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Estimating the Early Postmortem Interval in Domestic Canines

Keith William Proctor
University of Tennessee - Knoxville

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To the Graduate Council:

I am submitting herewith a thesis written by Keith William Proctor entitled "Estimating the Early Postmortem Interval in Domestic Canines." I have examined the final electronic copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Comparative and Experimental Medicine.

<div align="right">John C. New, Major Professor</div>

We have read this thesis and recommend its acceptance:

Linden E. Craig, Murray K. Marks, Karla J. Matteson

<div align="right">Accepted for the Council:
<u>Carolyn R. Hodges</u></div>

<div align="right">Vice Provost and Dean of the Graduate School</div>

(Original signatures are on file with official student records.)

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ESTIMATING THE EARLY POSTMORTEM INTERVAL IN

DOMESTIC CANINES

A Thesis
Presented for the
Masters of Science
Degree
The University of Tennessee, Knoxville

Keith William Proctor
May 2007

DEDICATION

I dedicate this thesis to my wife Michelle and my daughter Riley for their support, love,

and understanding.

ACKNOWLEDGEMENTS

ABSTRACT:

The estimation of time of death (TOD) can be used to aid law enforcement officers in solving criminal cases involving a death. By determining the period from the TOD until the time the body is discovered, forensic investigators can potentially link or rule out a suspect. A great amount of research has been conducted for the purpose of establishing a reliable and accurate means of estimating TOD in humans. In contrast, most animal studies have centered on techniques that might be applied to human forensic cases or to aid wildlife officers in prosecuting criminals who violate conservation laws. However, little research has been conducted concerning the estimation of TOD in companion animals, especially canines. Estimating TOD in companion animals can aid investigators in solving animal abuse cases involving the death of a pet and/or the murder of an owner that coincides with the death of the pet.

The objectives of this study are to take selected TOD measures and apply them for the purpose of expanding the current scientific knowledge concerning TOD determination in canines. Such information should be useful in animal cruelty/abuse investigations by providing a practical and inexpensive quantitative methodology of estimating TOD, maximize the probability that investigators with limited experience will collect useful data, and aid in teaching animal cruelty/abuse investigators proper forensic techniques for handling and collecting data in the field. The measures chosen for this study include postmortem temperature declines in the brain, liver, rectum, and external ear canal and analysis of changes in the concentration of vitreous humor potassium after death. Recording data for these measures are relatively easy, inexpensive, and have been

shown in many studies to be the least controversial and most accurate means for

estimating TOD. This study documented that body temperature declines measured in the

rectum, liver, brain, and external ear canal can be documented using relatively

inexpensive and readily available instruments. Further, this study confirms the work of

others that changes in K+ concentration in the vitreous humor of the eye is a reliable

measure for use in estimation of TOD in dogs.

TABLE OF CONTENTS

TABLES

FIGURES

INTRODUCTION:

Time of death (TOD) is the time or moment at which an individual dies. The estimation of TOD can be used to aid law enforcement officers in solving suspected criminal cases involving a death. By determining the postmortem interval (PMI), which is the period of time from TOD until the time the body is discovered; forensic investigators can potentially determine if a suspect could have committed the crime or whether a suspect can be ruled out. Because of this, research has been conducted for the purpose of establishing a reliable and accurate estimate of TOD in humans. Most animal studies have centered on techniques that might be applied to human forensic cases. A few animal studies have been conducted to specifically aid wildlife officers in prosecuting criminals who violate conservation laws. However, little research has been conducted concerning the estimation of TOD in companion animals. An ability to estimate TOD of companion animals is important because many households own companion animals and a crime may be committed in a home that involves the death of a companion animal. Estimating TOD in companion animals can aid investigators in cases involving animal abuse that results in the death of the pet and/or a murder of an owner that coincides with the death of the pet. These are important issues that until now have not been adequately addressed. The objectives of this study are to 1) add information to the scientific literature regarding estimation of TOD in canines, 2) aid animal cruelty/abuse investigations by providing a practical and inexpensive quantitative methodology of estimating TOD, and 3) maximize the probability that investigators with limited experience will collect useful data.

Many factors and techniques for estimating TOD of humans have been evaluated. Some of these are used to determine the time since death of a person who has been dead for quite some time, while others are better suited for estimating the early PMI or rather time since death in a recently deceased individual. While the two main methods currently used to estimate the early PMI are changes in body temperature after death and analysis of electrolyte concentration in vitreous humor (VH) of the eye, other methods and techniques have been evaluated and used. Each method has been used in numerous human and some animal studies with varying degrees of success. Most have been applied to humans. Only a few have been applied to animals and there are even fewer published reports of these variables and techniques being evaluated in dogs. Estimation of TOD is not an exact science and there are many factors that can affect the usefulness of certain TOD forensic techniques. Environmental temperature, time of day, season of the year, state of decay of the body, and other factors are challenges to the development of a reliable means for estimating TOD, yet some methods and techniques have proven to be beneficial for TOD estimation in some species and need to be evaluated in canines.

CHAPTER I

REVIEW OF THE LITERATURE:

TEMPERATURE-BASED STUDIES:

Body temperature is very important in estimating TOD and has been used for many years as a means for estimating the early PMI. After death the body cools at a standard rate and a prediction curve founded on temperature-based algorithms can be established with relatively accurate results for up to about 60 hours after death [2-4, 7, 13, 21-22, 29, 37, 40-41, 47, 56].

Once a human death is discovered, the scene is processed by homicide investigators [40]. The scene is photographed, sketched, and evidence collected, while, in most cases, the body is left undisturbed for many hours, during which temperature data can be collected [40]. "Because physical contact with the body is necessary in order to pronounce death, at that time the opportunity is provided to place a temperature probe and set up a compact recording device without disturbing the body or evidence" [40, p. 35].

There are problems in using temperature as a method for estimating TOD. In life as well as in death, there are many factors that can alter body temperature readings [2-4, 7, 13, 19, 21-22, 29, 37, 40-41, 47, 56]. Sometimes, the temperature of the body increases soon after death due to exothermic processes in which cells die slowly over several hours. [40]. "During that time tissues starved of oxygen switch to anaerobic respiration; cells lyse; the motility of smooth muscle slowly declines; the sustained

contractions of *rigor mortis* begin; chemical processes occur; bacterial activity

proliferates intestinally, and in the case of ante-mortem bacterial illness, systemically"

[40, p. 32]. Other factors affect the cooling processes of the human body. Some of these

are insulation of the body, exposure to liquids, the ratio of surface area to body mass,

temperature gradient between ambient and body temperature, and ambient humidity [40].

