

DETERMINE THE ESTIMATED POST-MORTEM INTERVAL FROM THE DEVELOPMENT OF FLIES' LIFE CYCLE IN WISTAR RATS IN THE TOBA DISTRICT

Henry Thomson Jamiler Hutasoit¹, Panusunan Simatupang^{2,3}, Adriansyah Lubis^{2*}

¹Forensic and Medicolegal Medicine Study Program, Faculty of Medicine
University of North Sumatra, Medan, Indonesia

²Department of Forensic Medicine and Medicolegal Sciences, Faculty of Medicine, University of North Sumatra, Medan, Indonesia

³Porsea Regional General Hospital, Toba Regency, North Sumatra, Indonesia
Email: Julipasaribu85@gmail.com

ABSTRACT

Flies are the most important species in forensics, the length of death can be determined by identifying changes that occur such as identifying flies found on corpses. The time between flies laying eggs and forming a developmental stage can be used to estimate the time of death. Larval development varies from place to place depending on weather, temperature, and humidity, and so far this research on larval development has never been carried out in Toba Regency. This study used descriptive observational research with a prospective study design. Variables were measured at the same time as the research took place. The study used primary data to determine the genus and length of fly larvae on the carcass of Wistar rats, using 7 (seven) samples of Wistar rats aged 3-4 months and male with a weight of 150-250 grams. This study aimed to obtain data on the development of fly larvae and their relationship to the length of death in Porsea, Toba district. The relationship. From the study results, the development of the life cycle of flies in Porsea District, Toba Regency, namely after a few hours the flies landed on dead mice, and eggs were found in the mice after a few hours to 24 hours. Estimated time of death for one to two days, found first instar larvae with a size of 2 mm - 4.5 mm. Estimated duration of death of two to three days found second instar larvae with a size of 5.2 mm - 13.2 mm. Estimated time of death of four to seven days found in third instar larvae with a size of 11 mm - 20.1 mm. Estimated duration of death of eight to nine days found prepupa with a size of 8.8 mm - 12.4 mm. The estimated length of death is ten to thirteen days pupae with a size of 6.5 mm to 12 mm. Flies are found after thirteen to fourteen days. The conclusion from the study: The estimated length of death on average in Toba Regency is 13-14 days with the identification of the type of fly found *Sarcophaga* sp. and *Chrysoma* sp.

Keywords: identification of changes, larval length time of death.

1. INTRODUCTION

The crime rate is predicted to increase, both globally and nationally. The recorded global crime rate in the form of murder in 1990 was 362,000 cases to 464,000 cases in 2017. This figure is predicted to continue to increase until 2030. Meanwhile, the crime rate in Indonesia since 2016-2018 was 357,197 cases in 2016, up to 336,652 cases in 2017, and 294,281 cases in 2018. In contrast to the national crime rate which tends to decrease, the crime rate in Toba Regency increased in 2017-2019, namely 266 cases in 2017, 372 cases in 2018, and 389 cases in 2019. This crime rate is used to see the level of crime vulnerability in a city over a certain period.

The discovery of a corpse which is occasionally reported is one of a crime victim, although it cannot always be categorized as a victim of a murder crime. To find out the exact cause of someone's death, it is necessary to identify the body. The corpse identification process can take the form of an external examination and an internal examination. The external examination consists of an insect examination carried out by a forensic entomologist. Through this examination, the type, cause, mechanism, and time of death can be determined.

The time of death can be determined by identifying the changes that occur, such as identifying flies found on a corpse. Flies are the first living creatures to land on a corpse, sometimes within minutes of death. Flies are also the most important species in forensics. Apart from that, flies are also found in large numbers so it is more likely that flies can be used to predict the time of death. The time between flies laying eggs until they form a developmental stage can be used to estimate the time of death.

Identification of fly species is done by measuring the length of the larvae. It is estimated that 36-48 hours after death, fly larvae will be visible, marking the start of the decomposition process. This method has been used in all regions or places in the world, such as the results of reports or research by Wang et al (2019), Metcalf (2019), Laksmi (2013), Sharma et al (2018), Bonacci et al. (2017), Badenhorst and Villet (2018), Manik (2018), and Pai (2007).

Factors that influence larval growth are factors that can indirectly determine the length of time of death, such as the presence or absence of free air, environmental temperature, corpse temperature, and the location of the carcass indoors or outdoors. Another determining factor is the geographic location of an area which can influence the size of the larvae and the type of fly larvae. Not only using larvae, determining the age of adult flies can also be used to determine the time of death.

