



**UNIVERSITI PUTRA MALAYSIA**

***ESTIMATION OF EARLY POSTMORTEM INTERVAL IN DOGS IN  
MALAYSIA***

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FPV 2015 29**

**ESTIMATION OF EARLY POSTMORTEM INTERVAL IN DOGS IN  
MALAYSIA**

**BY  
TAN WEI MIAO @ RACHEL**

**A paper presented, in partial fulfillment of requirement for the degree of  
Doctor of Veterinary Medicine, to the Faculty of Veterinary Medicine,  
Universiti Putra Malaysia**

**MARCH 2015**

It is hereby certified that we have read this project paper entitled “Estimation of Early Postmortem Interval in Dogs in Malaysia” by Tan Wei Miao @ Rachel and in our opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD4999 - Project.

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## DEDICATION

This project is dedicated to all the animals that have suffered injustice  
and  
to those that seeks justice for them.



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First and foremost, I am most grateful to both my supervisor and co-supervisor, Prof Dr. Noordin Mohamed Mustapha and Dr. Ibrahim Abdulazeez Okene for their invaluable knowledge and guidance. Despite their busy schedule, they always find time to answer my 'What, How and Why'. For that, I can never thank you.

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**LIST OF ABBREVIATIONS**

$^{\circ}\text{C}$	Degree Celsius
ATP	Adenosine triphosphate
IACUC	Institutional Animal Care and Use Committee
PMI	Postmortem interval
TPE	Temperature plateau



**ABSTRAK**

Abstrak kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 - Projek **ANGGARAN TEMPOH KEMATIAN ANJING DI MALAYSIA**

Tan Wei Miao @ Rachel

2015

Penyelia: Prof. Dr. Noordin Mohamed Mustapha

Penyelia bersama: Dr. Ibrahim Abdulazeez Okene

Sela masa pasca kematian (PMI) adalah tempoh yang berlalu dari saat kematian sehingga masa subjek ditemui. Disebalik lambakan dokumen PMI, hampir kesemua adalah data mengenai iklim sejuk. Kadar penyejukan karkas, patologi, dan entomologi telah digunakan dalam kajian ini untuk menganggarkan PMI bagi iklim tropika. Patologi luaran diperhatikan and direkodkan secara berkala. Bedah siasat dilakukan selepas 24 jam untuk memeriksa keadaan patologi dalaman. Bagi kaedah berdasarkan suhu, suhu otak, hati dan rektum diukur setiap 60 saat selama 24 jam menggunakan kuar penganding suhu. Biopsi daripada otak, hati, dan otot epaksial dikumpul pada sela 0, 12, 18, dan 24 jam PMI. Sampel tersebut diproses secara rutin untuk histologi dan dilihat bawah mikroskop. Serangga yang mengelilingi karkas dikumpul dan diletak dalam 70% alkohol etil dan dikenalpasti dibawah mikroskop-stereo. Terdapat perubahan daripada keputusan patologi dalam masa 24 jam. Kadar penyejukan suhu daripada organ-organ mendedahkan lengkuk eksponen dengan model regresi. Formula ini boleh digunakan untuk menganggarkan tempoh kematian

dalam keadaan praktikal. Serangga yang dijumpai adalah tergolong dalam Order Diptera dan spesies yang dikenalpasti termasuk *Musca domestica*, *Chrysosoma megacephala*, dan *Megaselia scalaris*.

Kata Kunci: *sela masa, patologi, kaedah berdasarkan suhu, entomologi*



## **ABSTRACT**

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of requirement for the course VPD 4999 - Project

### **ESTIMATION OF EARLY POSTMORTEM INTERVAL IN DOGS IN MALAYSIA**

Tan Wei Miao @ Rachel

2015

Supervised by: Prof. Dr. Noordin Mohamed Mustapha

Co-supervised by: Dr. Ibrahim Abdulazeez Okene

Postmortem interval (PMI) is the period that elapsed from the moment of death until the time the body is discovered. Despite substantial documentation on PMI, most were those of the temperate regions. Carcass cooling rate, pathology and entomology were used in this study to estimate the PMI under tropical conditions. Postmortem changes were observed and recorded periodically while postmortem examination was done after 24 hours. The temperature of the brain, liver and rectum were measured every 60 seconds for 24 hours using thermocouple probes. Biopsies from the brain, liver, and epaxial muscle were collected at 0, 12, 18, and 24 hour PMI. The samples were then routinely processed for histology and viewed under light microscope. Life cycle stages of flies that surrounded the carcasses were collected, placed in 70% ethyl alcohol and identified. Rate of temperature drop from the three organs revealed an exponential curve with strong regression models which

were later used to generate algebraic formulas to estimate the PMI. Likewise, these changes correlated well with normal postmortem changes occurring throughout that period. Fly species of the *Musca domestica*, *Chrysoma megacephala*, and *Megaselia scalaris* were collected from the carcasses.

Keywords: *postmortem interval, pathology, temperature-based method, entomology*





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## 1.0 INTRODUCTION

Veterinary forensic medicine is fairly a new discipline that is still in its infancy stage. It refers to the application of medical and veterinary knowledge to the interpretation of evidence to the court of law. The task of a veterinary forensic pathologist is essentially similar to the medical forensic pathologist but the major difference is the former focuses on multiple species where as the latter deals with only one species. Veterinary pathologist may be called to assist in crime scene investigations or to be expert witnesses in cases of unlawful killing, death of livestock during transportation, poaching, and cases of neglect or deliberate injury of companion animals.

At the crime scene, one of the burning question that every veterinary pathologist will face is; 'When did the animal die?'. This is because estimating the time of death of the victim narrows down the 'window of opportunity' and thereby eliminating specific events and suspects. However, it is very difficult to pinpoint the exact time of death of the animal, and so a time frame termed as postmortem interval (PMI) is used instead.

The PMI is defined as the period of time that elapsed from the moment of its death until the body is discovered. There are many factors that influence PMI and one of the most important factor is the ambient temperature at the point of moment the animal died. This is owing to decomposition rate of the body that accelerates at high ambient temperature and decelerates at low ambient temperature. That being

the case, decomposition rate during winter in temperate countries will profoundly differs from that of the hot and humid climate of the tropics. Hence, the ambient temperature will significantly affect PMI estimation of the victimised dogs.

Although there had been a lot of research being done in estimating the PMI in dogs, these studies had been focus mainly in the temperate regions such as Poland, United Kingdom, and United States. In the recent years, there have only been a handful of studies in the tropics done on estimating the PMI in dogs. In Malaysia, these was conducted by Abdulazeez and Noordin (2010) using rectal and liver temperature where as Heng *et al.* (2009) used thoracic radiography in estimating PMI in dogs. Aside from temperature based and radiography imaging, there are many other methods that could be utilized in determining PMI in dogs in Malaysia including forensic entomology, histology, and gross pathology. Therefore, there is paucity in estimating PMI in dogs in Malaysia using temperature-based, histology, gross pathology, and forensic entomology methods.

Therefore, the objectives of this study are to i) establish and map the pattern of temperature decline of the brain, liver and rectum 24 hour postmortem, ii) determine the histological changes of brain, liver, and epaxial muscle at 0 hour, 12 hour, 18 hour, and 24 hour postmortem, iii) determine gross pathological changes of overall carcass 24 hour postmortem and iv) identify the types of carrion insects present around the carcass within 24 hour postmortem.

## 2.0 LITERATURE REVIEW

### 2.1 METHODS OF ESTIMATING POSTMORTEM INTERVAL

According to Munro and Munro (2013), there are two approaches to estimating the PMI: (a) measurement of change that takes place at a known rate such as rigor mortis, cooling of the body and putrefaction and (b) comparison of the occurrence of events known to have taken place at a specific time with the time of death such as extent of digestion of last meal. All methods of estimation of time since death must demonstrate quantitative measurement and include a mathematical description (Munro & Munro, 2013).

Techniques and procedures used to estimating the PMI in mammals and birds have been outlined by Edge (1984), Erlandsson (2003), Cooper and Cooper (2007), Merck (2007), Munro and Munro (2008), Okene (2010) and Sinclair *et al.* (2006) as cited in Munro & Munro (2013). Method for the estimation of the PMI includes temperature-based methods, post mortem chemistry, electrical stimulation of muscle and nerves, gross appearance of body (includes rigor mortis, eye shape, eye colour and luminosity, decomposition), histopathology and electron microscopy, radiology, DNA and RNA analyses, entomology, and environmental and associated evidence (Munro & Munro, 2013).

### 2.2 TEMPERATURE-BASED METHOD

Temperature-based methods are considered the most reliable in practice, the easiest to implement and the most likely to be of value especially in the period of 24

to 48 hours after death (al-Alousi *et al.*, 2002). The main principle of determining PMI is a calculation of measurable data along a time-dependent curve back to the start point (Henssge & Madea, 2007).

Most of the research had been focused on human forensic casework. Henssge (1998) then developed a nomogram which was used as a standard method by forensic medicine specialist to read the time of death. In 1962, Marshall and Hoare proposed a two-exponential model which reveals that the body does not cool down according to Newton's law but slower that turned out to be of sigmoid character as cited in Kaliszan *et al.* (2009). Following that, more mathematical models have been proposed for the estimation of PMI.

