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A study of insects on decomposition stage of pig carcasses at Masarang Forest, North Sulawesi, Eastern Indonesia

Jimmy Rimbing*, Reity Engka, Erwin Kristanto and Frangky Rorong

Faculty of Agriculture, Sam Ratulangi University, Manado, Indonesia, 95115

ABSTRACT

To investigate the colonization of Insects on the pig (*Sus scrofa Linnaeus*) carcasses at Masarang forest, present study was carried out in two ways: pig carcasses weighing 35 kg are laid on the ground, and 2 kg of fresh pork pieces are hung at a height of 2.5 m. Decomposition of pig carcasses weighing 35 kg is found in the stage of fresh, bloat, decay, and skeletal. The forensic insects found in carcasses consist of 6 orders, 23 families, and 32 species. The main insects found on pig carcasses are Diptera and Coleopetara. Dominant insects consist of *Chrysomya megacephala*, *Ophyra albuquerquei*, and *Nicrophorus vespilloides*. The relative abundance of insects starts at the stage of fresh, in its peaks at the stage of decay, and decreases at the stage of skeletal. The *C. megacephala* came earlier on the pig carcasses, and it became dominant insect from the stage of fresh to decay, aw well as it can be a bioindicator for post-mortem intervals. Likewise, *N. vespilloides* become dominant and a bioindicatorat at the stage of decay. Larvae of *N. vespilloides* as predators and meat-eating necrophagous cause the stages of decomposition to be shorter. Variations in species and relative abundance of forensic insects at the stage of decomposition are in the stage of decay. The relative abundance of the highest imago of *N. vespilloides* are found on pork pieces. Staphylinidae, *Gauropterus* sp. become dominant at the stage of skeletal, and it can be used as an indicator.

Key words : Carcasses, decomposition, insects, Masarang, pigs.

INTRODUCTION

Insects can be attracted to vertebrate animals after the death of the animal to eat and lay their eggs. Forensic entomology is knowledge about insects to solve criminal problems (Kokdener, 2016; Albushabaa, 2016). Each species of the insect comes in a different time at the decomposition stages of pig carcasses. The insects' behavior can be attracted to the carcasses because of the presence of olfactory stimulus, vision, color and the presence of other insects that first come to the carcass (Fisher *et al*., 2001). There are approximately 60 families of insects playing an important role in the ecology of the carcasses. Knowledge of patterns and succession of insect's species to estimate post-mortem intervals (DeMotte and Wells, 2000; Tomberlin *et al*., 2012). Determination of the time of death is very important in determining the final situation of the victim, so that criminals can be discovered. Determination of the time of death through the approach of a forensic entomology in estimating the appropriate post-mortem interval can be used as an alternative tool in solving criminal cases. Post-mortem interval is the time between death and corpse discovery. Each species of the insect coming at the stages of the carcass can be different species, thus the insects coming at the decomposition stage of the carcass can be different as well. Succession of certain groups of insects can be used as a sign for post mortem interval estimates (Tomberlin *et al*., 2012). The primary purpose of the use of insects in the investigations of criminal case is to determine post-mortem intervals based on insect activity (Amendt *et al*., 2010; Kokdener, 2016).

The Calliphoridae was first the insects making colonization on pig carcasses. Even hours after the death of the pig, that insect had been in that place for up to two or more weeks (Amendt *et al*., 2010). Insects coming for succession on pig carcasses are influenced by climate, microclimate, geographic climate, topography, altitude, vegetation, and soil type (Al-Mesbah, 2010). Insects found in vertebrate carcasses usually indicate certain species and only live in certain areas (Goff, 2009). Knowledge about

*Corresponding author's E-mail: jimmyrimbing@gmail.com

regional insects is needed so that the post-mortem interval method can be implemented properly and correctly (Amendt *et al.*, 2010). The distribution of insect species is very helpful for obtaining information about insects in a geographical area. In Indonesia, it is still very minimal study of forensic insects involved in an ecosystem like forest ecosystems. Forensic insects are very useful in estimating post-mortem intervals from corpses and other uses such as parasitoid insects and predators that are useful in agriculture. The use of rabbits as bait forensic insects has been found in only six species in Iraq, including *Chrysomya albiceps*, *African Sarcophaga*, and *Saprinus* sp. (Albushabaa, 2016). The use of pig (*S. scrofa*) can reveal types of insects and forensic insect populations. The study examined the insects making colonization on pig carcasses, and insects are on the decomposition stages.

