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CHARACTERIZATION OF SAPROPHAGE INSECTS FORENSIC ENTOMOLOGY INDICATORS USING LOCAL PIGS

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Abstract

Forensic entomology is the science of insects which is used to analyze cases related to forensics, namely death. This is based on the close relationship between humans and insects who live side by side. Insects can be a factor in determining the Postmortem Interval (PMI) so that it is useful in the forensic investigation process. Medicolegal insects are insects that are used as evidence in death cases. This research aims to obtain the diversity of insect species, the morphological characteristics of insects, the characteristics of insects in experimental pigs that died from hanging, poisoning and stabbing and based on the stage of decomposition. The results of this research examine temperature, soil pH, air humidity and the decomposition process of local pigs. The results of research on the decomposition stage in 3 deaths (hanging, stabbing, and poisoning) showed differences in the time of death, where the hanging carcass decomposed more quickly than the stabbed and poisoned carcass. In the decomposition stage, the first insect encountered is the order (Diptera) where these insects damage internal organs such as the brain and liver and also damage body cells. The temperature results of local pig carcasses placed outdoors ranged from 32.2-34.5^{0}C, while indoor carcass temperatures ranged from 26.77-33.43 ^{0}C. The degree of acidity (pH) of carcasses placed outdoors ranges from 5.13-8.53 while indoor carcasses ranges from 6.0-7.77. The environmental temperature of animal bodies placed outdoors ranges from 26.35-39,74^{0}C, while humidity ranges from 57.79-86.10%. The temperature of animal bodies in the room ranges from 27.54-31,52^{0}C, while humidity ranges from 71.50-85.37%.

Keywords: Forensic entomology, insects, decomposition

INTRODUCTION

Forensic entomology is the science of insects that is used to analyze cases related to forensics, namely death. This is based on the close relationship between humans and insects that live side by side. Insects can be one of the factors in determining the Postmortem Interval (PMI) so that it is useful in the forensic investigation process. Medikolegal insects are insects that are used as evidence in death cases. Usually related insects are meat-eating insects. The problems that exist in Indonesia, especially in the field

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of Forensics, have an impact on the improvement of technologies that are able to uncover criminal cases within the National Police. Forensics comes from the Latin forensis which means "from outside" or "public place". Forensics can be interpreted as a field of science that is used to help the process of enforcing justice through the process of applying science or forensic science plays an important role in revealing the truth in judicial cases, revealing the truth in the investigation of certain cases if needed through a bioforensic science approach (Evand H*, et al*,. 2022).

In this case, the role of forensic entomology greatly contributes to uncovering scientific evidence based on biological approaches, from the physical to molecular approaches. In Indonesia, there are many criminal cases that occur, ranging from terrorism, murder, theft or rape. Along with the number of cases, identifying the perpetrator or victim and determining the time of occurrence has always been a difficult problem for the authorities to solve. The most common biological evidence found in criminal cases is body fluids that can be in the form of blood, sperm, saliva, bones, cigarette butts, and all parts of the human body that can be used as clues in forensic DNA examinations (Kasenda Aurelie B. *et al*,. 2021).

Insects are animal organisms with the most species diversity on planet earth. As many as 3/4 of the animal species that have been successfully identified belong to the class of insects (hexapods) or known as insects. The abundant diversity of insect species is due to its short life cycle, relatively small body size, and very high adaptability. Insects also have specific food preferences. Saproba insects are a group of insects that live on various types of animal carcasses. In many research reports, insects found in animal carcasses are affected by the process of death of the animal. Animals that die killed by a sharp object prick, strangled or poisoned will have a different initial insect. However, it is necessary to study the identification of insects in animal carcasses to obtain the characteristics and diversity of species. Forensic entomology research is very important to support and complement other fields of forensic science (Hadi, *et al*,. 2022).