Portsea is one of the sub-district cities in Toba Regency and is the second largest city after Balige (the capital of Toba Regency). With a fairly large area (37.88 km² or 1.87% of the total area of Toba Regency), the height of the area is 905 – 1,200 meters above sea level, the distance to Balige is around 19 km, geographical location (2024'-2037' North Latitude and 99003-99016 ' BT) and the population in 2019 was 14,220 people and with a population density of 375.40 people/km², this city may be free from crime.

2. METHOD

This research is research using descriptive observational research with a prospective research design. Variables are measured at the same time as the research takes place. Research using primary data to determine the genus and length of fly larvae on Wistar rat carcasses.

This research was conducted in one of the sub-districts in the Toba Regency area, namely in Portsea District. Portsea District is a sub-district city and is the second largest city after Balige. The research was carried out from July-August 2020.

The population in this study was Wistar rats. The samples in this study were Wistar rats that had been adapted for a week. There are 7 Wistar rats.

This research consists of two variables, namely independent and dependent variables. The independent variable in this study is the length of death while the dependent variable in this study is the development and type of fly. The sample used in this research must meet the inclusion and exclusion criteria. The inclusion criteria in this study were white mice that were purchased and adapted for 1 (one) week. The exclusion criteria in this study were that mice died before the research was carried out.

The way this research works is as follows:

1. Place 7 Wistar rats in a row.
2. The mice were adapted to their respective places for a week. During the week of adaptation, Wistar rats continued to be fed as usual.
3. The rat was euthanized in the normal way (neck dislocation) and given a 5 (five) cm-long incision in the right abdomen, then kept in a container and placed in the cage.
4. Wait until the flies land on the carcass and lay eggs. Observations begin at this stage.
5. If changes are visible in the sample, the larvae are measured. Measurements begin by placing the larvae in hot water and alcohol so that they die, then measuring them using millimeter paper and recording them.
6. Identify the type of fly larvae using a microscope. Identification of fly types is carried out in the laboratory.

3. Result

Characteristics of the Research Sample

The research determined a sample of 7 (seven) Wistar rats aged 2-3 months, weighing 150-250 grams and male. Maintained adaptation for 1 (one) week from 15 July 2020 to 21 July 2020, then on 22 July 2020, the cervical spine was dislocated after which the right abdomen was cut and placed in an open container, and placed in Portsea District, Toba Regency.

Characteristics of the Research Sample

The research determined a sample of 7 (seven) Wistar rats aged 2-3 months, weighing 150-250 grams and male. Maintained adaptation for 1 (one) week from 15 July 2020 to 21 July 2020, then on 22 July 2020, the cervical spine was dislocated after which the right abdomen was cut and placed in an open container, and placed in Portsea District, Toba Regency.



Pic 1. Wistar rat adaptations



Pics 2. GPS location where the research was conducted

The number of samples in this study was 7 (seven) Wistar rats aged 3 - 4 months and weighing 150-250 grams which were placed in the Porsea District area, Toba Regency.

The estimated time of death can be determined by observing the fly's life cycle from starting as an egg then becoming a first instar larva, then becoming a second instar larva, and then becoming a third instar larva, where this can be distinguished microscopically by looking at the spiracles (spiracular slits). The first instar larva is newly formed, then if you observe the slit spiracles there are two, meaning it is instar II, then you observe three spiracles and it is very clear, which means it is instar III larvae.

Then the third instar larva will develop into a prepupa then develop into a pupa and then become an adult fly. Sometimes the flies do not lay their eggs on the carcass, but the larvae hatch directly in the mother's body (ovoviviparous).

In this area, flies of the type *Sarcophaga* sp, *Chrysoma* sp, and *Chrysoma megacephala* were found. In the fly type, *Sarcophaga* sp. is found in many highland areas, similar to previous research conducted in Semarang. *Sarcophaga* sp. is very important to forensic entomology because it can be the first, or one of, the first arthropods to arrive at a corpse. The species has strong wings and can fly in bad weather when other arthropod species cannot fly. *Sarcophaga* is most often associated with corpses found at high altitudes and inside buildings (Byrd and Castner 2001).

The estimated time of death studied in Porsea District, Toba Regency was under one day, which can be determined by flies a few hours landing on dead mice, and after several hours to 24 hours, eggs were found on the mice. An estimated time of death of one to two days can be determined if first instar larvae with a size of 2 mm - 4.5 mm are found. An estimated time of death of two to three days can be determined if second instar larvae with a size of 5.2 mm - 13.2 mm are found. The estimated time of death is four to seven days if third instar larvae with a size of 11 mm - 20.1 mm are found. An estimated time of death of eight to nine days can be determined if a prepupa with a size of 8.8 mm - 12.4 mm is found. An estimated time of death of ten to thirteen days can be determined if pupae with a size of 6.5 mm to 12 mm are found. Flies were found after thirteen to fourteen days, which can also estimate the time of death. So one fly life cycle from egg to fly in the Toba Regency area takes thirteen to fourteen days.