MATERIALS AND METHODS

Forensic research on pig carcasses has been carried out at the Masarang forest, Tomohon, North Sulawesi, Eastern Indonesia. The research site is located (07°06'79,9" N, 01°46'62,6"E), and the altitude of the place is 789 m above sea level. During the study, the air temperature at the Masarang forest was 18.440C ± 1.06 at 07.00 WITA (the central Indonesian time zone), and the humidity was 85.70% ± 5.49. Meanwhile, at daytime of 14.00 WITA, it was 27.820C ± 1.23, and the humidity was 70.12% ± 5, 82. The sample of insects' collection associated with pig carcasses was carried out at 08.30-11.00 WITA. There are two methods to get a forensic insect in the field. The first method is by placing the whole white local pig (*S. scrofa*) weighing 35 kg on the ground. Pig weighing 35 kg is used as a model of pigs' decomposition. The second method is by hanging a slice of fresh 2 kg of pork, and it will be kept for 3 days. Meanwhile, the pig weighing 35 kg is kept starting from the stage of fresh to skeletal to obtain data on insect species and populations. The local pig carcasses are placed in cages of 4 × 2.5 × 4 m in the open field. That pig is placed in cages to keep it away from the vertebrate animals as carnivore's animal. Data collection of the species and populations of the insect on pig carcasses was carried out starting from the stage of fresh to the skeletal. There are two methods in collecting sample and population of forensic insect species. The first method uses insect nets with a diameter of 35 cm, and the second method uses visual observations for 15 min. on the insects being uncaught with the nets. Forensic insects collected from insect nets are put in a bottle with 12 cm of diameter and 20 cm of height. Then, it will be killed to investigate the species and population of the insect.

Sweeping with insect nets carried out for 5 times of a double swing, and it repeated 2 times at 1 h intervals. The collection of each sample of the insect is carried out an observation on the insects' species coming to the pig carcasses, so that the sequence of forensic insects coming to the pig carcasses laid on the field is discovered. Uncaught insect by insect nets, especially Coleoptera, are then collected sufficiently based on morphology, and they were preserved with 70% alcohol for identification purposes. 0.1 kg sampling of carcasses' meat of pig weighing 35 kg was carried out for the decomposition stages. Sampling of meat to collect larvae in pork, then bred in the laboratory until it becomes an imago. 2 kg of fresh pork chop was wrapped in a fine wire, then hung on wooden poles with a height of 2.5 m from the ground. For 3 days, the pork pieces are placed in the open field. Forensic insect sampling was performed 1 time with insect nets, and visual observation was conducted on the third day. Sweeping by insect nets over the pork is carried out in 3-double swings. After the insect sampling was taken, then the 2 kg pork cut was sent to the laboratory of Faculty of Agriculture, Sam Ratulangi University, and it was put in gauze cages for 10 days to collect the data of insect larvae.

RESULTS AND DISCUSSION

Forensic insects found on pig carcasses starting from the stage of fresh to skeletal at the Masarang forest consist of Diptera, Coleoptera, Hymenoptera, Hemiptera, Blattoidea and Collembola (Table 1). The analysis of Chi-square on the relative abundance of forensic insects based on the order has a significant effect. $\chi 2 = 49.40$; df = 5; p = < 0.000. The highest relative abundance of insects is found in Diptera and Coleoptera. The dominant population abundance on carcasses is Diptera and Coleoptera (de Souza *et al.*, 2008; Grisales *et al.*, 2010; Sukchit *et al.*, 2015). Both insects are indicators for post-mortem intervals (Albushabaa, 2016; Sukchit *et al.*, 2015). Decomposition of carcasses associated with necrophagus insects as meat eaters. The main insects as decomposition of pig carcasses in the Masarang forest are Calliophoridae, Muscidae, and Silphidae (Fig. 1).

The decomposition stages of pig carcasses in this experiment consisted of the fresh stages,

Table 1. Insects associated with pigs at Masarang Forest.

