquart) (14.7%), *Hemipyrellia ligurriens* (Wiedemann) (5.9%), *Hemipyrellia tagaliana* (Bigot) (3%) and *Isomyia viridaurea* (Wiedemann) (0.5%) (Figure 4). While there were two species of Sarcophagidae collected in this study namely *Parasarcophaga misera* Walker (67%) and *Seniorwhitea princeps* (Wiedemann) (33%). For the other families collected, species were not identified in this study.

For beetles residing in the buffalo dung, three families were revealed including Hydrophilidae (80%), Staphylinidae (12.4%) and Scarabaeidea (7.6%). Two beetle families were considered eudominant in this study and the observation was supported by the effective number of species. Ecological indices for beetles were shown in Table 5. Two species of Scarabaeidae were identified where *Liatongus femoratus* (Illiger) being more abundant than *Onthophagus babirusoides*.

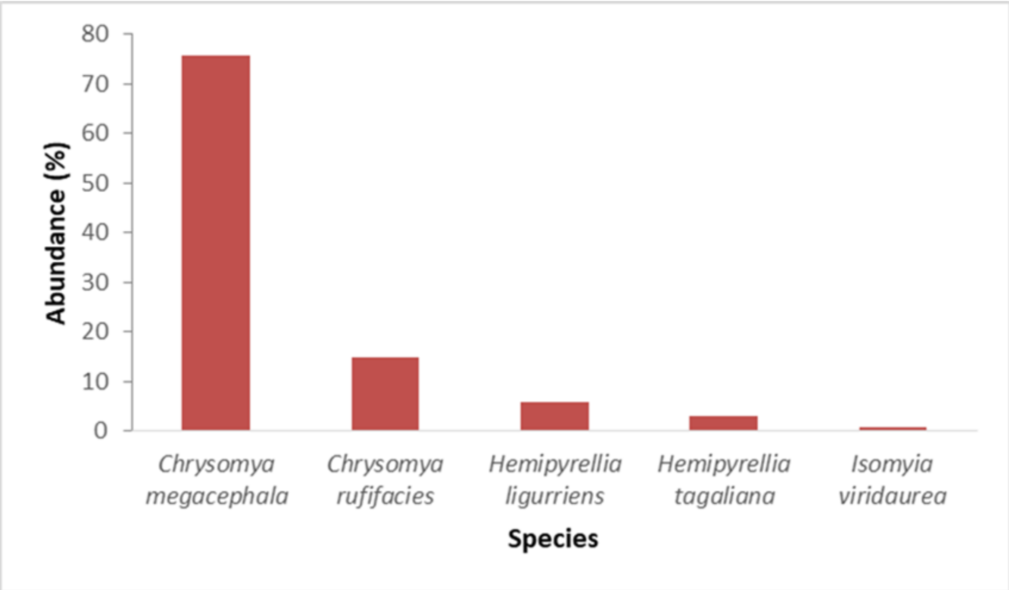

Figure 4 The abundance of Calliphoridae according to species in percentage (%) collected from the buffalo dung in Beranang, Selangor, Malaysia

Table 5 Ecological indices by beetle's Family collected from 140 buffalo dungs at Beranang, Selangor, Malaysia

Family	**Number**	**Dominance**	**Tischler scale**
Hydrophilidae	168	80.00	Eudominant
Staphylinidae	26	12.38	Eudominant
Scarabaeidae	16	7.62	Dominant
Ecological index	**Value**		
Simpson	0.661133787		
Shannon	0.633308111		
Richness	3		
Evenness	0.576461885		
Biodiversity Index	0.014285714		
Effective Number of Species	2		

Diptera larvae residing in buffalo dung were collected and reared in the laboratory until the adult stage. The emerged adult flies were later identified as *Allosepsis* sp. (Diptera: Sepsidae). Similarities and differences between the current and the previous studies on coprophilic Diptera associated with animal dung are summarised in Table 6.

Table 6 Comparison of coprophilic Diptera associated with buffalo dung with cattle and horse dung in Malaysia

Result	**Cow dung (Heo *et al*., 2010)**	**Horse dung (Heo *et al*., 2015)**	**Buffalo Dung**
Location of study	Sentul Timur, Kuala Lumpur (3°11’ N 101°41’ E)	Tanjung Rambutan, Perak (4°41’13” N 101°09’31” E)	Beranang, Selangor (2°54’11”N 101°52’59”E)
Total family collected	16	9	14
Most abundant Diptera	Sepsidae (43.8%)	Sphaeroceridae (93.8%)	Sepsidae (64.96%)
Biodiversity in Muscidae	12 species	4 species	11 species
Total blood-sucking species	6 species	4 species	3 species
Most dominant blood-sucking species	*Musca inferior*	*Musca conducens*	*Musca conducens* *Musca crassirostris* *Stomoxys calcitrans*
Percentage of blood-sucking muscid collected	51.5%	29.4%	18.3%
Larvae found in dung	*Allosepsis indica* Psychodidae *Musca inferior*	*Musca conducens* *Neomyia gavisa*	*Allosepsis* sp.

DISCUSSION

The present study found that the Sepsidae was the most abundant Diptera (~65%) associated with buffalo dung. This result is in-line with Heo *et al*. (2010) where sepsids were the most dominant dipterans on cattle dung in Sentul Timur, Kuala Lumpur. Sepsidae is dung scavenger, coprophagous and plays an important role in the dung ecosystem (Hanski, 1991; Pont & Meire, 2002). Additionally, sepsids are potentially harmful to humans because they are carrying pathogens and their close association with human (i.e., high degree of synanthropy) (Pont & Meire, 2002). Larvae of Sepsidae in the dung can serve as a food source for other predators such as dung beetles and ants (Sowig, 1995).