Site of temperature reading, collection techniques used, time of day, season of year, and

morphological differences are other factors that can affect the accuracy of the estimation

[2-4, 7, 13, 19, 21-22, 29, 37, 40-41, 47, 56]. Most authors agree that determining the

core body temperature (the central or innermost temperature of the body) provides the

most accurate estimate of the TOD [40]. By using the core body temperature,

environmental changes would have a lesser impact on body cooling, thereby increasing

the usefulness of temperature as a means of estimating TOD. The temperature recording

sites that have been used to provide the core body temperature are the external ear canal,

nasal cavity, liver, rectum, brain, and thigh muscle [2-4, 7, 21, 37, 40-41, 47, 56].

External Ear Canal and Nasal Temperature Sites in TOD Estimation:

HUMAN STUDIES:

Measurements from the external ear canal and nasal cavity are easy to perform

and do not disturb the body, clothing, or crime scene [41]. However, few studies have

been conducted to examine external ear canal and nasal cavity temperature. Of these,

temperature readings for the nasal cavity were obtained by way of a thermocouple

connected to a recording device that was inserted to a depth of 2 to 2.5 cm [41]. Ear canal

temperature was obtained by inserting a thermocouple or thermistor into the ear canal

until contact was made with the tympanic membrane (i.e., ear drum) [7, 41]. Once

4

contact was made, the thermocouple or thermistor was withdrawn slightly to prevent the distortion of temperature readings by contact with the ear drum [7, 41]. One study found the estimated TOD greater than the actual time of death in the 5 corpses examined [41]. There appeared to be a larger difference between estimated and actual TOD when using the nose than when using the external ear canal [41]. The authors of this study speculated that the discrepancy was due to many factors including initial differences in temperature at death between the external ear canal and nasal sites [41]. Respiration prior to death can also affect the temperature of the nose due to humidity and ambient temperature [41]. Another source of error is the assumption that the cooling curve can be approximated using a single exponential equation instead of a more complex equation [41]. Presence of a temperature plateau or initial delay in cooling could affect the estimated TOD [41]. Both the external ear canal and the nose are greatly influenced by environmental conditions, which should be taken into account when using them as sites for core body temperature estimation [41]. However, because this study was limited in scope to only five corpses, the conclusions could be subject to considerable error.

Another study involving 138 human corpses, which looked at only external ear canal temperature, found this measure to be statistically significant in estimating TOD [7]. This study looked at external ear canal temperature as a means of estimating TOD compared to other methods such as the use of vitreous potassium (K+), blood log sodium/potassium ratio (Na+/K+), cerebral spinal fluid (CSF) K+ and log chlorine (Cl-).

Rectal temperature and external ear canal temperature were found to have the best correlation coefficient for estimating TOD [7]. However, a multivariate equation combining many of the above methods showed an even higher level of correlation [7].

This study established an equation for estimating TOD as a function of ambient temperature and external ear canal temperature. The results showed that the method using external ear canal temperature provided the best quality/simplicity ratio of all methods studied and could be used at crime scenes under similar conditions as those under which the study was performed [7]. The authors also cautioned that there are limitations encountered when using external ear canal temperature to estimate TOD. This method cannot be used if there is otorrhagia (i.e., blood in the ear) [7]. Also, if the body is lying on one side with one ear lying on the ground, the other ear should be used [7]. The external ear canal temperature method does not appear to be effective in cases where the post-mortem interval is longer than 15 hours [7]. Further, atmospheric factors like rain, humidity, sun, and wind may cause errors in estimation based on this method and should be studied further [7].

A letter to the editor of the Forensic Science International journal listed the limitations of using external ear canal temperature in estimating TOD in the previous study [47]. Blood from the internal carotid artery is sent to the tympanic membrane as well as the hypothalamus by which core body temperature is regulated. Readings from the ears of the living are said to be correlated with core body temperature, however, canal temperature is dependent on local metabolic processes, which in turn, are dependent on time of day, emotional state, and age. "Moving air within the canal results in heat loss with reported temperature changes of 0.04°C per 1°C ambient temperature change." Thus external ear canal temperature can differ from the core body temperature by 3°C or more at any one time. Therefore, it cannot be assumed that the ear canal temperature at death was 37°C. In fact, it cannot be assumed that both ears are the same temperature and since

many bodies are moved after death, there is no reliable way to determine which ear should be used for temperature analysis [47].

Another factor that may affect the accuracy in using external ear canal temperature to estimate TOD is the depth that a thermometer is inserted. Since the insertion depth is dependent on the operator, the temperature readings can vary from operator to operator and each time the reading is taken. If the thermometer is not close to the ear temperature when inserted, it can cause the canal temperature to change, so an operator will only get one chance to gain an accurate temperature reading. If the temperature probe is inserted too deeply, the tympanic membrane can be damaged and possibly cause bleeding into the canal. Blood, CSF, water, otitis externa (i.e., ear infections), and wax can also cause discrepancies in the temperature readings. The ear canal could not be used in most cases of head injury, body immersion, and fire [47]. Many of these same limitations can be applied to using the nasal cavity temperature; therefore, these limitations should be taken into consideration before using either ear canal or nasal cavity temperature to estimate TOD.

ANIMAL STUDIES:

No published animal studies were found that used external ear canal temperature as a means of estimating TOD and only one animal study was found that used nasal temperature. This study, conducted on deer, found nasal temperature to be statistically significant in estimating TOD [56]. None of the limitations proposed in human studies were addressed in this study. No known published studies of this type have been conducted on canines.

Rectal, Liver, and Brain Temperature Sites in TOD Estimation:

HUMAN STUDIES:

Most TOD temperature studies use rectal temperature as at least one means of estimating the core body temperature, which is used to plot a temperature curve for the purpose of estimating TOD. The rectum is thought to be well insulated by the intestines and is located somewhat toward the center of the body. Rectal temperature is usually obtained using a rigid thermocouple attached to a data logging device such as a computer or stand alone module [3-4, 22]. The thermocouple is inserted into the rectum to a depth of approximately 8 cm [22]. In one study, rectal temperature measurements were used in a TOD nomogram, a graphical representation developed by the authors for the purpose of aiding investigators in the field [22]. Using this technique, investigators can plot temperature measurements of the rectum and ambient temperature, which is the basis for estimation of TOD [22]. In this study, it was determined that body build, as defined by height, weight, and body fat, did not influence TOD estimation [22]. One problem in using rectal temperature is that there appears to be a rectal temperature plateau within the first 6 hrs post mortem due to environmental temperatures and body factors. The temperature of the rectum does not fall and can actually increase because of environmental factors, but can be influenced by type of clothing, post-mortem metabolism, and pre-mortem muscular activity [7]. For example, obesity, high ambient temperature, and thick clothing can reduce the rate of rectal temperature decrease. The depth to which temperature measuring devices are inserted into the rectum can also significantly affect the reading [3, 22].