Many of the existing mathematical models of the postmortem temperature decay curve perform well in linear and even single exponential regions of the curve but develop problems at the start of the cooling curve and at the end of the cooling curve (Smart & Kaliszan, 2012) due to the presence of temperature plateau (TPE). However not all of the temperature reading will show TPE. Experiments within the head of pigs, dogs, and even humans have shown a complete or only a residual TPE (Smart & Kaliszan, 2012). In other studies, TPE was only recognised in dealing with rectal temperatures (Smart & Kaliszan, 2012). According to Henssge, the presence of TPE would be dependent on several factors such as location of temperature measuring site, ambient conditions, surface insulation, size of body, and also cause of death as cited in Smart & Kaliszan (2012). However, in a study done in the tropics (average ambient temperature of 29<sup>0</sup>C) by Abdulazeez and Noordin (2010)

measuring the liver and hepatic temperatures shows irregular temperature drop pattern with no evident of TPE. In another study in beagles, TPE was not recorded in the liver and rectum (Erlandsson and Munro, 2007).

### 2.3 HISTOLOGICAL METHOD

Decomposition change is due to the autolytic and putrefactive effects on the body after death. Autolysis occurs when there is a cessation of metabolic processes that preserve the integrity of cells (Cummings *et al.*, 2011). According to Erlandsson and Munro (2007), examining the specific histological changes may help in refining the postmortem interval. However, according to Janssen (1984), it is difficult to differentiate postmortem changes and antemortem injury using light microscopy. In a study by Tomita *et al.* (2004), light microscopic changes during early postmortem period are organ specific for up to 24 hour postmortem.

### 2.4 GROSS PATHOLOGY

After an animal dies, the body undergoes a variety of changes that includes livor mortis, rigor mortis, algor mortis and decomposition. These changes are important in estimating the PMI as they occur in a regular sequence (Salam *et al.*, 2012). Livor mortis or lividity is most useful in determining the body position at time of death and whether the body has been transferred (Merck & Miller, 2013). In human forensics, lividity might be seen as early as twenty minutes to several hours after death and fixation occurs in eight to twelve hours (Merck & Miller, 2013). In a study in India with a high ambient temperature from 26.6<sup>0</sup>C to 46.5<sup>0</sup>C, complete rigor lasted for an average duration of 18 hours and disappeared by 30 hours (Dalal

*et al.*, 2006). Pink teeth can also be seen due to livor mortis. In human forensics, rigor may appear two to four hours after death and would reach complete rigor mortis in six to twelve hours depending on perimortem factors, cause of death, and the temperature conditions (Merck & Miller, 2013). However, in hotter conditions, rigor mortis in humans may disappear within 24 hours or less (Di Maio & Di Maio, 2001). According to Erlandsson and Munro (2007) in a study in beagle dogs, rigor mortis of the hind limbs and jaw remained detectable from 24 hours to seven days.

## 2.5 FORENSIC ENTOMOLOGY

Medicolegal entomology is a discipline of forensic entomology, which refers to the insects and related arthropods as the first witness in order to estimate the postmortem interval (Ahmad *et al.*, 2011). Although medicolegal entomology is primarily being used in human forensics, carrion insects feed on dead organic matter irrespective whether it is on human or animal (Anderson, 2013). Necrophagous insects or carrion are attracted to the carcass for all or part of their life cycle.

There are three main areas in which medicolegal entomology can be of value in an animal cruelty investigation: when animal is found dead inside a residence, when an animal is found dead outdoors, and when live animal is found to have been colonized by insects (Anderson, 2013). In Malaysia, flies that are of forensic importance on human corpses or animal carcasses are made up of three major families which are *Calliphoridae*, *Sarcophagidae* and *Muscidae* (Heo *et al.*, 2011). However, principally, these medicolegal insect species do not occur on the carrion at

the same time but in succession patterns and some variations are observed in different geographical area (Ahmad *et al.*, 2011).



### 3.0 MATERIALS AND METHODS

#### 3.1 SOURCE OF ANIMALS

All animal procedures were carried out in accordance of the approval by Universiti Putra Malaysia Institutional Animal Care and Use Committee (IACUC), FYP-2014/FYP.031. Three local breed dogs, weighing 12.5kg to 18kg with short hair coat were included in this study. These dogs were scheduled for humane euthanasia after utilization in a scientific research.

#### 3.2 TEMPERATURE-BASED METHOD

Following intravenous euthanasia with sodium pentobarbitone, the carcasses were placed on left lateral recumbency on the examination table in the necropsy laboratory.

Temperature of the brain, liver and rectum were recorded using three Pt100 P-series thermocouple probes connected to 2-channel continuous temperature P-670 series temperature data logger. The ambient temperature were recorded with a %rF P-series probe (the probes and data logger were obtained from Dostmann electronic GmbH, Wertheim-Reicholzheim, Germany).

The rectal probe was inserted 8 cm into the dog's rectum. The liver probe was inserted 4cm following a stab incision at a point between the ninth and tenth ribs mid-ventral of the right thoraco-abdominal wall. The brain probe was inserted at a depth of 3.5 cm through a trephined hole in the calvarium at the intersection of lines

drawn from the lateral canthus of the right eyes to base of the right ear and lateral canthus of the left eye to base of the left ear. The temperature was recorded every 60 seconds for 24 hours postmortem. The set up of the probes and data logger is as in Appendix (Plates 13-14).

### 3.3 TEMPERATURE DATA ANALYSIS

The results from the P-670 data logger were analysed with the DE-Graph® software version 1.3.3 build 144 to obtain the temperature data that were recorded. The data were then transferred to Microsoft Excel® 2013 software. From there, the data were generated into graphs to look at the cooling curve of the three organs. Based on the single exponential proposal on temperature drop pattern by Kaliszan *et al.* (2005), the ambient temperature was regarded as the singular factor that affects the fall of organ temperature. Therefore, single exponential model is chosen to represent the equation for estimating the PMI in this study.

This model was chosen because according to Kaliszan *et al.* (2005), this model correctly predicts the increasingly larger differences between the actual and the calculated time of death. Furthermore, there is no practical advantage to using a far more complex model such as the double exponential model or the triple exponential model.

The algebraic form of exponential equation is

$$y = ae^{bx}$$

The single exponential equation used in this study is shown below:

$$T - T_a = ae^{-bt}$$

where,

T = temperature of any organ at any point in time after death  
 $T_a$  = ambient temperature at time of organ temperature measured  
 a = y-intercept  
 b = coefficient of the rate constant of temperature drop of organ  
 t = time of death

From the Microsoft Excel 2013®, the differences between the organ temperature and average ambient temperature were calculated,  $T - T_a$ . The mean of the difference between the organ temperature and ambient temperature ( $\sum T - T_a$ ) was plotted against time (hour).

The exponential equations and coefficient correlation ( $r^2$ ) for each of the graph plotted were generated using Microsoft Excel 2013®. Coefficient a and coefficient b were derived from the curve fit and tabulated. The coefficient a and coefficient b were then inserted into the equations to generate the single exponential equations for each organ.

### 3.4 BIOPSY COLLECTION

For the sample collection, brain, liver, and epaxial muscle biopsies were obtained at 0 hour, 12 hour, 18 hour and 24 hour postmortem. Biopsies of the cerebral cortex of the brain were collected using a 7cm hollow metal tube with a diameter of 3mm attached to a 10ml syringe (Appendix, Plate 11). Each biopsy was made at four different sites. For liver, biopsies were obtained from four different sites at each time interval. After liver biopsies were taken, the incision was sutured using simple continuous method with nylon sutures. The epaxial muscle was biopsied using open biopsy technique and the samples were collected along the

spinal vertebrae at the designated time intervals. The samples were then processed via routine histopathology processing and stained with Haematoxylin and Eosin.

### 3.5 GROSS PATHOLOGY

The changes of the external gross post mortem changes of these carcasses were observed periodically. The main focus of observation includes rigor mortis, livor mortis, changes of the eyes and teeth. After a 24 hour lapsed, postmortem was carried out to observe the internal gross pathology focusing on thoracic and abdominal region.

### 3.6 FORENSIC ENTOMOLOGY

Insects that surrounded the carcasses were captured from time to time throughout the duration of the study period. Insects, eggs, and larvae that were present around the carcass were collected and preserve in 70% ethyl alcohol. The insects were then identified under stereomicroscope and using identification keys.

## 4.0 RESULTS AND DISCUSSION

### 4.1 TEMPERATURE-BASED DATA

#### 4.1.1 Measurement of ambient temperature

The principle of determining PMI using this method is the calculation of measurable data along a time-dependent curve back to the start point (Henssge & Madea, 2007). Figure 1 shows the plotted ambient temperature during the study period.