RESEARCH METHODS

This research was carried out from May to July 2023 with observation times in the morning, afternoon and night. The research on the process of death to decomposition was carried out in Kolongan Village, Talawaan District, North Minahasa Regency and sample identification at the Biology Laboratory of the Department of Biology, UNIMA.

Sample Taking and Preparation Techniques

Insect samples from local pigs that are in the process of decay are also samples used from different individuals and the number of samples is 3 where local pigs are used. Each sample was given different treatment.

Experimental Animals Used

The experimental animal used was a local pig *(Sus scrofa domesticus)* from Batu Village, South Likupang District, North Minahasa Regency. The indoor research uses a closed room measuring (2 x 4 x 2) m, with floor mats and Hemiptera, Aranea, and Lepidoptera. Decomposition stage of carrion indoor

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faster than outdoor. In the early stage to the decay stage, insects that came on carrion outdoor and indoor were Diptera (Calliphoridae, Tachinidae, Muscidae, and Sarcophagidae. On the post decay and skeletonization stage the insect that come were Coleoptera (Staphylinidae, Chrysomelidae, Scarabeidae, and Silphidae). A total of 3 local pigs were sacrificed their lives with different treatments. The animal carcass is placed in a glass box (40 cm x 80 cm), each animal carcass is covered with a cable as a fastener measuring (1.5 m). To avoid disturbing carnivorous animals, wire holes still allow insects to enter. The animal's body was left until bones remained.

Tool: Spear, Hook strap, Kg Scale. Material: Pig try, Potassium.

Insect Collection and Observation

Insect collection and observation are carried out day and night. Pre-adult insects (eggs, larvae, pupa, nymphs) and adult insects that do not fly by manual means are using soil and moisture measuring instruments, air temperature measuring instruments, and body temperature measuring instruments.

Insect Presence

The collected premature insects (larvae and pupa) were then processed as dried specimens using laboratory stainless steel elbow tweezers and stored in bottles containing 70% alcohol and labeled (Hadi *et al*., 2011).

Insect Identification

Identification of insects was carried out using the identification key Spradbery (2002), Amendt *et al.*, (2010), and by matching with existing specimens at the University of Alberta's Forenscis Entomology Laboratory.

Measurement of soil Temperature and pH

Animal carcasses Measurement of body temperature, soil acidity (pH) and environmental temperature is used to determine the decomposition process. Temperature measurements are taken daily during the day and night.

Data Analysis

Data on environmental temperature characteristics, duration of animal death, humidity, animal body temperature, and soil pH were tested for correlation analysis. The data on the number and type of insects are described and displayed in the form of tables and graphs.

RESULTS AND DISCUSSION

Decomposition Stage of Animal Corpses Tested by Local Pigs

The speed of decomposition of carcasses placed indoors is faster than outdoors. The process of decomposition speed in the room is greatly influenced by environmental conditions, namely temperature, environmental humidity and larval activity of members of the Order Diptera that feed on tissues without being disturbed by rain.

Types of Adult Insects Coming to the Bodies of Local Pigs

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Based on the results of monitoring around 600 adult insects on the bodies of animals outdoors. The insect consists of 6 orders, namely Diptera, Coleoptera, Hymenoptera, Hemiptera, Arachnida, and Lepidoptera. The decomposition processes of the body that can be recognized in the decay of corpses are autolysis, decay, and decomposition of skeletal bones (diagenesis). The autolysis process is a natural breakdown, the body's cells are digested by enzymes, including lipases, proteases, and carbohydrates.