There are other situations under which the proposed rectal temperature nomogram should not be used without the use of corrective factors. These situations include bodies that are near a strong source of heat, have a suspicion of hypothermia, fever at time of death, change of location from place of death, and uncertain severe changes in temperature conditions between death and examination. In cases that have unusual cooling conditions such as the body being covered, clothes removed or put on the body after death, and an open windows at a crime scene will also influence the usefulness of the nomogram [22]. Except for the limitations of the variables above, the use of the nomogram showed a good level of reliability and accuracy [22].

Brain and liver temperatures have also been used for the estimation of TOD with good results. Two studies found both sites to be preferable to rectal temperature in estimating TOD [3-4]. Few studies have used the brain and liver as potential temperature sites due to the difficulty of accessing these sites under field conditions [2-3]. The liver is located in the true center of the body and may be considered more accurate in estimating the core body temperature. However, the liver can respond quickly to heating and cooling processes, but more slowly than other parts of the body [3].

The brain is also considered a good site for estimating the core body temperature. The shape of the head is globular and relatively uniform in humans with few variations in the size of the head between individual humans [3]. Clothing plays little role in brain temperature changes and hair, while variable among individuals in area of coverage and length, also plays little role in brain temperature changes [3]. The brain is insulated uniformly by CSF which protects it, within limits, from environmental temperature effects [3]. Thus, it has been shown that the rectum responds fastest to environmental

9

temperature changes, followed by the liver, and finally the brain, which responds the slowest and is therefore the preferred site for the estimation of the core body temperature [3].

Brain and liver temperature readings have been collected using microwave probes on the surface of the skin or by direct thermocouple readings by placement of the instrument in the liver and/or brain tissue. The thermocouple can be attached to a data logging device which can record temperature readings every few seconds [2-4]. Unlike thermocouples, microwave probes have the unique advantage of measuring the temperature of internal organs without disturbing the body, but both techniques have been used with good results [2-4]. Whichever method is employed, the use of a data logging device is essential in plotting a temperature cooling curve, due to the great number of readings possible and reduction of recording errors inherent with multiple manual temperature collections. While the brain appears to be the best site to estimate the core body temperature, liver and rectum are also useful sites and should be considered when formulating a TOD estimation study.

ANIMAL STUDIES:

No known published studies of this type have been conducted on animals or specifically in canines.

Thigh Muscle Temperature in TOD Estimation:

HUMAN STUDIES:

No known published studies of this type have been conducted on humans.

ANIMAL STUDIES:

In studies of white-tailed deer, the temperature of the central portion of the thigh muscle [specific muscles not defined by authors] is used to estimate the core body temperature [21, 56]. The reasoning behind this may be that the thigh muscle is large and surrounded by insulating tissue. The muscle is easy to reach and obtaining temperature readings is fairly easy with little need for technical training. Readings can be obtained through a thermocouple inserted into the center of the muscle mass of the thigh and connected to a data logging device [21]. Thigh temperature was found to be statistically significant in predicting TOD when used in conjunction with other TOD measures such as nasal temperature, rigor mortis, and/or pupil diameter [21, 56]. Due to the ease in which temperature readings can be collected, the thigh should be considered a promising site to use in the estimation of the core body temperature and ultimately the prediction of TOD. No known published studies of this type have been conducted on canines.

Multiple Temperature Sites in TOD Estimation:

HUMAN AND ANIMAL STUDIES:

Few published studies are limited to any one site for temperature recording. Most studies have found that by using multiple temperature sites TOD estimation can be improved and any potential bias from any one site can be minimized [3-4, 7, 13, 37, 41, 56]. While some sites may be better than others, each site has advantages and disadvantages and there is no consensus for a single best site [4]. Brain temperature may correspond best to core body temperature, followed by the liver and then the rectum, but other sites have been used to estimate TOD and have been shown to be equally significant [3-4, 7, 13, 21-22, 29, 37, 40, 56]. A greater degree of accuracy can be

achieved by sampling multiple sites [4]. No known published studies of this type have been conducted on canines.

Effect of Hair Coat in TOD Estimation:

HUMAN STUDIES:

No known published studies of this type have been conducted on humans but there have been studies in which clothing or other materials covering the body may be an appropriate simulation of hair coat. Such coverings have been shown to delay the rate of core body temperature cooling [7, 22, 41].

ANIMAL STUDIES INCLUDING CANINES:

No known studies of this type have been conducted on animals except in canines. One difference between human skin and animal skin is the extent and properties of hair. Canines have hair coats of different lengths, colors, and densities. The effects, if any, that canine coat has on the core body temperature in dogs, has not been studied extensively. Though many studies determined human thermoregulatory and temperature response to environmental conditions, only one canine study included this variable [12]. The dogs (n=7) used in this study were all Newfoundland Landseers, which have a long dense coat with sharp divisions between black and white areas and uniformly pale colored skin [12]. Hair coat temperature was measured using an electronic thermometer. Hair coat humidity, which can affect temperature variation, was also measured using a specially designed catheter attached to a humidity sensing capacitor. A heat source was used to simulate different heating conditions [12]. There were few physical differences (i.e. coat length or density) noted between sites with differing coat color (i.e. black and white areas). No statistically significant differences in temperature and humidity changes were

12

found between the white and black areas on the dogs, suggesting that color plays little role in affecting body temperature changes. The conclusions of this study were that the hair coat in dogs acts as a protective shell, is effective in maintaining homeostasis, and hair color does not influence body temperature. Therefore, while hair coat temperature measurements may vary, the inner core remains relatively stable [12].