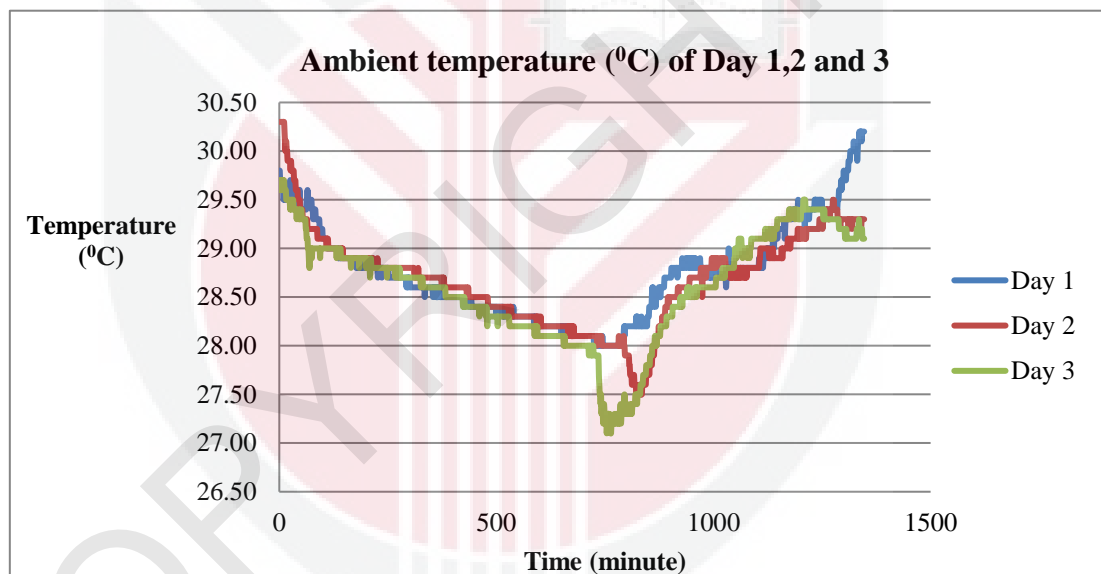


Figure 1. The ambient temperature changes during the study period.

The ambient temperature of the room drops during the night and starts to rise again during the day. Therefore, there will be fluctuation in the ambient temperature, causing variation in the calculation of the single exponential equation. Therefore, the average ambient temperature from the maximum and minimum ambient temperature is fixed as the ambient temperature,  $T_a$ . The maximum, minimum and average

temperatures were  $28.7^{\circ}\text{C} \pm 0.31^{\circ}\text{C}$ ,  $27.4^{\circ}\text{C} \pm 0.35^{\circ}\text{C}$  and  $28.7^{\circ}\text{C} \pm 0.32^{\circ}\text{C}$ , respectively. Hence, the ambient temperature,  $T_a$  is fixed at  $27^{\circ}\text{C}$ .

#### 4.1.2 Cooling curve of brain, liver, and rectum

Figures 2-5 show the cooling curve of the brain, liver and rectum and their combination, respectively.

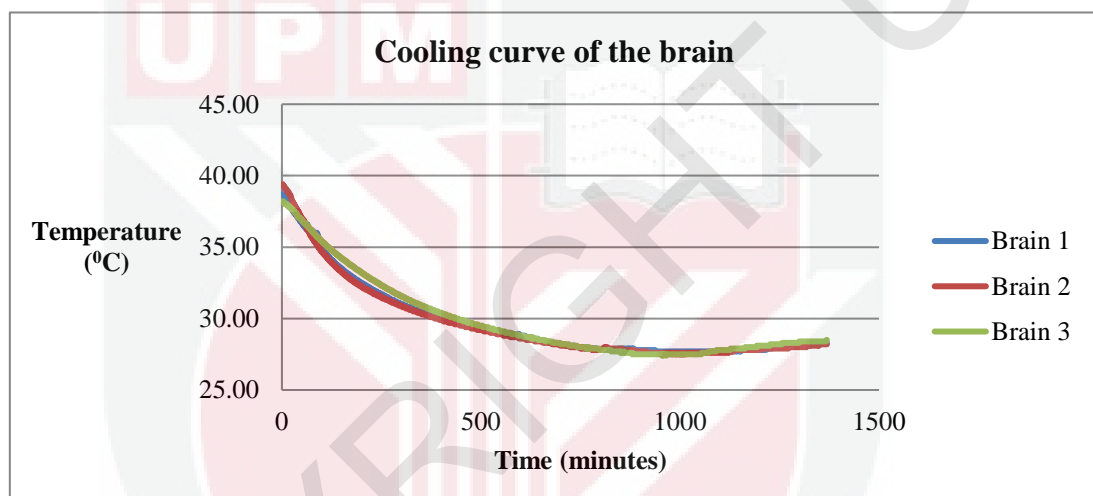


Figure 2. The cooling curve of the brain over the 24 hour period postmortem

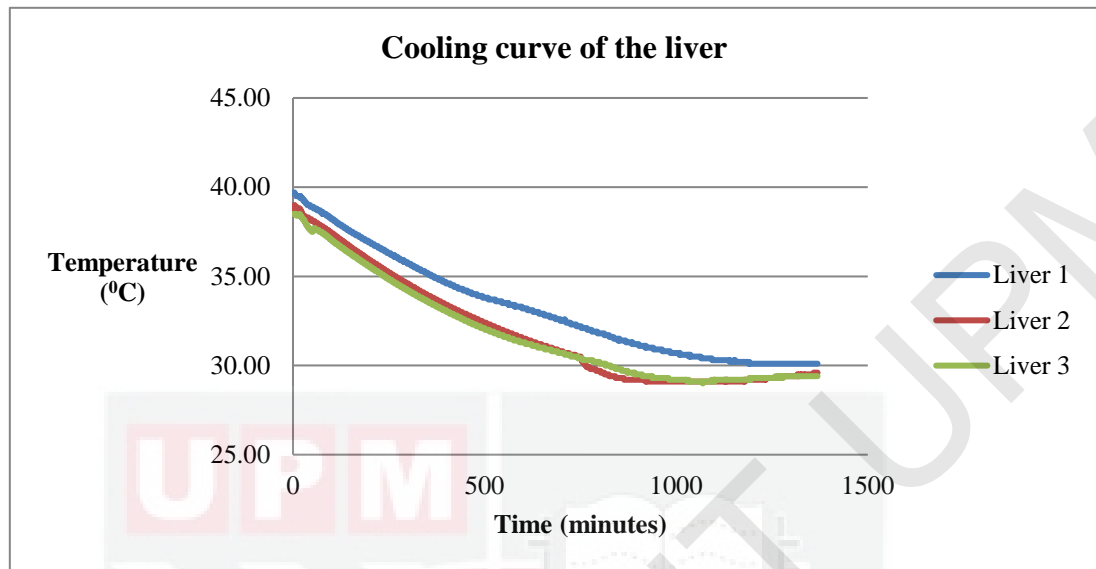


Figure 3. The cooling curve of the liver over the 24 hour period postmortem

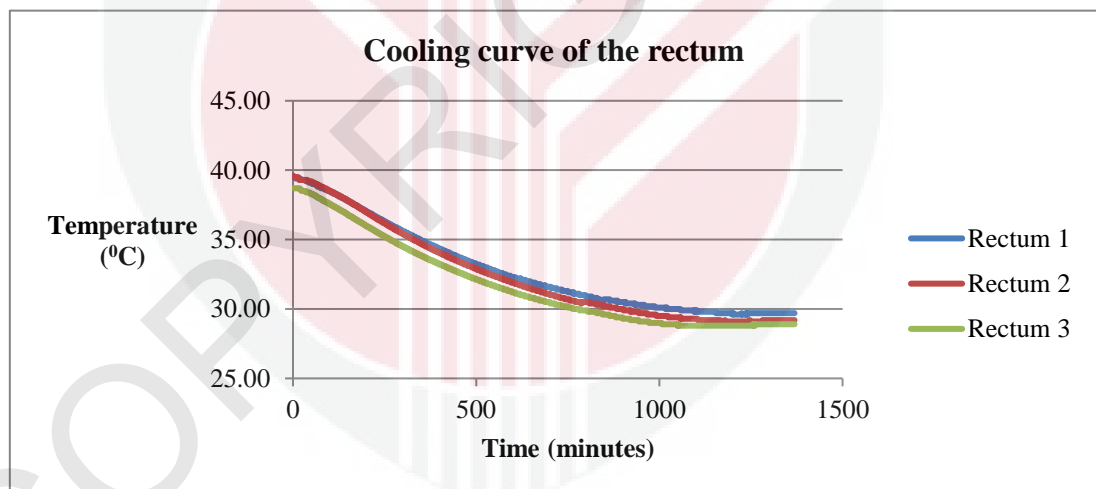


Figure 4. The cooling curve of the rectum over the 24 hour postmortem

When mapped, the cooling rate of these three organs showed a comparable pattern (Figure 5). However, the cooling pattern of the rectum in all three dogs show a short initial temperature plateau for nine to ten minutes prior to descending into a rapid and slow cooling phase leading to a sigmoidal curve phase. This finding is

similar with a study done in pigs at an ambient temperature of 19.5°C to 22.5°C by Kaliszan *et al.* (2005). Experiments with pigs, dogs, and humans had shown a complete absence or only a residual temperature plateau within the head (Smart & Kaliszan, 2012).

The occurrence of temperature plateau depends on the presence or absence of various intrinsic and extrinsic factors (Al-Alousi, 2000). Smart & Kaliszan (2012) had shown that the temperature plateau occurs due to several possibilities; anaerobic glycolysis, residual brain activity that continues after death, and also pathophysiological vital reactions.