Order	Family / Species	Trophic category	State of decomposition (Day period)								Population abundance	
			Fresh (0-1) day		Bl (2-5) days		Dec (6-10) days		Skeletal (11-30) days			
			L	I	L	I	L	I	L	I	A	RA (%)
Diptera	Calliophoridae											
	Chrysomya megacephala	Nec		23		76	√	58		2	159	24.16
	Chrysomya albiceps	Nec		1		3		7			11	1.67
	Chrysomya pinguis	Nec				1		3			4	0.61
	Chrysomya sp	Nec						3		1	4	0.61
	Muscidae											
	Ophyra albuquerquei	Nec				17	√	63		1	81	12.31
	Ophyra sp	Nec						4			4	0.61
	Hydrotaea sp	Nec						1		3	4	0.61
	Antherigona sp	Nec						5			5	0.75
	Sarcophagidae											
	Sarcophaga sp	Nec/Para						2			2	0.30
	Piophilidae											
	Piophila sp	Nec/Sap						7			7	1.06
	Paraphiola sp	Nec/Sap						5		4	9	1.36
	Fannidae											
	Fannia sp	Nec						2			2	0.30
	Phoridae											
	Megaselia sp	Nec/Para				5	√	20			25	3.79
	Dolichopodidae	Sap								2	2	0.30
	Stratomyiidae	Sap				2					2	0.30
	Ephrydidae	Sap				1					1	0.15
	Sepsidae	Nec						2			2	0.30
	Cecidomyiidae	Nec								1	1	0.15
	Syrphidae	Sap								1	1	0.15
Coleoptera	Silphidae											
	Nicrophorus vespilloides	Nec/Pre					168	1	4		173	26.29
	Diamesus sp	Nec/Pre					13				13	1.97
	Histeridae											
	Saprinus sp	Pre						5		3	8	1.16
	Dermestidae											
	Dermestes sp	Nec/Pre						2			2	0.30
	Staphylinidae											
	Gauropterus sp	Pre/Sap								12	12	1.82
	Scarabaeidae	Sap								3	3	0.45
Hymenoptera	Formicidae			8		2				9	19	2.88
	Oecophylla sp	Omn		22		9				15	46	6.99
	Encyrtidae	Para						2			2	0.30

contd...

Table 1 contd...

Order	Family / Species	Trophic category	State of decomposition (Day period)								Population abundance	
			Fresh (0-1) day		Bl (2-5) days		Dec (6-10) days		Skeletal (11-30) days			
			L	I	L	I	L	I	L	I	A	RA (%)
Hemiptera	Coreidae	Inch								2	2	0.30
	Pyrrocoridae	Inci								1	1	0.15
Blattodea	Blattidae	Omn		45		4					49	7.44
Colembolla	Unidentified	Sap								2	2	0.30

Informations: L=Larvae, I=Imago, Bl=Bloat, Dec=Decay, A=Abundance, RA=Relative Abundance, Necro=Necrophagous, Sap=Saprophagus, Pre=Predator, Para=Parasitoid, Omn=Omnivorous, Inci=Incidental, √= Larvae of Diptera

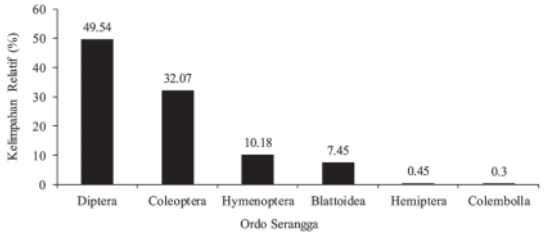

Fig. 1. Percentage of forensic insects on pig carcasses.

bloat, decay and skeletal. Al-Mesbah (2010) stated the decomposition process in rabbits consisted of 4 stages of decomposition. The analysis of Chi-square showed significant relative abundance of insects at the decomposition stages of the carcass $\chi 2 = 55.92$; df = 3; $P < 0.000$. The highest relative abundance of insects is found in the decay stage. Based on the types of insects visiting the carcass, it was dominated by Diptera and Coleoptera. Diptera are found at all stages of decomposition of pig carcass while other insects are only found at certain stages of decomposition. The fresh stage is the beginning stage of decomposition of pig carcasses, and morphologically, there has been no change and no odor. The fresh stage starts at death and ends at the beginning of the stage of bloat. The *C. megacephala* visited the pig carcasses earlier, and they are found at all stages of decomposition with a dominant population, so that it can be a bioindicator at the fresh, bloat, and decay stages. de Souza *et al*. (2008), an earlier insect found for the fresh stage was *Pheidole* sp. and *Camponatus* sp. in rabbits. Calliophoridae insects are usually the first group to arrive after death (Fisher *et al*., 2001; Sukchit *et al*., 2015).