Table 1. Decomposition stage of the carcass of local pigs placed indoors

Beginning of death	Bloating	Decay	Post-decay	Bone-phase
1-2 days of early *Rigor Mortis* Bulging	2-3 days of bulking Liquid discharge Smell Hair loss	3-4 days the body breaks down and breaks down Fluid discharge, bone tissue discharge gas Exit of the instrumental organ Larvae spread Remaining skin	5-6 days dry body Remaining skin and hair	7-30 days of hair and bones remaining

Table 2. Decomposition stage of local pig animal carcasses placed outdoors

Beginning of death	Bloating	Decay	Post-decay	Bone-phase
1-2 days of early *Rigor Mortis* Bulging Stiff smell	2-3 days of bulking Liquid discharge Smell Strong smell Hair loss	3-5 days of broken and damaged body Fluid discharge, bone tissue discharge gas Exit of the instrumental organ Larvae spread	6-7 days dry body Remaining skin and hair	8-30 days of hair and bones remaining

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Figure 1. Animals of pigs that were hanged, stabbed and poisoned
(Source : Prepared by the Author, 2023)

Figure 2. Animals of pigs that were hanged, stabbed and poisoned
(Source : Prepared by the Author, 2023)

This process occurs most quickly in internal organs such as the brain and liver. Sub nutrients are released that become a food source for bacteria. The process of decay is tissue damage by bacteria. This causes gases such as hydrogen sulfide, sulfur dioxide, carbon dioxide, methane, ammonia, hydrogen, and carbon dioxide to be released. Along with this occurs anaerobic fermentation when volatile propioic acid and butyrate are formed.

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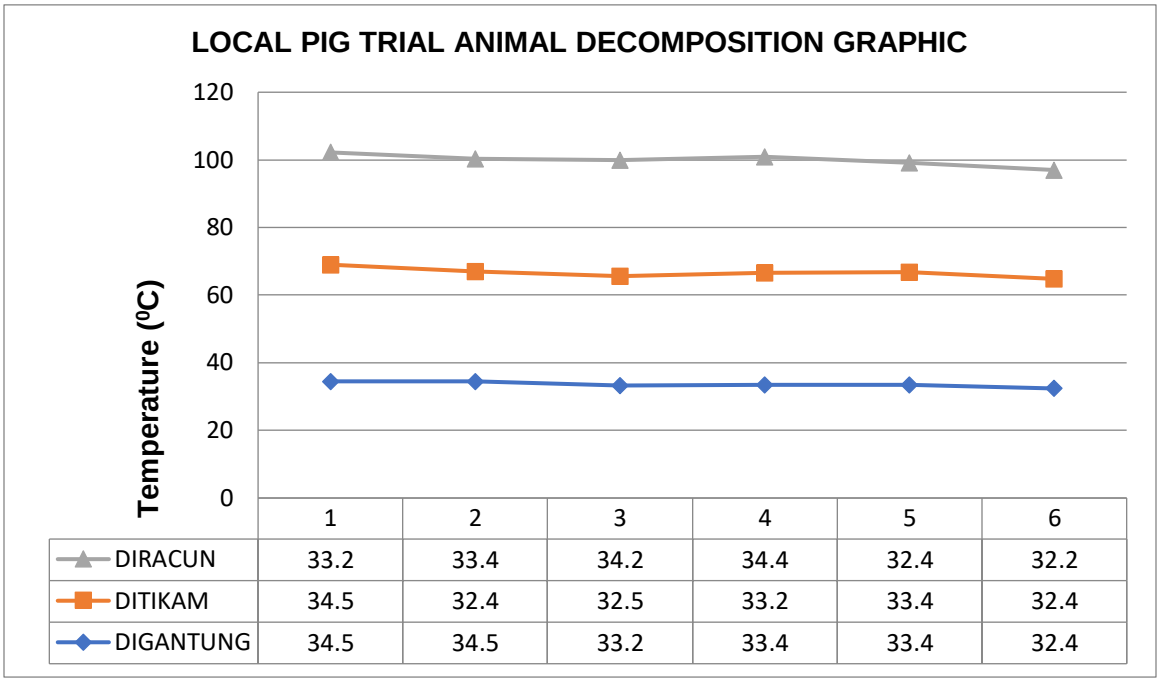