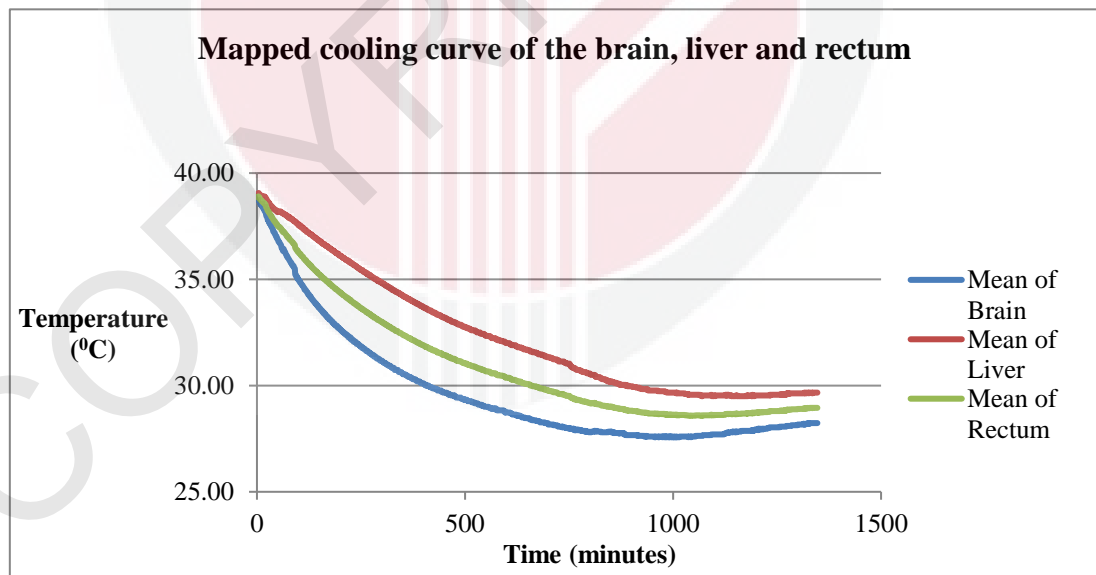


Figure 5. The cooling curve for brain, liver and rectum temperature over the 24 hour period postmortem.

Based on Figure 5, brain shows the greatest rate temperature drop whereas liver and rectum cooled at almost similar rate. Brain temperature shows the greatest rate of temperature drop, which was consistent with previous study in dogs by Proctor *et al.* (2005). This occurs as a consequence of the anatomical localization of each temperature site that differs in terms of thermal conductivities. Essentially, body is not a mass of uniform substance but rather constitutes tissues of different thermal properties that influence the cooling curve (Al-Alousi, 2002).

#### 4.1.3 Exponential curve fitting

Figures 6-8 show the  $T - T_a$ , i.e. the difference between each mean organ temperature ( $T$ ) and ambient temperature of  $27^{\circ}\text{C}$  ( $T_a$ ), was calculated and plotted for each dog.

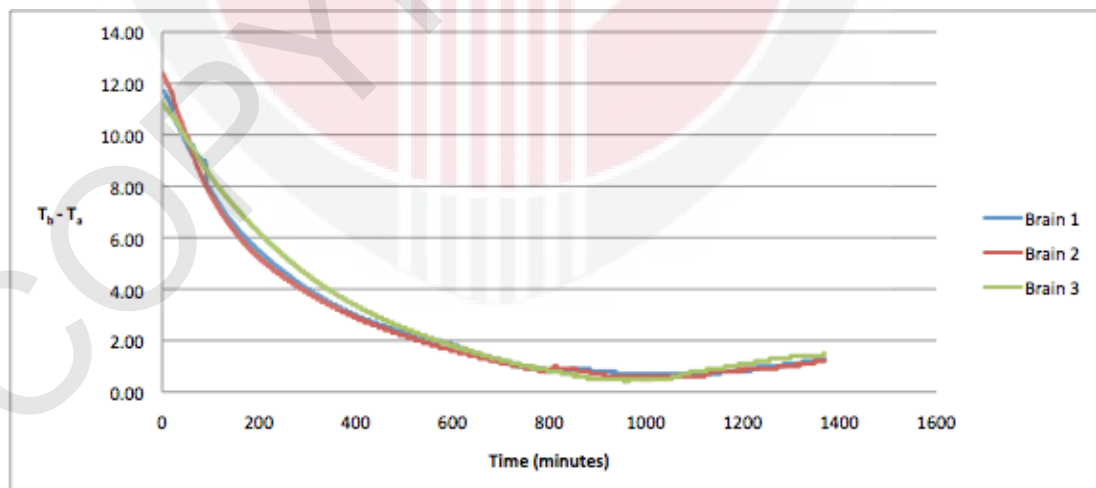


Figure 6. The recording of the difference between brain cooling temperature and the mean minimum ambient temperature ( $27^{\circ}\text{C}$ )

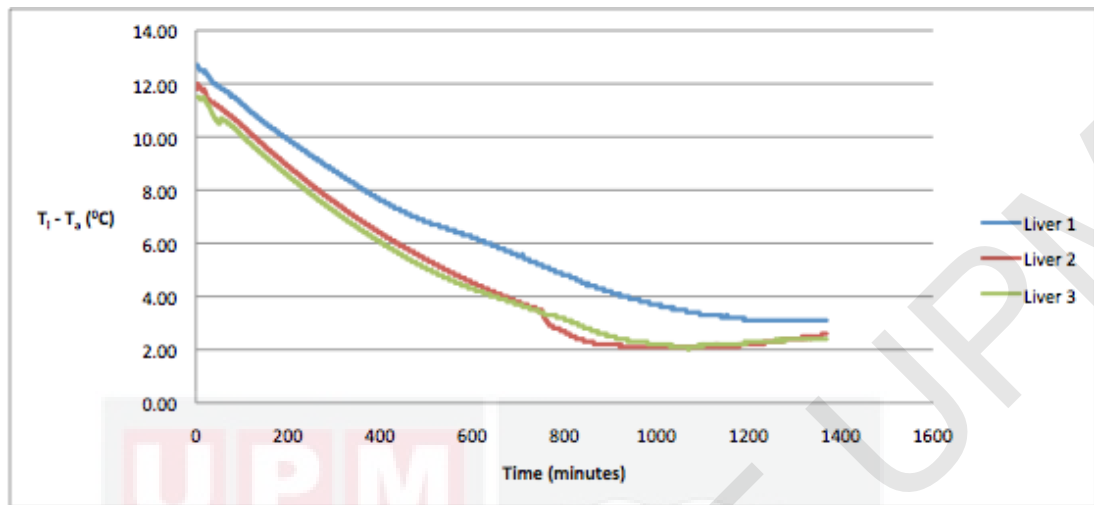


Figure 7. The recording of the difference between liver cooling temperature and the mean minimum ambient temperature ( $27^{\circ}\text{C}$ ).

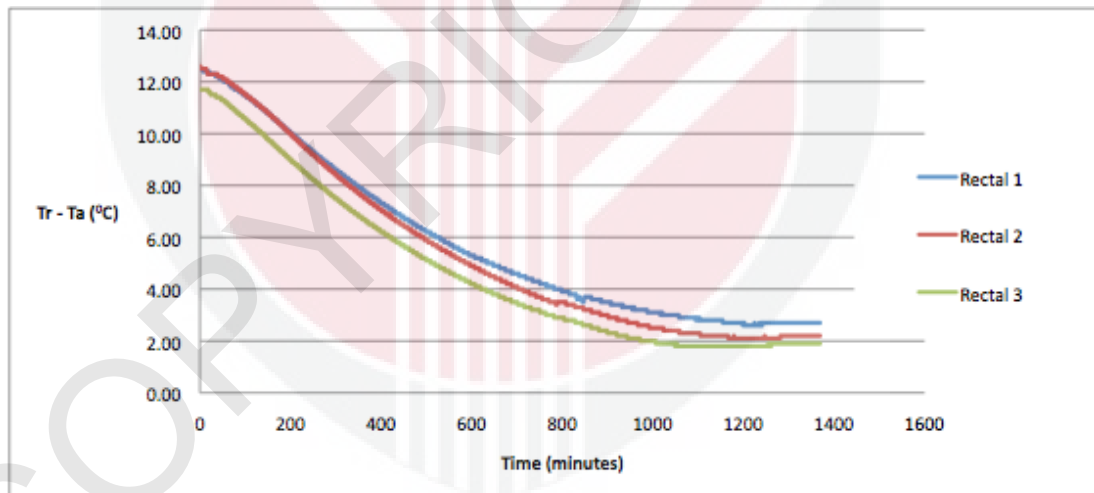


Figure 8. The recording of the difference between rectal cooling temperature and the mean minimum ambient temperature ( $27^{\circ}\text{C}$ ).

Figures 9-11 show the mean of the differences for each organ were calculated and plotted against time. The exponential curve was fitted in and the correlation coefficient, coefficient a, and coefficient b were obtained.