The bloat stage begins from the bloating until the beginning of deflating the abdomen and the beginning of the decomposition process changes. The first part of the stomach becomes bloated, then the whole body becomes bloated. This stage is the beginning of decay and odor but is still around the carcasses. The odor is attractive to insects, resulting in an increase in population type and abundance compared to the fresh stage. This stage is for the most part the forensic insect spread in all parts of the body, but the dominant population is found in the ears, nose and stomach where the skin begins to detach from meat. The relative abundance of populations classified as high at the stage of bloat is *C. megacephala* and *O. albuquerquei*, so that both insects can be a clue to the post-mortem interval at the stage of bloat. Both insects have laid eggs at the stage of bloat. It has been proven by the presence of larvae at the stage of decay. Byrd and Castner (2010) stated that flies lay eggs on the carcass after the smell of the carcass and prefer to lay their eggs in the area already colonized by Calliophoridae. During the stage of bloat, it is assumed imago of *N. vespilloides* has laid their eggs. It was discovered that it was found larvae of *N. vespilloides* at the beginning of the decay stage.

The decay stage of the biomass of the carcasses seems to start to decrease until it is left behind by the bones, and it was seen that the liquid of pig carcasses falls to the ground. The main characteristic of this stage is the relative abundance of larvae of *N. vespilloides* is high. The strong odor becomes lighter after entering the skeletal stage. In addition, it was also after the decay stage of carcasses becomes black and the separation of the skin with flesh and bones. This stage has the richest insect species compared to the fresh, bloat, and skeletal stages. The decay stage is shorter, thus affecting the type and abundance of insect populations on pig carcasses. Grisales *et al*. (2010) stated the

decomposition stage of pigs weighing 25 kg was in 6-15 days. Whereas, what happened in Masarang was that the decomposition stage is short. The decay stage is the main factor influencing the increase of species and abundance of insect populations. Larvae of *N. vespilloides*, *Diamesus* sp. and *O. albuquerquei* have succession patterns occurring at the stage of decay, thus *C. megacephala* succession was at the stage of bloat and decay.

The decay stage is relatively short because of the presence of *N. vespilloides*. These insects at larval stage greedily ate pig carcasses. The larvae of *Nicrophorus* sp. greedily ate meat and survived until the stage of decay, after no meat on the carcasses; the larvae leave the carcasses (Kocarek, 2003; Matuszewski *et al.*, 2008). Population of larvae of *N. vespilloides* rapidly increases during the decay stage, but at the end of the decay stage, the *N. vespilloides* begin to decrease because the meat begins running out, resulting in a drastic decline in the population at the skeletal stage. Grisales *et al.* (2010) stated that the predatory insects Silphidae, *Anotylus* sp. and *Oxelytrum discicolle* were found on pig carcasses, but the population was very low. In winter, the decomposition of carcasses becomes longer, while in summers are shorter (Albushabaa, 2016; Sukchit *et al.*, 2015). The position of the carcasses at the Masarang forest were exposed by sunlight, and there was a rainy season which is part in accelerating the decomposition process, but the main factor influencing the decomposition stage becoming shorter was due to the presence of larvae of *N. vespilloides* at the beginning of the decay stage. The temperature and humidity factor at the Masarang forest is shown in the development and growth of insects, thus the larvae are more active in eating pig carcasses. If the temperature and humidity are not suitable for the activity, the process of larvae of *N. vespilloides* in eating the carcasses' meat will be hampered, thus the decay stage will be longer. Insects are strongly influenced by temperature and humidity, especially regarding the life cycle, development, and distribution of insects (Sarthak *et al.*, 2016).

