



**UNIVERSITI PUTRA MALAYSIA**

***DETERMINATION OF POST-MORTEM INTERVAL (PMI) VIA  
IMMUNOHISTOCHEMICAL LOCALISATION AND EXPRESSION OF  
BIOGENIC AMINE (CADAVERINE)***

**FRANKIE LAU PICK PING**

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SERDANG, SELANGOR**

**2016**

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**FRANKIE LAU PICK PING**

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**CERTIFICATION**

It hereby certified that I have read this project paper entitled “Determination of Post-Mortem Interval (PMI) via Immunohistochemical Localization and Expression of Biogenic Amine (Cadaverine)”, by Frankie Lau Pick Ping and in my opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Final Year Project.



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## DEDICATIONS

This thesis paper is dedicated to the One Almighty God who make anything possible,

To my father and mother,  
My grandmother and grandfather,  
My brothers and sisters,  
Lee Chee Yien

And to all my beloved teachers who guided me through the education pathway.

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**LIST OF ABBREVIATIONS**

|     |                             |
|-----|-----------------------------|
| %   | percent                     |
| °C  | degree Celsius              |
| BSA | bovine serum albumin        |
| cm  | centimetre                  |
| DPX | dibutyl phthalate xylene    |
| H&E | Hematoxylin and Eosin       |
| IBD | interlobular bile ducts     |
| IHC | immunohistochemical         |
| kg  | kilogramme                  |
| mL  | millilitre                  |
| mM  | milimolar                   |
| PBS | phosphate buffered solution |
| PMI | post-mortem interval        |
| µl  | microlitre                  |

**ABSTRAK**

Abstrak kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999-Projek Tahun Akhir

**PENANGGARAN TEMPOH KEMATIAN MELALUI PEWARNAAN  
IMUNOHISTOKIMIA DAN KEPEKATAN AMINA BIOGENIK  
(KADAVERINA)**

Oleh

**Frankie Lau Pick Ping**

**2016**

**Penyelia: Prof. Dr. Noordin Mohamed Mustapha**

Sela masa pascakematian (PMI) adalah tempoh sejak kematian sehingga jasad haiwan atau manusia ditemui. Penentuan PMI amat penting bagi penyiasat forensik, terutamanya dalam isu forensik yang melibatkan binatang atau manusia. Anggaran PMI yang tepat dapat dijadikan *prima facie* yang kukuh untuk penyiasatan pihak pendakwa. Banyak parameter PMI telah dikaji oleh saintis dari seluruh dunia. Namun, kebanyakan data adalah dari negara-negara barat. Maka, kajian ini bertujuan untuk mencadangkan parameter baru untuk penanggaran PMI melalui perwarnaan imunohistokimia dan kepekatan amina biogenik (kadaverina). Sampel tisu otak, hati dan otot epaksial dari tiga ekor anjing telah diperolehi pada sela 0, 12, 18, dan 24 jam pasca kematian. Sampel telah diproses untuk prosedur perwarnaan imunohistokimia menggunakan antibodi kadaverina dengan histologi H&E. Setiap sampel dianalisis

menggunakan perisian profil immunohistokimia (Image J IHC Profiler) bagi menentukan kepekatan kadaverina pada setiap titik masa pasca kematian. Keputusan menunjukkan perbezaan keertian pada kepekatan kadaverina antara setiap titik masa pasca kematian bagi otak ( $p = 0.000$ ) dan hati ( $p = 0.000$ ) sahaja. Peningkatan kepekatan kadaverina adalah tererti antara otak dan hati ( $p = 0.043$ ), hati dan otot ( $p = 0.008$ ) dan otot dan otak ( $p = 0.009$ ). Terdapat juga perkaitan yang tererti antara perubahan suhu dengan kepekatan kadaverina pasca kematian pada otak ( $r = -0.898$ ,  $N = 12$ ,  $p = 0.000$ ) dan hati ( $r = -0.958$ ,  $n = 12$ ,  $p = 0.000$ ) tetapi tiada bagi otot. Perubahan histology berdasarkan pewarnaan Hematoxylin-Eosin (H&E) turut berkait rapat dengan perubahan berdasarkan pewarnaan immunohistokimia pada sampel tisu sepanjang tempoh kematian. Kajian lanjut yang serupa perlu dilakukan dengan menggunakan amina biogenik lain seperti putresina dan spermidina. Secara kesimpulan, kadaverina dari otak dan hati boleh dijadikan parameter untuk penganggaran PMI.