OTHER STUDIES:

Vitreous Humor in TOD Estimation:

HUMAN, ANIMAL, AND CANINE STUDIES:

Electrolyte concentration and biochemical analysis of various body fluids have been investigated in many human and animal studies. The biochemical changes that certain body fluids undergo after death can be examined and utilized for the purpose of estimating TOD [1, 3, 10, 12, 15-16, 23, 25-27, 29, 32-33, 35-36, 38-39, 42-43, 48, 51-52, 55]. While blood, serum, synovial fluid, and cerebrospinal fluid (CSF) have been used in some studies, intraocular fluid, or more specifically VH, is preferred [1, 24, 33]. Vitreous humor is a viscous solution found in the vitreous chamber of the eye. The biochemical components that make up VH are relatively stable over a prolonged interval after death and analyses of some of these components have been shown to be important indicators for estimating TOD [1, 10, 14-15, 23-25, 27, 35-36, 38, 43, 48, 55]. Vitreous humor has a relatively large volume (approximately 2 ml in humans and 1.7 ± 0.86 ml for the canine eye), it can be obtained fairly easily postmortem, and it tends to be less susceptible to contamination and putrefactive effects for at least72 hours [1, 8, 20, 24, 35]. Also, the eye in a corpse is often undamaged, even in cases of head trauma; therefore, VH can be readily obtained for analysis in most cases [1].

13

Vitreous humor can be obtained using a 1 to 12 ml syringe with a 15 to 25 gauge needle [1, 9-10, 14-15, 23-25, 27-28, 31-33, 35-36, 38-39, 43, 48, 51-52, 55]. A puncture is made 5-6 mm caudal to the cornea and the VH is withdrawn into the syringe using slow suction [1]. After collection, samples are often centrifuged and the supernatant frozen for later analysis [1, 9-10, 14-15, 23-25, 27-28, 31-33, 35-36, 38-39, 42-43, 48, 51-52, 55]. In some studies VH is filtered prior to analysis, but the specific technique depends on the equipment used to analyze the solution [35, 55]. Analysis can be performed using a flame photometer (FP), ion specific electrode (ISE), or capillary zone electrophoresis (CZE).

Ion specific electrode analysis has the advantage over FP in that the VH sample does not need to be filtered, diluted, or centrifuged before analysis, which reduces the steps required between sample collection and analysis, thereby decreasing the chance for contamination, sampling error, or improper handling [32, 38, 42]. The ISE analysis method also has the advantage of being easy to use, allows testing immediately after collection without additional preparation, has the ability to analyze samples without destroying them which permits retesting if needed, and tends to generate a steeper, more accurate slope in regression analysis than FP [32, 38, 42].

Capillary zone electrophoresis has been shown in recent studies to provide an even more accurate analysis of VH samples than either ISE analysis or FP because it is based on a technique in which the sample components are separated and measured as is done in ion chromatography [28, 51-52]. Flame photometry and ISE are both based on non-separative techniques so they are less accurate and more subject to error [28, 51-52].

While VH samples must be diluted with a barium aqueous solution prior to analysis with CZE, only a very small amount of VH is needed to perform an analysis [28, 51-52].

In previous studies, biochemical components of VH analyzed for the purpose of finding correlation to TOD are potassium, sodium, magnesium, calcium, phosphorus, chloride, ammonia, barium, creatine, urea nitrogen, glucose, lactate, and hypoxanthine. The most important electrolyte analyzed for the purpose of estimating the early PMI is vitreous K+. The increase in K+ after death has been found to have a high correlation with TOD and has been shown to be accurate in estimating TOD from two hours up to 120 hours postmortem [1, 9-10, 14-15, 23-25, 27-28, 31-33, 35-36, 38-39, 42-43, 48, 51-52, 55]. After death, K+ concentration in the eye increases, particularly in the retina, as a result of "…the energy breakdown and the related cessation of active transport and selective membrane permeability" [52, p. 273]. In fact, "…the autolysis of the vascular choroids and retinal cells are thought to be responsible…" for this rise [1, p. 530]. While phosphorus has also been found to increase in proportion to time after death and sodium has been found to decrease proportionally after death, K+ remains the electrolyte that has the strongest statistical correlation to TOD and therefore is most often used [1, 9-10, 14-15, 23-25, 27-28, 31-33, 35-36, 38-39, 42-43, 48, 51-52, 55].

However, the average vitreous K+ values vary greatly among species. For example, in humans, the normal vitreous K+ values are between 2.6 to 4.2 milliequivalents/liter (meq/l) [1]. In white-tailed deer, the average normal vitreous K+ values are about 6.00 meq/l, while in canines the normal vitreous K+ values have been determined to be between 5.0 to 9.7 meq/l with a mean value of 6.6 meq/l [1, 23, 37].

Such discrepancies are problematic when comparing K+ levels between species. Therefore, inferring values determined in humans to other animals is not valid.

Some studies have uncovered specific problems in using vitreous K+ to estimate TOD that make its overall effectiveness questionable. These studies show that there can be differences in the electrolyte concentrations between the right and left eye [9, 31, 42]. While these reports raise questions about the effectiveness of using the concentration of vitreous K+ for estimating TOD, other studies have not found a difference between eyes [1, 14, 38-39, 51]. One way in which this particular problem can be overcome is by sampling and averaging the results of the VH collected from both eyes [32]. The authors of one study state, "While there are differences of single potassium values taken at identical times, the statistical parameters of potassium concentration with time since death for each eye do not differ from those of the mean value of both eyes: there are identical regression lines" [31, p. 235]. The use of CZE in sample analysis has been shown to result in negligible differences between eyes [28, 51-52].

Other factors can possibly influence the effectiveness of using vitreous K+ for estimating TOD. Some of these factors are ambient temperature, wind, moisture, sex, age, race, weight, body size, body handling, alcoholism, technical errors, lack of standardization of equipment, sampling errors, methods of analysis, sample handling and transport, sample storage, modality of death, and ante-mortem pathologies [1, 14-15, 23-25, 27, 31-32, 35-36, 38-39, 43, 48, 51-52, 55]. Not all of these can be eliminated, but most can be minimized by reducing the possibility of contamination, improving collection, storage, and analytic techniques, and using CZE for sample analysis [28, 51-52]. While there are still discrepancies among studies over whether or not there are

differences in the vitreous K+ levels between eyes, with proper attention to collection and analysis, using the average K+ values for both eyes, and analyzing samples using ISE or CZE methodologies, between eye differences can be minimized.