	1	2	3	4	5	6
DIRACUN	33.2	33.4	34.2	34.4	32.4	32.2
DITIKAM	34.5	32.4	32.5	33.2	33.4	32.4
DIGANTUNG	34.5	34.5	33.2	33.4	33.4	32.4

Figure 3. Decomposition of Local Pig Animals

Discussion

In this study, the existence of adult insects in the early stages of death to the decay stage was dominated by fly insects members of the Order Diptera (Calliphoridae, Muscidae, Tachinidae, Sarcophagidae) and ants who are members of the Order Hymenoptera. In the post-decay stage and the bone phase is dominated by insects from the order Coleoptera (Silphidae, Chrysomelidae, Scarabeidae, Staphylinidae) and insects from the Order Hymenoptera (Formicidae) come to the body of the animal from the beginning of death until the bone phase. Fly larvae are found from the beginning of death to the post-decay stage. At the stage of decay, the larvae of the flies form very numerous colonies and begin to spread. The larval stage (L3) at this stage begins to leave the animal's body heading into the soil crevices or cracks around the carcass and is ready to become a pupae. The number of larval colonies begins to decline after decay, this is because the amount of food begins to decrease due to the beginning of drying of the carcass. The decrease in the number of larvae in carcasses is due to competition in foraging. This competition occurs both between species and within a single species. In addition, fly eggs do not hatch due to the condition of the carcass that has dried.

The pupa stage begins to be found in the post-decay stage. The stage of C. megacephala fly eggs to hatch takes 9-10 hours. The larval stage becomes pupa and adults take 9-10 days at a temperature of 34ºC (Gurafi and Mohamed, 2015). Byrd and Castner (2010) stated that the existence of the remaining premature stages, namely larval skin, pupae skin, and peritropic membrane, is very important to know the type of insect colony that comes to the animal's body if no premature insects are found. The type of insect that comes to the animal's body is also influenced by the location and condition of the animal's body (Singh *et al*., 2016; Pastula *et al*., 2013). The activity of microbes and cells in animal carcasses greatly affects the

pH value, besides that the pH value is also affected by the activity of larvae in the animal carcass. The development of microbes in animal bodies is very influential in the decomposition time (Finley *et al*., 2015).

The acidity level in the decaying carcass will decrease and then be relatively settled. The temperature of the carcasses of local pigs placed outdoors and indoors ranged from 26.77-37.23 ^{0}C. The temperature of the animal carcasses also had a great influence on the development of premature insects. Hofer *et al*. (2017) stated that the temperature of animal carcasses greatly affects the arrival of insects and the determination of the time of death. In addition, temperature also greatly affects the time insects take to lay eggs on animal carcasses (Ody *et al*., 2017; Bugelli et al., 2017).

Environmental temperature and humidity greatly affect the microbial activity of animal and insect corpses so that it will affect the speed of decomposition of carcasses (Wang e*t al.,* 2016). The development of C. megacephala fly larvae is optimal at a temperature of 33 ^{0}C, the higher the temperature, the faster the change in the premature stage of C. megacephala fly insects. Meanwhile, Ody *et al*., (2017) stated that Calliphoridae flies will lay eggs on animal carcasses at ambient temperatures ranging from 16-35 ^{0}C. The role of insects that come to animal carcasses plays a very important role and as an indicator in determining the time of death.

CONCLUSION

The decomposition of animal bodies indoors lasts for six days, while outdoors for seven days. In the early stages of death to decay, the insect populations that come on the bodies of animals outside and indoors are the order Diptera (Calliphoridae, Tachinidae, Muscidae, and Sarcophagidae). Post-decay and bone phase : the insects that come are the order Coleoptera (Staphylinidae, Chrysomelidae, Scarabeidae, and Silphidae).Hymenoptera insects (Formicidae) come from the beginning of death to the bone phase. The estimated time of death based on insects is greatly influenced by several factors, including the cause of death and the location of the animal's body. Therefore, further research involving several variables is needed.

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