The changes that occur in flies in the highlands take longer than in the lowlands, whereas, in previous research in Medan, which is a lowland area, the time required for changes in flies to occur in one cycle took ten to twelve days. Where this is the average, namely that larvae complete the third instar phase taking five to six days before pupating (Smith and Whitman 1992), although other research shows the duration of larvae is seven to nine days at a temperature of 25 ° C (Pape 1996)

The reason for this difference is that larvae are strongly influenced by geographic natural conditions such as temperature, humidity, and rainfall. Higher temperatures generally accelerate larval development, especially during the third instar.

In this study, temperature, humidity, and rainfall levels were not measured and we looked at the types of fly life cycles based on the species of flies that landed. So that the next research will be carried out specifically regarding the factors in this cycle.

4. Conclusion

The estimated time of death can be determined by paying attention to the development of the fly's life cycle. Based on the research results and discussion above, it can be concluded as follows:

- a) From research conducted in Porsea District, Toba Regency, the estimated time to death from one fly life development cycle on average in Toba Regency takes thirteen to fourteen days.
- b) The types of flies identified in Porsea District, Toba Regency are *Sarcophaga* sp, *Chrysoma* sp, and *Chrysoma Megacephala*

Acknowledgments

Thank you to the Head of Toba district North Sumatra and the Head of the Forensic Medicine and Medicolegal Study Program, FK USU.