Many studies have examined ways to improve the accuracy in estimating TOD based on vitreous K+. In addition to using ISE or CZE analysis, some studies have examined the statistical regression equations based on the data collected to improve accuracy in estimating TOD. One study found that TOD estimation could be greatly improved by making vitreous K+ the independent variable and time the dependent variable used in regression analysis [39]. Most studies make K+ the dependent variable and time after death the independent variable. The authors state "… according to the mathematical approach to determine the regression line, the method previously used to obtain the value of the unknown is incorrect, giving a line adjusted to [K+] instead of to PMI, which could lead to serious errors in estimating PMI, and the estimation of the confidence interval is not correct on this formulation" [39, p. 210].

Other studies have used vitreous K+ levels in conjunction with other measures to improve TOD estimation. One study showed that by taking into account the length of the terminal episode, or rather how long it took for death to occur, and urea concentration, TOD estimation accuracy could be improved [9]. Another study showed that hypoxanthine levels when used in conjunction with vitreous K+ levels, can improve accuracy in estimating TOD [24]. The use of artificial neural networks, a computerized chemo-metrical method of analysis, in combination with CZE analysis can also improve the accuracy of estimating TOD [10].

Overall, the use of vitreous K+ to estimate time of death has many benefits yet has been shown in some studies to have serious drawbacks. Therefore, it is important to use other techniques in combination with vitreous K+ to estimate the early PMI.

Synovial Fluid in TOD Estimation:

HUMAN STUDIES:

Synovial fluid, the fluid which lubricates articular joints of the musculo-skeletal system has been used in human studies to estimate TOD and can be used in the same way as VH. This fluid has been thoroughly studied by rheumatologists and many printed resources are available for joint fluid analysis. However, few focus on medico-legal aspects. Most often the focus has been on alcohol concentration, cause of death, and drug distribution rather than TOD. One study [33], describes the fluid of the knee as being contained within the suprapatellar pouch and protected from the detrimental effects of putrefaction; therefore the components that make up synovial fluid are relatively stable over a prolonged PMI. Synovial fluid is collected by puncturing the suprapatellar pouch. The viscosity of the fluid makes aspiration difficult. After collection, the sample is either frozen or immediately centrifuged and can be analyzed by flame photometry. Potassium concentration in synovial fluid, as in VH, increases after death [1, 10, 14-15, 23-25, 27, 33, 35-36, 38, 43, 48, 55]. While K+ concentrations were found to be slightly higher in VH than synovial fluid, each fluid shows nearly equal concentration increases in K+ during the PMI. Therefore, synovial fluid may be used instead of or in conjunction with VH for estimating TOD [33]. One disadvantage of using synovial fluid is its viscosity which makes it harder to collect than VH. Proper placement of the needle in the suprapatellar pouch is potentially more difficult than collection of vitreous humor.

18

No known published studies covering synovial fluid as it applies to TOD have been conducted on animals including canines.

Pupillary Changes in TOD Estimation:

HUMAN STUDIES:

No known published studies of this type have been conducted on humans.

ANIMAL STUDIES:

Pupillary changes have been used to estimate TOD. After death, the pupil contracts due to body temperature change and the development of rigor mortis [21, 56]. This change can be measured (to the nearest 1/10 mm) with dial calipers [21]. Pupil diameter has been shown in some animal species such as deer to be significantly associated with a specific time of death, especially when used in conjunction with other TOD estimation techniques [21, 56]. Alone, pupil diameter has only a marginal predictive value, thus it should only be used in combination with other methods [56]. No known studies of this type have been conducted on canines.

Osmolality of the VH in TOD Estimation:

HUMAN STUDIES:

Due to the potential of using VH in predicting the PMI, methods other than K+ concentration measurement in VH have been studied. One method thought to perhaps aid in TOD estimation was the measurement of the osmolality in the VH. Osmolality is the measure of the pressure of a substance determined by the number of solute particles in the solution rather than the weight, shape, or charge of the particles [50]. This method of VH analysis is easy to perform, and since the sample is not destroyed when determining

osmolarity, its performance will not prevent other more destructive tests from being performed.

One study attempted to determine if osmolality could be used to predict TOD [50]. This study found that osmolality had no correlation to the PMI. Osmolalities were found to have a wide range and varied greatly. The authors' state: "The numerous factors inherent in natural disease, immediate response to injury, and autolytic processes following death undoubtedly contributed to this variation" [50, p. 390]. This study concluded that osmolality should not be used for the estimation of TOD. Even so, this study is important in understanding the many factors that may alter the usefulness of certain TOD estimating methods. Some of the same factors that limit the efficacy in using osmolality to estimate TOD may affect aspects of VH analysis and should be taken into consideration. In fact, some of the factors that are thought to cause variation in osmolality analyses may be reasons that there are discrepancies found among various VH studies.

ANIMAL STUDIES:

No known published studies of this type have been conducted on animals including canines.

Rigor Mortis Studies in TOD Estimation:

HUMAN AND ANIMAL STUDIES:

Onset and length of rigor mortis, the stiffening of skeletal muscles and joints after death, has been shown to be significantly associated with TOD [21, 26]. Rigor mortis is caused by calcium ions being pumped into muscle cells, which in turn causes the muscles to contract and the joints to be locked into place due to the force of the muscles [18]. The muscles remain contracted and the joints remain locked until the muscles begin to break

down during the decay process which can last up to 4 days at room temperature and up to 28 days at freezing temperatures. Development of rigor mortis is retarded by low temperatures (i.e., 25° C or less) and progresses rapidly at temperatures at or above 37^oC [26, 54]. Rigor mortis is a biphasic process in which the early onset of rigor occurs in red skeletal muscle fibers, which contract slowly after stimulation, while the late onset of rigor occurs in white skeletal muscle fibers, which contract quickly after stimulation [26].

Rigor mortis follows a physico-chemical process which takes place in all muscles simultaneously. Therefore, it is hypothesized that rigor mortis should progress more rapidly in muscles with little mass [26]. Hence, joints surrounded by small muscle mass would become immobilized sooner than joints surrounded by larger muscle mass [26]. For example, in humans, rigor has long been thought to progress down the body from the temporomandibular joint. A small joint such as the temporomandibular joint, which is surrounded by a small volume of muscle mass, should, therefore, become immobilized sooner than large joints surrounded by larger muscle masses [26].

This hypothesis was refuted by one study which found that the volume of the muscle did not influence the resolution or progression of rigor mortis. This study demonstrated that postmortem biological changes and rigor mortis in muscles do not progress in all muscles at the same time, and there are no differences in resolution and development of rigor mortis between differing muscle volumes [26]. Muscle volume had no effect on rigor mortis progression. Temperature does, however, have an effect on rigor mortis progression. The study found that since temperature decreases at different rates in different joints based on joint size, smaller joints cool faster than larger joints [26]. For example, muscles in the wrist, hands, feet, etc. cool at a faster rate than muscles in the

21

shoulder or hips. Therefore, joint temperature may affect the sequence of rigor mortis development [26].