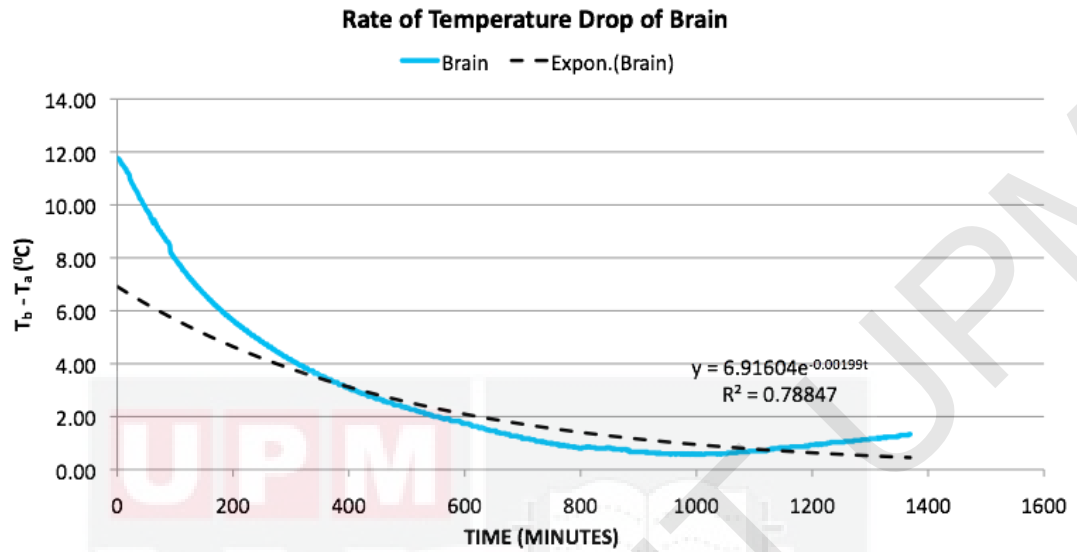


Figure 9. The curve-fit model for the average temperature differences ( $T_b - T_a$ ) for the brain, also showing the exponential equation and correlation coefficient values (Coefficient data:  $a = 6.92$ ,  $b = -0.00199$ , standard error = 0.113 and correlation coefficient = 0.788)

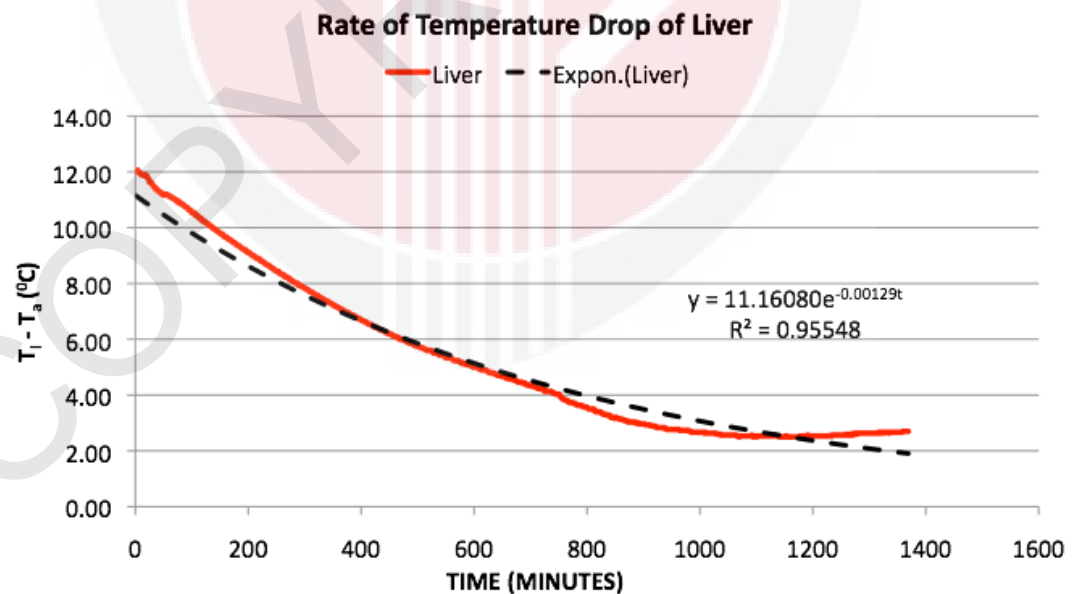


Figure 10. The curve-fit model for the average temperature differences ( $T_l - T_a$ ) for the liver, also showing the exponential equation and correlation coefficient values (Coefficient Data:  $a = 11.16$ ,  $b = -0.00129$ , standard error = 0.467 and correlation coefficient = 0.955)

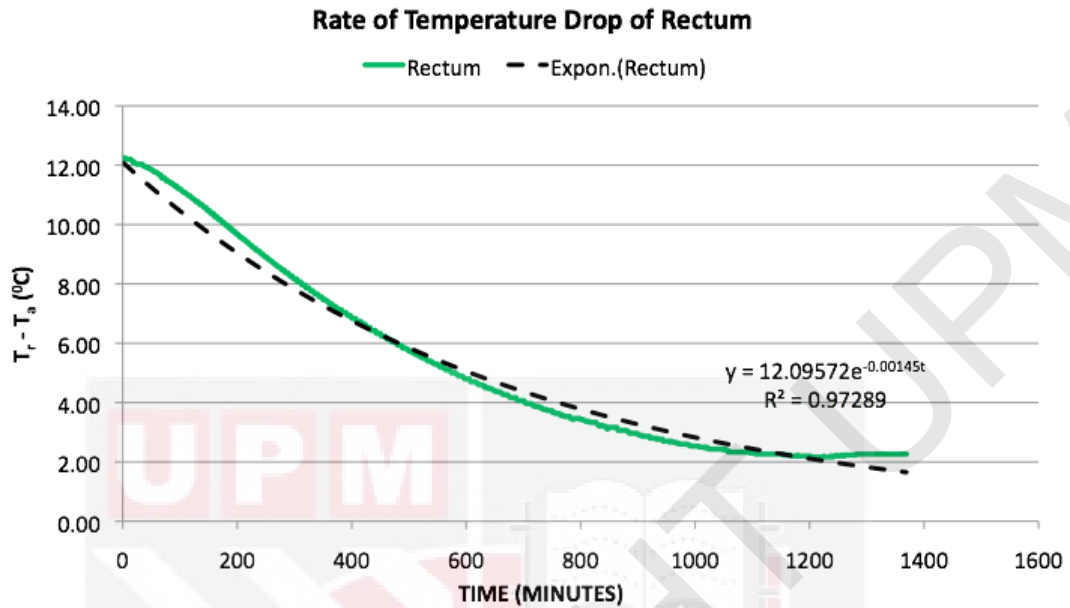


Figure 11. The curve-fit model for the average temperature differences ( $T_r - T_a$ ) for the liver, also showing the exponential equation and correlation coefficient values (Coefficient data:  $a = 12.10$ ,  $b = -0.00145$ , standard error = 0.533 and correlation coefficient = 0.973)

Table 2 shows the data for coefficient  $a$ , coefficient  $b$  and the single exponential equation generated for each tissue. The curve of best fit for each graph has a high value of correlation coefficient of  $r > 0.78$  with a marginal standard error of  $S \geq 0.113$  recorded for all organs. Therefore, the usage of exponential curve highly correlated with the graphs.

Coefficient a and coefficient b are inserted into the single exponential model,  $T - T_a = ae^{bt}$  to generate the equations for each organ. All the data were tabulated in the table below.

Table 2. Data for coefficient a, coefficient b and the single exponential equation generated for each site.

Site	Coefficient a (y-intercept)	Coefficient b (Mean rate constant of temperature change per hour)	Single exponential equation $T - T_a = ae^{bt}$
Brain	6.92	-0.119	$T - T_a = 6.92e^{-0.119t}$
Rectum	12.10	-0.087	$T - T_a = 12.10e^{-0.087t}$
Liver	11.16	-0.077	$T - T_a = 11.16e^{-0.077t}$

Coefficient a is also the y-intercept and coefficient b is the mean rate constant of temperature change. It is evident that the brain shows the highest mean rate constant of temperature change (per hour) where as rectum and liver shows almost similar value mean rate constant of temperature change (per hour).

From the single exponential equation, the time of death, t can be calculated. For the ease of calculation and practical use, the single exponential equation can be converted into its algebraic form making time of death, t the subject. To calculate for t, the time of death,

$$\begin{aligned}
 (T - T_a) &= ae^{bt} \\
 (T - T_a)/a &= e^{bt} \\
 \ln [(T - T_a)/a] &= bt \\
 \mathbf{t} &= \mathbf{(1/b)\ln[(T - T_a)/a]}
 \end{aligned}$$

This method of calculation can be applied to all the equations to obtain the time of death, t.

Nevertheless, it must be noted again that these single exponential equations were generated are based on dogs with weight ranging from 12.5kg to 18kg at an ambient temperature of 27°C.

Among these three temperature sites, the most practical site to obtain the animal's body temperature at time of death is from the rectum provided that the site was not compromised or contained valuable evidence. It is not recommended to take liver temperature due to possibility of iatrogenic damage to the organ (Merck & Miller, 2013). It appears that for the brain temperature, a more elaborate procedure is required and may not be appropriate in the field or applicable in cases of gunshot wound to the head of animal.

Validation using dog carcasses with known PMI is required to determine the accuracy of the single exponential equations generated. Kaliszan *et al.* (2005) had shown that by using a single exponential equation, the precision of the estimation of time of death decreases functionally with time, therefore validation using dog carcasses with known time of death is required.

## 4.2 HISTOLOGY

The changes of the histological structure was observed across 0 hour, 12 hour, 18 hour, and 24 hour postmortem. When all of these individual histological changes of individual tissues were compiled, a set of markers for refining the PMI was created.

### 4.2.1 Brain

Plate 1 shows the changes in the brain during the experimental period. At 0 hour postmortem (Plate 1a), nucleus of neurons and glia were still discernible. Swelling of neurons were seen at 12 hour but the elongated (Plate 1b), tapered form was still retained. By 18 hour postmortem (Figure 1c), there were fragmentation of the nucleus of neurons and vacuolization of the cytoplasm of the glial cells. By 24 hours postmortem (Figure 1d), nuclei of neurons were no longer visible along with clumping of cytoplasm of the neurons.