Domination of larvae of *N. vespilloides* among forensic insects was found in the decay stage of pig carcasses, so that the species becomes a bioindicator in estimating post-mortem intervals. The presence of *N. vespilloides* has significantly reduced the process of decomposition pig carcasses at the Masarang forest, but it has a negative impact on the diversity of species and populations of forensic insects at the Masarang forest. This study should have been found on pupa of *Megaselia* sp. on the pig carcasses, but it was not. It was not found due to the presence of the predators, *N. vespilloides*, *Diamesus* sp. and *Saprinus* sp. The *N. vespilloides* and *Diamesus* sp. were as predators for eggs, larvae and small sized beetles (Sikes, 2008). Sampling of at weighing 0.1 kg at the decomposition stage was found larvae of *Megaselia* sp., *C. megacephala* and *O. albuquerquei*. The *Megaselia* sp. becoming a pupa on the surface of the pig carcasses in a laboratory. Almost all types of insects are found at the stage of decay. It was because it is a source of food and habitat for them. Succession of forensic insects generally occurs at the stage of decay. The population and types of insects start at a fresh stage and reach a peak at the decay stage. Then, it declines at the skeletal stage. Insects collected from the pig carcasses at the Masarang forest can provide a concept as a forensic insect database on corpses at the Masarang forest in relation to a legal investigation. The dominant forensic insects at the Masarang forest consist of *N. vespilloides, C. megacephala* and *O albuquerquei*. Those three insects become the main clues from the stage of fresh to decay.

The skeletal stage is characterized by the presence of bone and no more visible meat, slightly dry, and odorless. The dominant insects at the skeletal stage were Staphylinidae, *Gauropterus* sp. The *Oecophylla* sp. became dominant at this stage, but it had less effect on the decomposition of pig carcasses. The *Gauropterus* sp. on pig carcasses was only found at the end of the stage of decay and skeletal. Generally, the Staphylinidae is a predator of agricultural crop pests, so that in the agricultural crop ecosystem, to maintain and increase its population, it is through conservation by using pig carcasses bait. In addition to being a predator, the *Staphylinidae* acts as a saprophagus insect (Kocárek, 2003; Sikes, 2008). It is assumed that the *Gauropterus* sp. can survive for a long time at the skeletal stage because of the presence of organic matter and dead insects on the pig carcasses. *Gauropterus* sp. can be a bioindicator in pig carcasses at the Masarang forest at the skeletal stage. The imago of *N. vespilloides* and *Diamesus* sp. were found in a slice of 2 kg pork with relatively high abundance compared to pig carcasses placed on the ground. The relative abundance of insect populations significantly affected $\chi 2 = 125.5$; df = 5; P = < 0.000. The highest relative

Table 2. Relative abundance of the imago of forensic insect on pig carcasses weighing 2 kg.

S. No.	Order	Family	Species	Relative abundance (%)
1.	Diptera	Calliophoridae	*C. megacephala*	63.71
			C.albiceps	2.81
		Muscidae	*O. albuquerquei*	7.25
		Phoridae	*Megaselia* sp	2.89
2.	Coleoptera	SIlphidae	*N. vespilloides*	20.29
			Diamesus sp	2.89

abundance of forensic insect populations is found in *C. megacephala* (Table 2).

If the vertebrate carcasses have been separated from each other and new events occur within a few days, the imago of *N. vespilloides* and *C. megacephala* can be indicator for post-mortem intervals. An experiment of a slice of fresh pork are a clue to get the abundance of the population of imago of *N. vespilloides* and *Diamesus* sp. in forest ecosystems. Various research reports only reveal about the population of larvae of *N. vespilloides,* while the imago was not found in vertebrate carcasses. The imago prefers red, fresh pork starting to decay. The surface of meat having black color in outer of the meat and white inside is assumed to be less preferred by imago of *N. vespilloides* and *Diamesus* sp. as food. It was proven by the pig carcasses placed on the ground not attractive for the imago of *N. vespilloides and Diamesus* sp. An observation on skin of a pig carcass, it was not eaten by imago, but only the red part of the was eaten by the imago. Silphidae including the adult *Nicrophorus* sp. rarely eat rotten meat (Kocarek, 2003). It is in accordance with the experiment on pig carcasses weighing 35 kg showing that an imago of *N. vespilloides* was only found located under pig carcasses at the decay stage.

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