Morphological and functional differences among different joints may also affect onset and resolution of rigor mortis. Each joint looks and acts differently in their function and that is the reason for variation in rigor mortis development in muscles in different joints. Pre-rigor load affects the resolution or onset of rigor mortis in long muscles, but does not affect the time for rigor mortis to reach full or even half extent. Because of this, the arm and leg positions at death do not affect the time for rigor mortis to reach half or full extent, but it can affect the onset or resolution [26]. All of these variables should be carefully considered when choosing the muscles and joints used to measure rigor mortis.

Rigor mortis can be measured by using an isometric sensor or by using a device that can duplicate the flexion of the joint in which rigor mortis occurs [21, 26]. In a study involving twenty-eight white-tailed deer, a device was constructed from two pipes, attached by a bolt, and designed to fold to form a 90° angle. The diameters of the pipes were large enough to allow them to slide over the foreleg of the deer with the angle made by the two pipes positioned at the wrist joint. Using a torque wrench on the bolt head connecting the two pipes, a measure of rigor mortis was obtained. No statistically significant differences were found between the left and right foreleg rigor measurements, therefore, measurements were obtained from both forelegs in each animal [21].

The results of this study showed rigor mortis to be significantly correlated to TOD but ambient temperature can affect rigor measurements which make them of questionable use under field conditions when ambient temperature is below 20° F (-6.67^{o} C) [21]. By itself, rigor mortis does not provide a high level of accuracy in predicting TOD, but

22

overall accuracy can be increased when rigor is used in combination with body temperature [21]. Due to these limitations, rigor mortis measurement alone may not be the most suitable method for estimating TOD. Of course this study was limited in scope and perhaps the selection of a different joint or the use of a better method for measuring rigor mortis may produce better results and additional studies should be conducted. At present, rigor mortis measurement should only be used after careful consideration and in combination with other measures. No known published studies of this type have been conducted on canines.

Hypostasis in TOD Estimation:

HUMAN STUDIES:

Hypostasis is the discoloration, or darkening, of the skin as blood settles in the deceased due to gravity and is discernible visually from 20 min to 2 hours after death [53]. It reaches maximum intensity at 6-9 hours and is fixed permanently at 3-5 days after death depending upon observable putrefaction changes, and can also be used to determine if a body has been moved after death from its original position [53].

Hypostasis has, until recently, received little attention and few studies have been published to determine the relationship between hypostasis and TOD. In order to explore its relationship to TOD, one study focused on the intensity (lightness) of the hypostasis in the skin of human cadavers as measured by a tristimulus colorimeter. The change in the lightness of the skin was found to decrease (become darker) as PMI increased. This shift was found to be most profound in the first 12 hours after death. However, hypostasis was found to be useful in estimating TOD for up to 48 hours. After this, all changes were found to be unreliable [53].

23

This was a preliminary study that demonstrated that colorimetry can be used to assess the color changes and intensity over time at various postmortem intervals. The results of this study have shown "…that there is a consistently predictable rate of alteration in the position, or distribution of hypostasis, which is dependent on how long the person has been dead" [53, p. 27].

There are, however, some factors that affect the usefulness in using hypostasis in estimating TOD. These factors include age, sex, body build and size, position of the body, cause of death, skin color, and environmental temperature [53].

ANIMAL STUDIES:

No known published studies of this type have been conducted on animals including canines. This technique may be less useful in estimating TOD in animals because of the presence of hair, which could impede or even prohibit the quantification of color change. Even so, the promising results of hypostasis in estimating TOD should be explored.

Skinfold Impedance in TOD Estimation:

HUMAN STUDIES:

There are no known published studies of this type that have been conducted on humans.

ANIMAL STUDIES:

Electrical impedance (EI) is the opposition in an electrical circuit to the flow of an alternating current [44-46]. In biological tissue, the equivalent electrical circuit is composed of a resistor, combined with a capacitor in parallel with another resistor.

Cytoplasmic impedance represents the first resistor and cell membrane represents the capacitor, while the second resistor is represented by the extracellular fluid.

This technique has been found to be correlated with TOD in rats [44-46]. The EI was measured in the skinfold of the lateral abdomen, due to its ease of access. After anaesthetization and death, hair covering the lateral abdomen was removed and the body was laid on a cork mat in a supine position. The skinfold was impaled by the electrodes into the cork mat in two places and left undisturbed throughout the experiment. Electrical impedance was measured at specified postmortem intervals. The results showed time-dependent postmortem changes in the electrical impedance and resistance in biological tissue and impedance was found to increase exponentially during the first 120 hours post mortem. The changes in electrical impedance reflect both autolytic and temperature changes during the early PMI. Impedance increased rapidly during the first 24 hours which could be due to temperature changes, while changes from 24 to 120 hours are mainly due to autolytic changes. The data collected in this study showed that skinfold impedance followed a time course that was predictable during the first five days after death and the promising results of this study warrant similar studies in humans to determine if skinfold impedance can be used to estimate the early PMI in human cadavers [44-46]. No known published studies of this type have been conducted on canines.

Electrical Excitability of Skeletal Muscle in TOD Estimation:

HUMAN STUDIES:

The technique of measuring the electrical excitability (EE) in skeletal muscle to determine TOD has been utilized since the late 19th century [30]. After death, the ability for muscles to contract decrease over time. By measuring this decrease, an estimated

TOD can be predicted. Most techniques used for measuring EE for the purpose of estimating TOD involve subjective grading based on observation of the strength and spread of the muscular contraction after excitation, but these grading systems are so subjective that they cannot be compared with one another. The use of a force transducer, which measures muscular contraction directly, in combination with electrical stimulation, can reduce this subjectivity.

This technique involves a force transducer, originally used in experimental cardiac surgery, being inserted into the muscle of the corpse by way of a hypodermic needle. Then the muscle is stimulated every half hour using a square wave generator until electrical excitability of the muscle has expired. The decrease in muscle contractions has a linear relationship with TOD and can be used in humans to estimate TOD up to 10 hours [30].

Two factors that affect the accuracy of EE to estimate TOD are the glycogen content of the muscle at TOD and the environmental temperature. Electrical excitability appears to last longer as environmental temperature decreases. Also, there is great inter-individual variability in the duration of electrical excitability [30]. More investigation is needed to determine if EE measurement in skeletal muscles is an accurate and reliable method for estimating TOD.