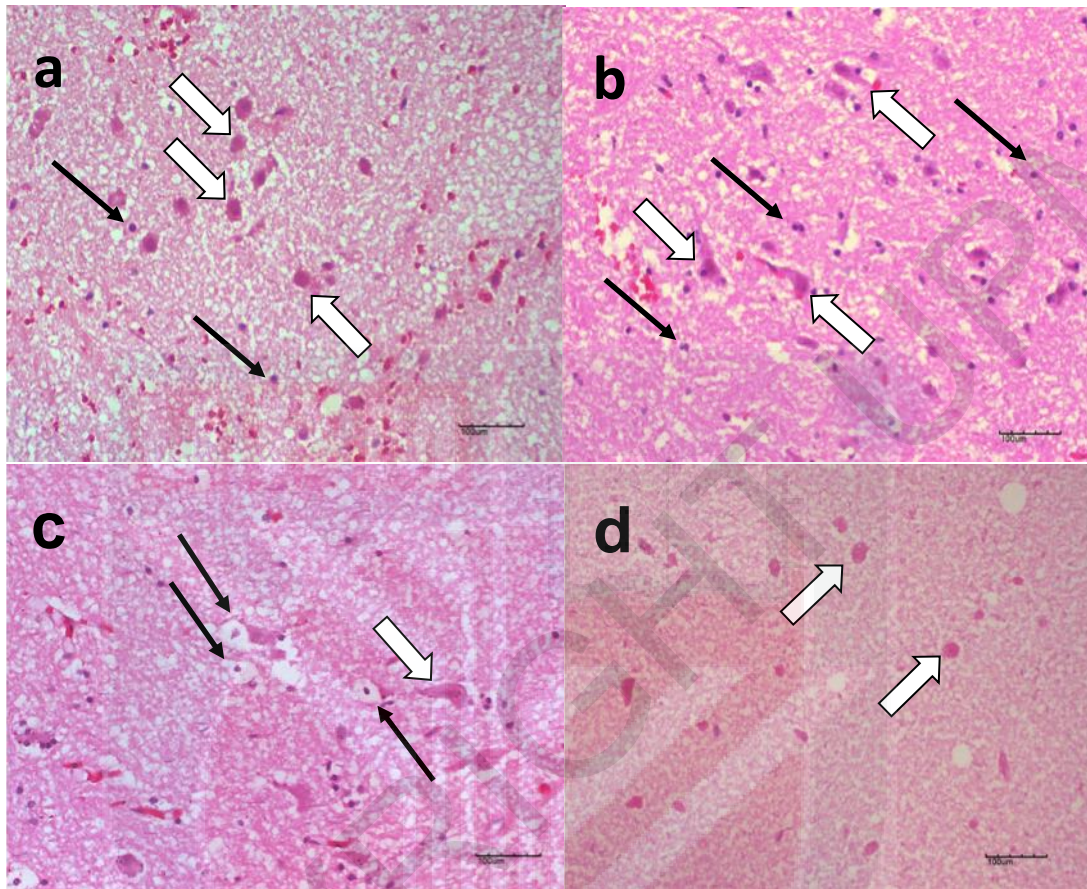


Plate 1. Photomicrograph of the brain during the experimental period. Histological changes that occur in the brain at 0 hour postmortem (a), 12 hour postmortem (b), 18 hour postmortem (c), and 24 hour postmortem (d). The white arrows indicate neurons and the black arrows indicate glial cells.

#### 4.2.2 Liver

The architecture of the portal triad which consists of the portal vein, hepatic artery branch, lymphatic vessel, and bile ductule was clearly seen at 0 hour postmortem. Nucleus of hepatocytes was also seen. By 24 hour postmortem, this architecture of portal triad was almost totally disintegrated. Nucleus of hepatocytes can no longer be seen and the hepatic cord arrangement became completely disarrayed.

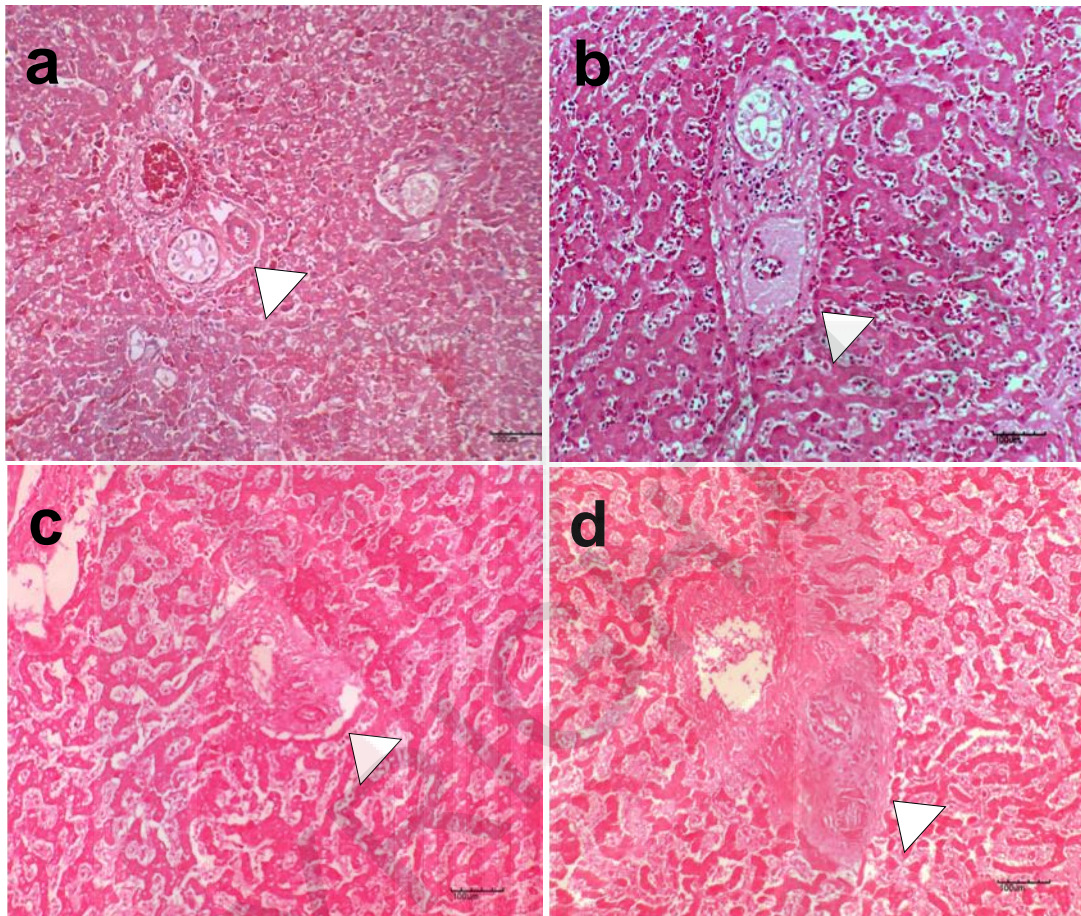


Plate 2. Photomicrograph of the liver during the experimental period. Histological changes that occur in the liver at 0 hour postmortem (a), 12 hour postmortem (b), 18 hour postmortem (c), and 24 hour postmortem (d). The field of interest is area surrounding the portal triad. The white arrowhead indicates the portal triad.

By 24 hour postmortem, hepatocytes had lost their distinct cell borders and there was complete loss of nuclei, all that remained were ghost-like cells. The onset of autolysis depends on the amount of hydrolytic enzymes present. Liver, which has more digestive enzymes compared to brain and muscle are more subjected to autolytic changes after death (Scarpelli & Iannaccone, 1990) as seen in this study.

#### 4.2.3 Epaxial muscle

Plate 3 shows the changes of the epaxial muscle during the experimental period. From the four time interval postmortem, not much histological changes were observed in the musculatures.

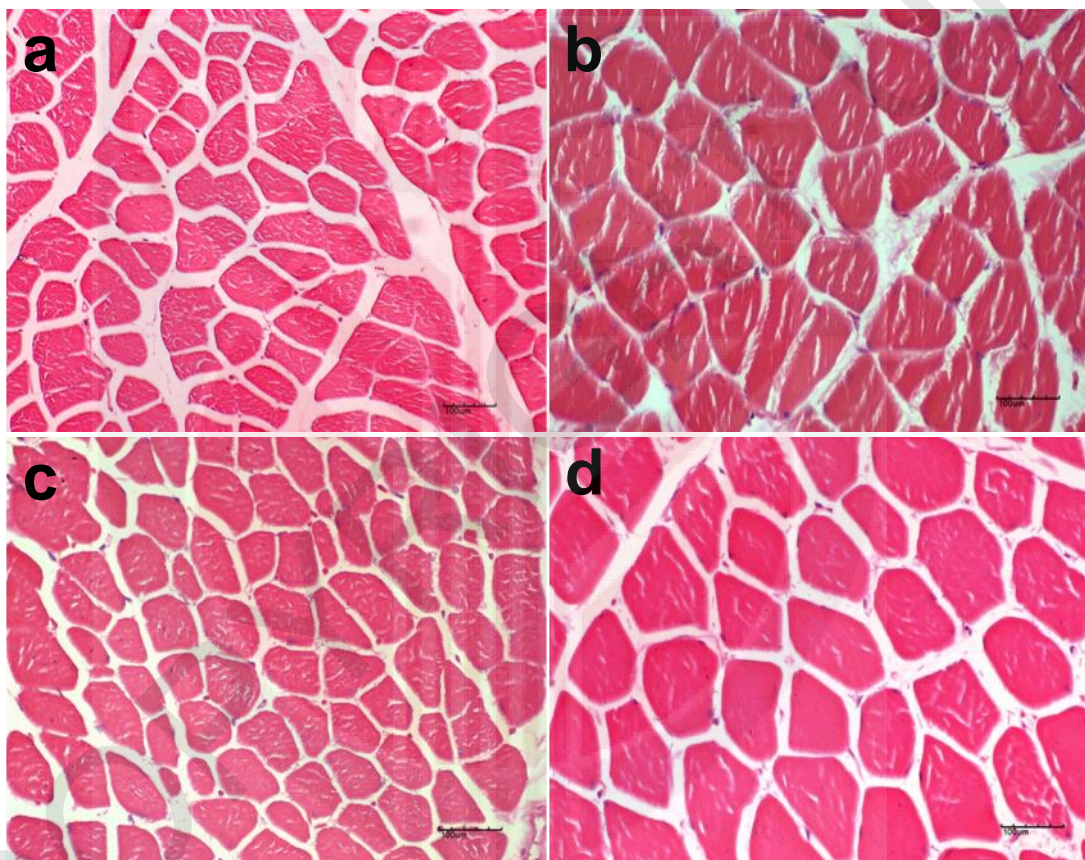


Plate 3. Photomicrograph of the epaxial muscle during the experimental period. Transverse section of histological changes that occur in the epaxial at 0 hour postmortem (a), 12 hour postmortem (b), 18 hour postmortem (c), and 24 hour postmortem (d).