Three blood-sucking muscid species and at least one species of *Tabanus* were recovered in this study. These hematophagous species are considered medical and veterinary important as they could potentially transmit diseases to the animals and humans (Van Emden, 1965; Lehane 2005). *Musca conducens* is a very common blood-sucking muscid found on and around cattle and it may feed on the wounds, and is capable in transmitting filarial worms called *Stephanofilaria dedosi* Chitwood to the cattle and buffalo (Radostits *et al*., 2006). Another muscid, *M. crassirostris*, breeds in cow and horse dung (Capinera, 2008) and they are vicious biters (Patton, 1922). Furthermore, these species serve as a mechanical vector for various viruses, bacteria, protozoan and metazoan parasites (Taylor *et al*., 2016). Interestingly, there was one report on myiasis caused by this species (James, 1947). The stable fly, *S. calcitrans*, is of hematophagous and is known to vector viruses (such as equine infectious anemia, African swine fever, West Nile virus, Rift Valley viruses), rickettsia (*Anaplasma, coxiella*), and parasites (*Trypanosoma* spp., *Besnoitia* spp.) (Baldacchino *et al*., 2013). Moreover, *S. calcitrans* serve as an intermediate host for helminths *Habronema microstoma* (Schneider) and may be involved in the transmission of *Onchocerca* and *Dirofilaria* (Baldacchino *et al*., 2013).

There are non-hematophagous Muscidae were collected in this study, notably the metallic coprophilic *Neomyia* spp. These species are common on horse and cow dung and the larvae are saprophagous and coprophagous. Their larvae also serve as food sources to many carnivorous larvae residing in the dung (Skidmore, 1985). It is noteworthy to mention that thus far eight species of *Neomyia* have been recorded in Malaysia: *N. rufitarsis, N. coerulea, N. indica, N. coeruleifrons, N. lauta, N. timorensis, N. diffidens* and *N. gavisa* (Van Emden, 1965; Heo *et al*., 2010, 2015).

The oriental latrine blow flies, *C. megacephala*, and hairy maggot blow flies, *C. rufifacies*, are both facultative myiasis agents in the wounds of animal and human (Wells, 1991; Baumgartner, 1993). Besides, they both are mechanical vectors for various pathogens (Chaiwong *et al*., 2014; Sulaiman *et al*., 2000) and their larvae are necrophagous and hence considered forensically important in criminal investigations (Sukontason *et al*., 2008). *Hemipyrellia ligurriens* and *H. tagaliana* are calliphorids in the tribe Luciliini (Kurahashi *et al*., 2007). Both larvae are necrophagous and have been reported on animal carcasses and human corpses (Kumara *et al*., 2012; Silahuddin *et al*., 2015).

Other than muscid and calliphorids, adult species of Sarcophagidae are occasionally collected from animal dung. The present study collected two species namely *P. misera* and *S. princeps*. Both species can be collected from the outdoor environment (Tan *et al*., 2010) and *S. princeps* have been reported to breed on human corpses found outdoor (Kumara *et al*., 2012).

The results of the current study shared several similarities with the previous study on coprophilic Diptera associated with cattle dung as in Heo *et al*. (2010). One of the similar results obtained was the most abundant family of Diptera where both studies indicate that Sepsidae was the most dominant family collected. Also, the larvae of sepsids were recovered from both cattle and buffalo dung. The diversity in the family Muscidae was quite similar between the two studies where 12 species and 11 species were collected from cattle and buffalo dung, respectively (Heo *et al*., 2010). One possible explanation to this phenomenon could be attributed to the similar structure and chemical composition between cattle and buffalo dung. A comparative study on nutrient parameters between cattle, buffalo, white rhinoceros and horse dung revealed that cattle and buffalo dungs shared similarities in moisture percentage (82% vs 78%), pH (6.88 vs 7.42), fibre percentage (34.75 vs 35.72) as well as nitrogen content (10.27 vs 9.38) (Jankielsohn, 1998). This observation further strengthened the guild concept that similar resources attract similar consumers where this interaction can be employed to predict possible ecological communities in an ecosystem based on the resource's physical and chemical properties (Simberloff & Dayan, 1991).

It is no doubt that there are similarities between animal dung and animal carcasses as both resources are unpredictable, nutritious and of ephemeral (Finn, 2001). Rapid accumulation of animal dung (i.e., establishment of a new animal farm) or carcasses (i.e., culling of animals or sudden mass death events) may introduce a huge influx of resources into the ecosystem on that particular temporal and spatial scale. There is a possibility that the copro-saprophagous communities may not be able to process these influxes of resource pulse efficiently and may eventually lead to environmental toxicity and pollution. Unprocessed dung and carcasses allow microbial proliferation over time, and increases its chances to disseminate further into the environment, and subsequently increase the risk of disease outbreak (Tomberlin *et al*., 2017).

CONCLUSION

This study revealed that the scavenger flies (Sepsidae) were the most abundant adult Diptera associated with buffalo dung. Also, this study revealed three blood-sucking muscid species associated with buffalo dung where *M. conducens*, *M. crassirostris*, and *S. calcitrans* were equally abundant hematophagous species in this study. Fly control should be targeted on these species in order to minimise animal injury or to reduce disease transmission incurred by these blood-sucking fly species. Limitations in this study include only one study site was selected, and not all specimens can be identified to species level. From the perspective of insect ecology and geographical distribution, we recommend more biodiversity surveys and documentations on coprophilic and hematophagic Diptera to be conducted on various animal dungs in both Peninsular and Malaysian Borneo.

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