ANIMAL STUDIES:

No known published studies of this type have been conducted on animals including canines

Nerve Conduction in TOD Estimation:

HUMAN STUDIES:

No known published studies of this type have been conducted on humans.

ANIMAL STUDIES:

There is a theoretical relationship between declining nerve conduction properties and TOD due to cessation of nerve metabolic activity. By measuring this decline in nerve conduction, it may be possible to estimate the PMI during the first few hours after death [16, 49].

Two preliminary studies were conducted in rats to determine if TOD could be estimated by this method [16, 49]. Both studies examined nerve conduction in the sciatic nerve of rats (n=12 and n=10, respectively) before and after death. The entire length of the right sciatic nerve was exposed and cleared of connective tissue. An electrode was placed at the most proximal distal parts of the nerve and square wave stimulating pulses were delivered. Data were recorded every 5 minutes for up to 60 minutes after death. The excitability of the rat sciatic nerve showed a reproducible mathematical relationship to the PMI. Neurological state, alcohol, and medication were found to possibly affect the accuracy of using nerve excitability to estimate PMI [49].

The results in humans may vary greatly from those found in rats. In humans, the excitability may last longer and therefore provide a longer PMI estimate, but such studies have not been done [16, 49]. No known published studies of this type have been conducted on canines.

Absolute Refractory Period in TOD Estimation:

Human Studies:

No known published studies of this type have been conducted on humans.

Animal Studies:

Absolute refractory period (ARP) is the interval following action potential in which a nerve cannot propagate another action potential when stimulated [34]. The rat sciatic nerve was used in a study to investigate the relationship between PMI and the duration of the ARP. In preparation for ARP determination, a rat was anesthetized and killed and the left leg was shaved and washed. The body was then placed on an electrically grounded table and one electrode was placed 2 cm distal to the sciatic notch of the biceps femoris muscle and a recording electrode was placed in the extensor digitorum longus muscle. A square wave was used for muscle stimulation and ARP was recorded every 5 minutes until no changes could be detected. The correlation between TOD and ARP was found to be strong and similar to the correlation between temperature and TOD. The correlation was strengthened when ARP and temperature data were combined. On the whole, the results from this study show that the use of ARP may improve the reliability and accuracy of other TOD techniques [34]. No known published studies of this type have been conducted on canines.

Pericardial Fluid in TOD Estimation:

Human Studies:

Pericardial fluid analysis has been studied as a method to estimate TOD. As with VH, K+ in the pericardial fluid has been found to be directly proportional to the time since death. Pericardial fluid has one distinct advantage over VH and CSF in that is can

be collected in relatively large quantities (10-15 ml) [8]. The volume collected allows more tests to be run with fewer restrictions based on the volume of available sample. The fluid is collected by opening the thoracic cavity, removing the sternum, opening the pericardium with scissors, and aspirating the fluid into a 10 ml syringe. After collection, the sample is centrifuged and frozen until analysis by flame photometry. While pericardial fluid shows an increase in K+ concentration after death, variations found in individual results at the same time after death limit the usefulness of this measure. The biochemical changes that occur in the pericardial fluid are neither constant nor predictable and do not allow for an accurate estimation of TOD. However, vitreous humor provides a more accurate estimation of TOD than pericardial fluid. For this reason, pericardial fluid analysis should not be used for the estimation of TOD.

ANIMAL STUDIES:

No known published studies of this type have been conducted on animals including canines.

Use of Blow Fly Eggs in TOD Estimation:

HUMAN STUDIES:

Until recently, entomology has been used to estimate TOD in the later postmortem period (e.g., minimum of several days after death) and is usually based on insect larvae age estimation or age estimation in other life stages [6, 11]. "Under favorable ecological conditions, the flies of the family Calliphoridae are attracted by corpses rapidly after death, and deposit their eggs almost immediately in natural openings" [11, p. 27]. While the age of these eggs is not widely used to estimate the early PMI, this technique should be considered as another method which can be employed by

29

the forensic investigator [6, 11]. Estimating egg age is difficult, but the best method

"…is to await hatchings and to estimate the time of laying according to the temperatures

during incubation" [11, p. 27].

One study conducted in the past three years used common blow fly, *Lucilia*

sericata (Diptera, Calliphoridae) eggs to estimate the early postmortem interval. This

study measured the hatching times of the blow fly eggs at different ambient temperatures.

Tables were created showing incubation periods at different temperatures, which were

then used to estimate the early PMI. Results obtained were promising with a precision in

estimating PMI of 2-100 hours [11].

ANIMAL STUDIES:

No known published studies of this type have been conducted on animals

including canines.

Computer Programs and Formulas to Aid in TOD Estimation:

HUMAN AND ANIMAL STUDIES:

Computer programs, temperature-based algorithms, and predictive mathematical

equations and models are used in combination with certain TOD indicator data to predict

a post mortem interval. Some of these programs or equations use only temperature-based

data, but some more complicated programs or equations use additional TOD indicator

data. Investigators in the field can input temperature readings, pupil diameter sizes, rigor

measurements, or other indicators, into the program/equation and acquire a TOD

prediction [3-4, 13, 21-22, 29, 37, 40]. The main goal of these studies was to develop

ways in which a simple computer program, cooling curve table, or field manual can be

written so that investigators at crime scenes can obtain a quick and accurate TOD

estimation with minimal training and with minimal disturbance of the body [3-4, 13, 21-22, 29, 37, 40]. The use of programs, equations, and models also have the added benefit of increasing the accuracy and reliability of TOD estimation by limiting the variations caused by environmental temperature and conditions, unknown body temperatures at time of death, and other potential sources of bias [3-4, 13, 21-22, 29, 37, 40]. Some of these referenced TOD formulas and equations are included in the appendix (Appendices 4-1 to 4-11). No known published studies of this type have been conducted on canines.

SUMMARY:

The two main methods most often used in human forensic cases to estimate the early PMI are body temperature changes after death (primarily using rectal temperature) and electrolyte concentration changes in the VH (typically K+ concentration). Many studies have been conducted to improve the accuracy of estimation of the early PMI and one lesson learned is that the science of determining early PMI is incomplete and subject to error. There are numerous potential measures for determining the early PMI and TOD and the overall conclusion is that no one method appears to be solely reliable. For temperature based TOD estimation approaches, the use of multiple recording sites gives a more accurate estimation for TOD than the use of only one site. It has also been shown that temperature based methods are more accurate than biochemical or physical ones, yet some combination of these techniques is the best approach.