There were no apparent changes seen in the skeletal muscle, most probably due to the lack of digestive enzymes at that region. Tomita *et al.* (2004) demonstrated that the ultrastructure of skeletal muscle showed the greatest delay in

postmortem change due to low level of hydrolytic enzymes in fibroblasts. Thus, this explains a similar finding as seen in the current study.

### 4.3 GROSS PATHOLOGY

#### 4.3.1 EXTERNAL

##### 4.3.1.1 Rigor mortis

Rigor mortis of all limbs and jaw were present throughout the 24 hour postmortem period. Since no observation was done from 0 hour to 12 hour postmortem, the onset of rigor mortis could not be exactly determined.

Rigor mortis is postmortem muscle contraction that immobilizes the joints of the body that occurs due to the depletion of adenosine triphosphate (ATP). This in turns causes the muscle's actin and myosin to stay locked together until it is broken by decomposition (Merck & Miller, 2013).

Since rigor mortis was observed at 24 hour postmortem interval, the decomposition has not set in. A longer study period with shorter time interval for this observation should be done to determine the total duration of rigor mortis of dogs in Malaysia.

##### 4.3.1.2 Livor mortis

Plate 4 shows the development of livor mortis during the experimental period. Livor mortis or lividity due to hypostatic congestion can be observed as early

as 18 hours after death. By 18 hour postmortem, all dogs showed some degree of skin discolouration mainly at the lower side of abdominal region and medial side of the hind limbs. There was a variability that ranges from purplish-red to greenish-blue in colour.



Plate 4: Photograph of the abdomen during the experimental period. *a*. Colour of abdomen at 0 hour postmortem. *b*. Distinct livor mortis changes can be seen at 24 hour postmortem.

Livor mortis occurs due to blood leakage from the blood vessels caused by haemolysis and breakdown of the blood vessel walls from decomposition (Merck & Miller, 2013). Since the dog was placed on left lateral recumbency immediately after euthanasia, blood pooled on the left side of the body following gravitational force.

#### 4.3.1.3 Eyes and Teeth

Plate 5 shows the eye of dogs during the experimental period. The upper eye (right eye) was dry by 24 hour. No teeth discolouration was observed within 24 hours after death in any of the three carcasses.

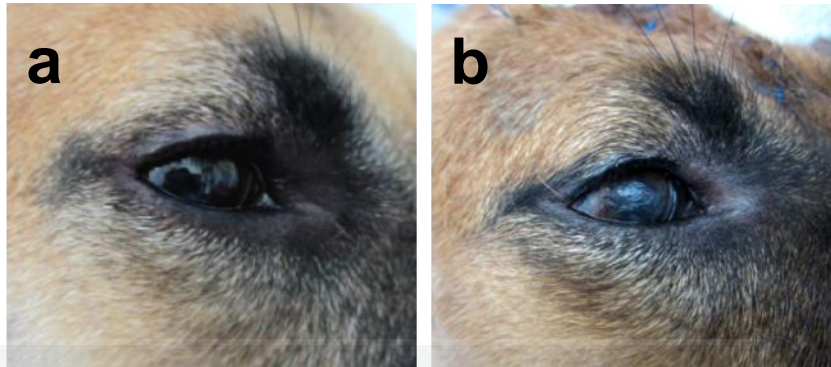


Plate 5: *a.* The eye at 0 hour postmortem interval was moist and shiny. *b.* The same eye at 24 hour postmortem interval was slightly cloudy and dry.

Corneal clouding occurs with increase in intensity until the cornea loses its turgor postmortem (Salam *et al.*, 2012). No colour changes were observed in the teeth, which was in accordance with the study done by Erlandsson and Munro (2007) that noted pink discolouration of the teeth by three weeks postmortem.

#### 4.3.2 INTERNAL

The postmortem changes of the body systems can be seen in Plates 6-7. The dogs were placed on left lateral recumbency after being euthanised. There was blood pooling on the lower side (left) of the thoracic cavity. There was also haemoglobin imbibition on the inner of the left thoracic wall. The colour of the lungs varied among the three carcasses. However, from the three carcasses, only upper side of the lungs (right) mostly showed discolouration (Plate 6).

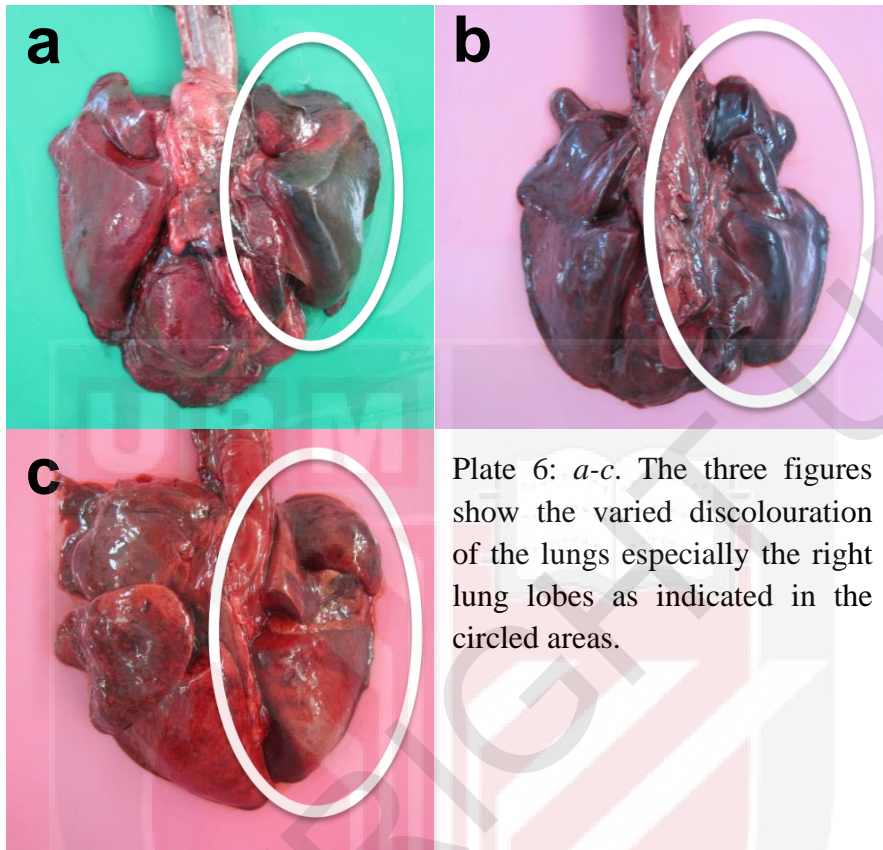
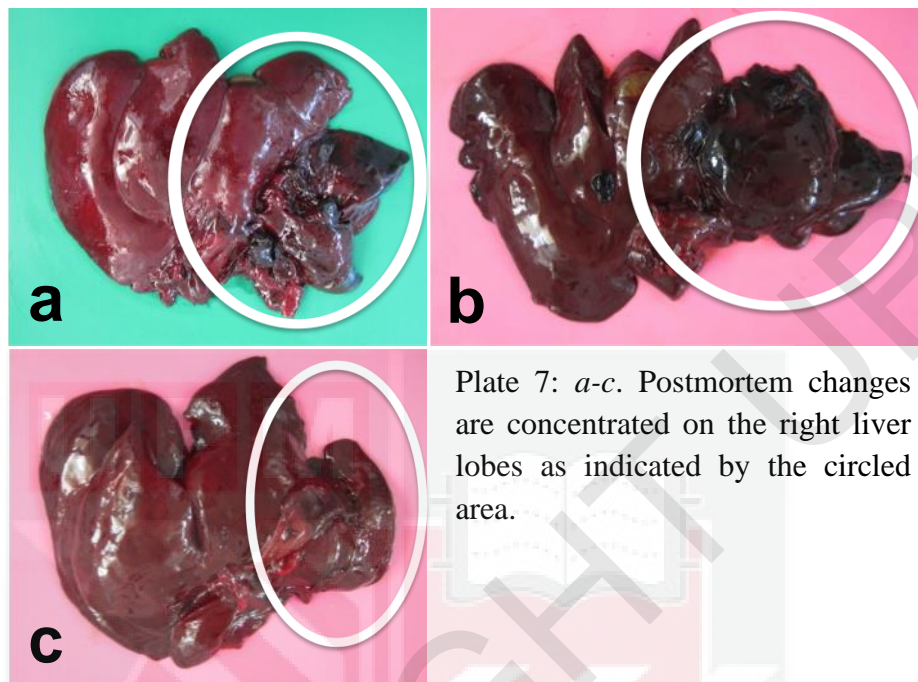


Plate 6: *a-c*. The three figures show the varied discoloration of the lungs especially the right lung lobes as indicated in the circled areas.