References

1. Badenhorst, R., Villet, M.H. 2018. The Uses of *Chrysomya Megacephala* (Fabricius, 1794) (Diptera: Calliphoridae) in Forensic Entomology. *Forensic Sciences Research*, 3(1): 2.
2. Barnett S, A. 2002. *The Story of Rats: Their Impact on Us and Our Impact on Them* Crows Nest NSW: Allen & Unwin.
3. Bonacci, T., Vercillo, V., Benecke, M. 2017. Flies and Ants: A Forensic Entomological Neglect Case Of An Elderly Man in Calabria, Southern Italy. *Rom J Leg Med*, 25: 283.
4. Toba Regency BPS. 2020. Toba Regency in Figures 2020. Balige: CV Graphic Release.
5. BPS. 2019. Crime Statistics 2019. Jakarta: BPS.
6. Budiyanto, A., Widiatmaka, W., Sudiono, S., Winardi, T., Mun'im, A., Sidhi. 1997. *Forensic medicine*. Jakarta: Forensic Medicine Section, Faculty of Medicine, University of Indonesia.
7. Byrd, J.H., Castner, J.L. 2001. *Forensic Entomology: The Utility of Arthropods in Legal Investigations*. New York: CRC Press.
8. Cammack, J.A. 2013. *Advancing Forensic Entomology in Indoor Environments: A Comprehensive Study From Colonization to Dispersal*. Dissertation. Raleigh, North Carolina.
9. Dahlan, S. 2007. *Forensic Medicine: Guidelines for Doctors and Law Enforcement*. Semarang: Diponegoro University Publishing Agency.
10. Dorland. 2002. *Dorland Medical Dictionary*. Ed. 29. Jakarta: EGC.
11. Gennard, D.E. 2012. *Forensic Entomology*. England: Wiley-Blackwell.
12. Gunn, A. 2006. *Essential Forensic Biology*. England: Wiley-Blackwell.
13. Idris, A.M. 2013 *Practical Guidelines for Forensic Medicine*. Jakarta: Sagung Seto.
14. Ihsan, I.M., Hidayati, R., Hadi, U.K. 2016. Effect of Air Temperature on Fecundity and Preadult Development of House Flies (*Musca Domestica*). *Journal of Environmental Technology*, 17(2): 100.
15. Isfandiari, A.B., Suharto, G., Kirana LB, S.K. 2009. Differences in the genus of dead Wistar rat fly larvae in the high and lowlands of Semarang. Thesis. Semarang: Faculty of Medicine, Diponegoro University.
16. Ismail, I. 2018. Various types and activities of decomposer fly (Diptera: Cyclorrhapha) on white rat carcasses as a forensic indicator. Thesis. Bogor: IPB.
17. Kumar, R., Shiv, K. 2019. Determining Time of Death Using Forensic Entomology. *International Medico-Legal Reporter*, 2(3): 7.
18. Laksmi, A.S., Watiniasih, N.L., Junitha, I.K. 2013. Prediction of Length of Death Based on the Presence of Insects of the Genus *Lucilia* (Calliphoridae) on Mouse (*Mus Musculus*) Carcasses in Mangrove Forest Locations. *Journal of Biology*, 16(1): 1.
19. Marquez-Grant, N., Roberts, J. 2012. *Forensic Ecology Handbook*. United Kingdom: Wiley-Blackwell. 2
20. Maley, K., Komasa, L. 2003. *VET 120 Introduction to Lab Animal Science*. Val Macer.
21. Mansjoer, A. 2005. *Medical Capita Selecta*. Jakarta: Media Aesculapius.
22. Metcalf, J.L. 2019. Estimating the Postmortem Interval Using Microbes: Knowledge Gaps and A Path to Technology Adoption. *Forensic Science International: Genetics*, 38: 211.
23. Muhammad Fernando Manik, M.F. 2018. Determining the Estimated Length of Death Time from the Development of the Fly Life Cycle in Wistar Rats in the Medan City Area. Medan Thesis: USU Faculty of Medicine. 2
24. Mullen, G.R., Durden, L.A. 2019. *Medical and Veterinary Entomology*. UK: Academic Press.
25. Pai, C., Jien, M., Li, L., Cheng, Y., Yang, C. 2007. Application of Forensic Entomology to Postmortem Interval Determination of a Burned Human Corpse: A Homicide Case Report from Southern Taiwan. *Formos Med Assoc*, 106(9): 792.
26. Primahatmaja, B., Sardjono, T.W., Lestari, N. 2014. Changes in Growth Speed of *Chrysomya* sp Fly Larvae on Rat Carcasses Containing Various Levels of Morphine. *FKUB Health Magazine*, 1(4): 190.
27. Rusidi, H.A., Yulianti, K. 2019. Description of the Genus and Length of Fly Larvae on Wistar Rat Carcasses with Different Geographical Locations in Bali. *Udayana Medical Journal*, 8(9): 1.
28. Santi, D.N. 2001. *Fly Control Management*. Medan: Faculty of Medicine, University of North Sumatra.
29. Sharma, A., Bala, M., Singh, N. 2018. Five Case Studies Associated with Forensically Important Entomofauna Recovered from Human Corpses from Punjab, India. *J Forensic Sci & Criminal Investigations*, 7(5): 001.
30. Sigit, S. H., Hadi, U. K. 2006. *Indonesian Settlement Pests Introduction to Biology & Control*. Bogor: Bogor Agricultural Institute.
31. Sirois, M.. 2005. *Laboratory Animal Medicine: Principles and Procedures*. United States of America: Mosby Inc.
32. Sucipto, C. D. 2011. *Tropical Disease Vectors*. Yogyakarta: Goysen Publishing.
33. Suckow, M.A., Steven, H.W., Craig, L.F. 2006. *The Laboratory Rat*. USA: Academic Pr.
34. Suharsono, Nuryadin, E. 2019. The Effect of Temperature on the Life Cycle of Fruit Flies (*Drosophila melanogaster*). *Bioexperiments*, 5(2):114.

38. Supriyono, Soviana, S., Hadi, U.K. 2019. Patterns of Insect Arrival on Animal Bodies as an Indicator in Forensic Activities. *Veterinary Journal*, 20(3): 418.
39. Switha, E.T., Anwar, C., Dalilah, Ghiffari, A. 2019. The influence of different carcass placement locations on the growth of fly larvae in rats (*Rattus norvegicus*). *Syifa' MEDIKA*, 10(1): 46.
40. United Nations Office on Drugs and Crime. 2019. Global Study on Homicide: Executive Summary, Vienna: UNODC.
41. Wanga, M., Chu, J., Wang, Y., Li, F., Liao, M., Shi, H., Zhang, Y., Hu, G., Wang, J. 2019. Forensic Entomology Application in China: Four Case Reports. *Journal of Forensic and Legal Medicine*, 63: 40.
42. Wardani, D.P.K., Mulyanto, A. 2019. Identification of Fly Larvae for Post Mortem Intervals on Rat (*Rattus Novergicus*) Carcasses Treated with CiuOplosan at the Science Techno Park, Muhammadiyah University, Purwokerto. *Herb-Medicine Journal*, 2(1):15.
43. Wistar Institute, 2020. Our History Philadelphia: The Wistar Institute. Available at: <http://www.wistar.org>, Accessed 28 May 2020.