Kata kunci: sela masa pasca kematian, patologi, amina biogenik, kadaverina, otak, hati, otot

**ABSTRACT**

Abstract of the project paper presented to the Faculty of Veterinary Medicine in  
partial requirement for the course VPD 4999 – Final Year Project

**DETERMINATION OF POST- MORTEM INTERVAL (PMI) VIA  
IMMUNOHISTOCHEMICAL LOCALISATION AND EXPRESSION OF  
BIOGENIC AMINE (CADAVERINE)**

by

**Frankie Lau Pick Ping**

**2016**

**Supervised by: Prof. Dr. Noordin Mohamed Mustapha**

Post- mortem interval (PMI) is the time since death until the moment the dead body of either an animal or human is discovered. The determination of PMI is of crucial importance in a forensic investigation as it would narrow down uncertainties with respect time and justifying a much more valid prima facie. Despite being extensive, most studies on PMI originated from the temperate regions. Thus, this study aimed to establish a parameter to estimate PMI via immunohistochemical localisation and expression of biogenic amine (cadaverine). Brain, liver and epaxial muscle tissue samples from three dogs were obtained at 0, 12, 18, and 24-hour post-mortem. The samples were then processed for cadaverine immunohistochemistry and H&E stained histology. Each sample was scored using an immunohistochemistry profiler software (ImageJ IHC profiler) to assess the expression of cadaverine at each interval. Results

demonstrated significant differences in expression of cadaverine in a time-dependent manner for the brain ( $p = 0.000$ ) and liver ( $p = 0.000$ ) but none in the muscle ( $p = 0.817$ ). These expression was comparable between the brain and liver but was much higher ( $p = 0.009$  &  $0.008$  respectively) than that of the muscle. There was significant correlation between temperature changes against concentration of cadaverine in the brain ( $r = -0.898$ ,  $N = 12$ ,  $p = 0.000$ ) and the liver ( $r = -0.958$ ,  $N = 12$ ,  $p = 0.000$ ) but again none was demonstrated in the muscle. Likewise, the post-mortem changes based on H&E correlated well with those of cadaverine immunohistochemistry throughout the assessment period. Similar study should be subjected to different biogenic amines such as putrescine and spermidine. In conclusion, brain and hepatic cadaverine serves as a potential indicator for the determination of PMI.

Key words: Post- mortem interval, pathology, biogenic amines, cadaverine, brain, liver, muscle



## 1.0 INTRODUCTION

The importance of post-mortem interval (PMI) is an upcoming parameter in veterinary science especially with regards to medicolegal issue. In accord with the Government Act 999 (2009) and establishment of Animal Welfare Act (2015) and various legal frame to protect animal welfare, warranting the need of a veterinary forensic team. To do so, one of the important question posed in sudden death inquiry is “When did the animal die?” Hence determining an accurate PMI will assist prosecution with strong evidence. The main principle of determination of the time since death is calculation of a measurable date along a time-dependent curve back to the start point (Henssge, 1986).

In general, there are various methods studied to estimate PMI through physical, chemical and biological parameters. Traditional methods since centuries ago are based on detection of phenomena happen near to the death including rigor mortis, livor mortis, and external post-mortem appearance of body. These physical parameters have their limitations due to several environmental and the host factors. There are changes, either destructive or conservative events that may accelerate, slow down or suppress some stages of tissue changes (Estracanhalli *et al.*, 2011). Biochemical detection is based on electrolyte changes in cerebrospinal fluid, vitreous humour and pericardial fluid (Kawamoto *et al.*, 2013; Chandrakanth *et al.*, 2013). Detection of temperature changes based on algor mortis, mechanical stimulation of skeletal muscle contraction based on rigor mortis, study of ultrastructure changes of certain organ post-mortem and maggot activity are examples of biological approaches to determine PMI (Mall *et*

*al.*, 2005; Tomita *et al.*, 2004; Warther *et al.*, 2012; Anders *et al.*, 2013; Villet & Amendt, 2011).

Traditional methods of determining time since death are based on naked eye observations of the gross changes in dead body to provide a rough approximation of post-mortem interval. These various gross changes in the body after death are cooling of the body, rigor mortis, loss of corneal reflex, post-mortem hypostasis, decomposition and other putrefactive changes. (Kushwaha *et al.*, 2009).

Most of the parameters established for human work rather than animal use. Munro & Munro (2008) described the particular difficulties for the animal pathologist particularly when they are more likely to be dealing with different species and more environmental factors to be take consider. There are limited studies established for veterinary use. Most literatures on this issue are reported or studied in the temperate (Edges, 1984; Erlandsson & Munro, 2007; Cooper & Cooper, 2007; Merck, 2007; Munro & Munro, 2008; Munro & Munro, 2011; Sinclair *et al.*, 2006). Recently three reports were documented from Malaysia (Heng *et al.*, 2009; Abdulazeez & Noordin, 2010; Okene, 2010). Determination of PMI used to estimate time since death rather than to determine time of death. Hence most of currently available methods focus on constructing a timeframe since animal death and the events happen post-mortem.

To date there is no definite method to pin-point PMI although various methods were studied. An estimation of PMI cannot be just merely based on a single parameter due to various factors. For example, post-mortem cooling or algor mortis has been established as standard in practice with wide applicability and reliability (Henssge &

Madea, 2007). The principle site used for temperature recording is the rectum although other body sites include brain, skin, axilla and internal organs. If there were circumstances where rectal temperature or any organ temperature cannot be established due to no rectum remaining or organ available for the examination.

Hence, most practical way suggested by Henssge & Madea (2007) estimation of time since death is via integration of different parameters into a single system which enable a much more accurate, reliable and applicable estimation of PMI in all cases. Thus, this study was undertaken to fulfil the following objectives:

- i. to determine and score the extent of biogenic amine expression overtime in tissues
- ii. to map the scores with previously obtained data on PMI using time and body temperature

It is hypothesised that biogenic amines are reliable and sensitive indicators of PMI in dogs which correlate well against body temperature changes.

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