The right division of the liver, which consists of the right medial, right lateral and caudate process of caudate lobe were mostly dark red to black in colour with a soft consistency. Postmortem changes in liver were concentrated on the upper side of the abdomen that is the right side of the body. On the other hand, the quadrate lobe, left medial lobe, and left lateral lobe were still reddish in colour and firm when palpated (Plate 7).



Internal gross pathology reveals that postmortem changes were more concentrated on the upper side of the organs. This could be explained by conduction of heat. Hot air which is less dense than cold air will flow upwards and accumulate on the upper side of the organs which propagates autolytic changes of the tissues.

The pattern of postmortem change in the early PMI concentrates on the upper side of the organs. Since animals were placed at left lateral recumbency after euthanasia, the postmortem changes were seen mainly at the right side of the organs especially lungs and liver. In addition to livor mortis, this could also be useful in determining how the body was placed within 24 hours postmortem.

Postmortem changes occur due to decomposition process that involves both processes of putrefaction and autolysis. The onset of decomposition is variable and it is dependent on the environmental conditions, body condition before death, and the cause of death (Merck & Miller, 2013). For that reason, estimation of PMI using gross pathological changes needs to be carefully interpreted and correlated with other findings.

#### 4.4 FORENSIC ENTOMOLOGY

Diptera are the insects of the greatest forensic interest and are important indicators of PMI especially in the first few weeks after death (Sharma *et al.*, 2013). Three types of insects were identified and they are all from the Diptera order.

Plate 8-10 shows the flies species seen scavenging the carcass during the experimental period. The first flies to appear around the carcass were the *Chrysosoma megacephala* from the *Calliphoridae* family. They are also known as the Oriental Latrine flies. They colonized the natural orifices of the oral cavity and the anal opening.

Next flies to appear at the scene were the scuttle flies, *Megaselia scalaris* from the *Phoridae* family. They are known as scuttle flies due to the erratic movement across a surface. Similar with the *Chrysosoma megacephala*, the scuttle flies were seen only in the oral cavity.

The last flies that were seen around the surface of carcasses were the common houseflies, *Musca domestica* from the *Muscidae* family. They only appeared in a small numbers and never observed in the natural orifices.

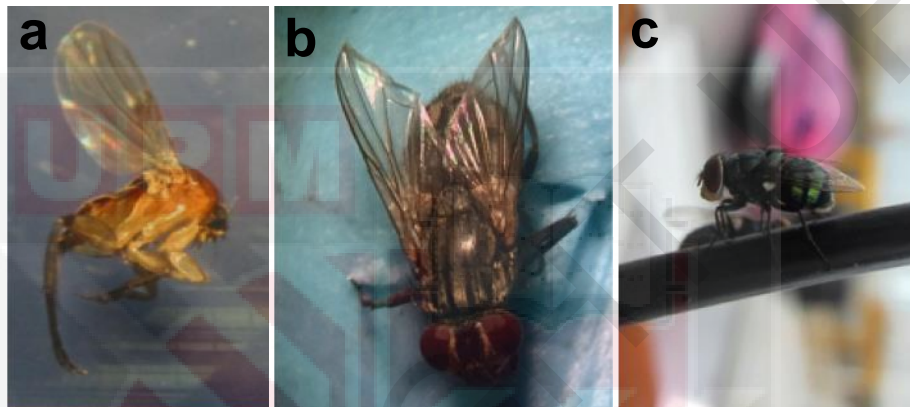


Plate 8: *a.* Scuttle flies, *Megaelia scalaris* *b.* Common houseflies, *Musca domestica* *c.* Oriental latrine flies, *Chrysosoma megacephala*

By 18 hour postmortem, batches of egg were seen on the gums, on the tongue, and on the hard palate. By the end of 24 hour postmortem interval, maggots were seen on the gum but the maggots were unable to be identified.

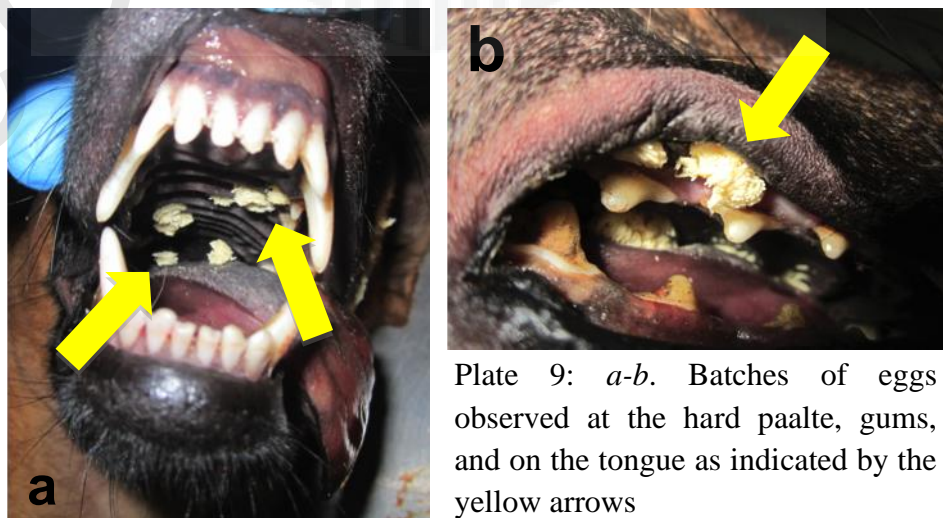


Plate 9: *a-b.* Batches of eggs observed at the hard palatte, gums, and on the tongue as indicated by the yellow arrows



Plate 10: Larvae of flies at the gums by 24 hour postmortem as seen in the circled area.

Among the three types of flies collected, *Chrysosoma megacephala* (*Calliphoridae*) were the first to arrive on the carcass and their offspring can provide an accurate estimate of the time the carcasses had been exposed to insects (Sharma *et al.*, 2013). Since the carcass was laid out in the evening, no insect activities were seen until the morning which was a few hours after the 12 hour postmortem. According to a study by Ahmad *et al.* (2011), adult flies were observed to move away from monkey carcasses during sunset or during the twilight zone period and do not oviposit at night. Thus, there is a possibility of a 12 hour or more time interval lapsed when using forensic entomology in estimating the postmortem interval in cases where the victim died in the evening or at dusk.

Apart from the type of flies that colonize the carcass, eggs provide crucial information on the estimation of PMI. Laboratory rearing of eggs of flies found on carcasses and studying the time of egg hatching or the emergence of the first instars can contribute to the determination of a short PMI (Sharma *et al.*, 2013). The estimate from the laboratory and experimental data shows the time of egg laying

within a period of 2 hours determines the time since death as flies from the *Calliphoridae* lays eggs immediately when exposed to carcasses (Sharma *et al.*, 2013).

Although the maggots found on the gums were never identified, a study done by Lee *et al.* (1989) and Kavitha *et al.* (2013) stated that maggots of *Chrysoma megacephala* reveal as the predominant maggots in 63% of human cadavers.

Houseflies are considered as an incidental visitor and the presence of that flies was only of minor significance in estimation of PMI (Goff, 1991). In Malaysia forensic cases (2005-2010), there were not a single case involved the presence of *Musca domestica* larvae or flies. It is also important to note that the arrival time of flies and the type of flies depends on several factors such as carcasses was indoors or outdoors, exposed to sun or shaded and geographical location.

## 5.0 CONCLUSION

Pattern of cooling rate for the brain and rectum shows an exponential drop whilst the rectum has a short initial temperature plateau giving rise to a sigmoidal shape. The brain also shows the greatest mean rate constant of temperature change which is probably due to the different anatomical localization of varying thermal conductivity. Gross pathology and histology shows distinct changes that could be tabulated into a set of markers that could be useful in refining the postmortem interval. Three types of flies from Diptera order which are *Megaselia scalaris*, *Chrysosoma megacephala*, and *Musca domestica* were identified to be present around the dog carcasses within the first 24 hours postmortem. The types of flies, eggs and larvae would prove an estimate of time the body is exposed to insects.

## 6.0 RECOMMENDATIONS

Validations of the single exponential equations for each organ are required to determine the accuracy of the equations. More studies are required to establish the postmortem interval in dogs of different weight ranges and also possibly in other species. Studies for late postmortem interval should be conducted also in the future.



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**APPENDICES**

Plate 11. The modified biopsy needle that was used to collect brain biopsy.



Plate 12. The two channel data logger P-670 model. The probe on the left is %rF P-series which was used to measure ambient temperature and the probe on the right is Pt100 used to measure the temperature of brain, liver, and rectum.



Plate 13. Picture above shows the set up of how the probes were placed into the brain, liver, and rectum. The probes are then connected to the data logger which records temperature reading every 60 seconds.



Plate 14. The incisions were sutured after the wedge liver biopsy were obtained.



Plate 15. Flies especially *Chrysoma megacephala* were seen around the oral cavity and nasal opening.