The aims of this research were to take selected TOD measures and apply them to the purpose of expanding the knowledge concerning TOD determination in canines. Canines were chosen because little TOD research has focused on them, and the fact that dogs are culturally important to humans since many households own at least one [5]. This

type of research could be beneficial for the prosecution of animal cruelty cases, teaching forensic techniques, and may be applicable to human forensic investigations in cases where there are human and canine deaths. The main measures chosen for this study included the decline of temperature of the brain, liver, rectum, and external ear canal and analysis of the K+ levels in the VH after death. Recording data on these measures are relatively easy, inexpensive, and have been shown in many studies to be the least controversial and most accurate means for estimating TOD.

CHAPTER II

MATERIAL AND METHODS:

FOCUS OF STUDY:

Sixteen adult domestic canines euthanized at a local animal shelter were used for the purpose of collecting data to estimate the early post-mortem interval (PMI) in domestic canines. Samples and data collected included: vitreous humor (VH) for the purpose of evaluating vitreous K+ concentration; temperature readings over time from the rectum, liver, brain, and external ear canal; hair coat samples to calculate density of hair; body mass estimation; and weight.

STUDY DESIGN & METHODS:

Measurements were taken from 8 adult male and 8 adult female mixed bred domestic dogs euthanized at a local animal shelter. No dogs were euthanized specifically for this study. The chair of the University of Tennessee IACUC determined that a protocol was not required for this study (letter on file and available upon request). All dogs were ≥ 6 months of age, based on lack of deciduous premolars [17]. After death was confirmed by auscultation, one over-the-counter (OTC) Digital Thermometer with Accu-Beep™ feature (Becton Dickinson (BD) Franklin Lakes, New Jersey, USA) (Appendix 1-1) was placed in the rectum to a depth of approximately 2 cm and a temperature measurement was recorded and the thermometer removed. Another BD Digital Thermometer was placed in the ear canal to a depth of approximately 2 cm and an aural temperature measurement was recorded and the thermometer removed. The OTC

temperature readings were recorded in Fahrenheit and converted to Celsius for data analysis. Immediately following the OTC temperature measurements, a VIP-T series probe (Omega Engineering, Inc., Stamford, Connecticut, USA) connected to a model OM-CP-QUADTEMP 4-channel continuous temperature measurement data logger (Omega Engineering Inc., Stamford, Connecticut, USA) (Appendix 1-2), was inserted in the dog's rectum, replacing the OTC thermometer, to a depth of approximately 5 cm. The body was then placed in a plastic container and transported to the University of Tennessee Veterinary Teaching Hospital (UTVTH). Once there, each body was weighed and placed on the left side on a table in a secure room with relatively constant temperature, humidity, and air flow.

An approximate 10 cm long incision was made in the abdomen between the 9th and 10th ribs halfway between the spinal column and costal arch to gain access to the liver. A second VIP-T series probe was inserted in the liver to a depth of approximately 4 cm and connected to the temperature data logger. Due to the limited temperature reading range imposed by using a normal OTC thermometer, a Hanna Instruments, Checktemp Digital Chemical Thermometer (Celsius Model) (Woonsocket, Rhode Island, USA) (Appendix 1-3) was used instead. It was placed in the liver to a depth of approximately 4 cm and about 5 cm from the VIP-T series probe. The incision was then closed around the probe and the chemical thermometer using two standard towel clamps (Adler Instrument Company, Norcross, Georgia, USA). Temperature was periodically and manually recorded from the chemical thermometer.

An approximately 1.5 cm incision was made in the scalp midway on a line running from the medial point of the left eye to the medial aspect of the right ear canal to

allow access to the skull. Using a surgical trephine (Adler Instrument Company, Norcross, Georgia, USA) (Appendix 1-4), a 0.8 cm diameter hole was drilled through the skull. A third VIP-T series probe was inserted through this opening into the brain to a depth of approximately 4 cm and then connected to the temperature data logger. The skin over the hole was closed around the probe using two standard towel clamps (Adler Instrument Company, Norcross, Georgia, USA).

A fourth and final VIP-T series probe was inserted to a depth of approximately 4 cm into the external ear canal secured to the ear using masking tape and then connected to the temperature data logger. The temperature data logger was set to record the temperature of each probe at five minute intervals.

Another Checktemp Digital Chemical Thermometer (Celsius Model) (Woonsocket, Rhode Island, USA) was placed in the rectum to a depth of approximately 4 cm and a rectal temperature reading was recorded. Environmental temperature and humidity readings were obtained and recorded using a temperature-hygrometer combination (Fisher Scientific, Suwanee, Georgia, USA, catalog number 11-661-13) (Appendix 1-5).

After the initial set-up was completed, a rectangular patch of hair was removed from the upper right thoracic region (between the 3rd and 6th vertebrae) of the body using hair clippers (WHAL Clipper Corporation, Sterling, Illinois, USA). All clipped hair was placed in a labeled zip-lock bag and stored for weight determination. The area of the site of hair removal was measured and recorded. Dimensions of the clipped site and weight of hair removed from the site were used to calculate hair coat density.

Vitreous humor was extracted at approximately 1.5 hours after death from each eye in separate syringes (not pooled) using a 1 ml syringe and an 18 gauge x 1.5 inch needle (Fisher Scientific, Suwanee, Georgia, USA). The needle was used to puncture the sclera and withdraw approximately 0.5 ml of VH from the posterior chamber of each eye. Labeled samples were refrigerated for later analysis.

Temperature readings from each VIP-T probe were recorded by the data logger every five minutes. Environmental temperature and humidity readings, using the temperature-hygrometer, and rectal and liver temperature readings using the two digital chemical thermometers were recorded by the author approximately every hour for the first seven hours after death. At approximately seven hours after death, a second collection of vitreous humor was obtained from both eyes (approximately 0.5 ml each), labeled, refrigerated and stored (approximately 48 hours) until analysis using the Hitachi 911 Automatic ion specific electrode analyzer (Boehringer Mannheim Corporation, Indianapolis, Indiana, USA) by the College of Veterinary Medicine's Department of Pathobiology Clinical Pathology Laboratory.

The body was left undisturbed overnight in the secure room during which automatic temperature readings were taken by the data logger. At approximately 31 hours after death, environmental temperature and humidity readings and rectal and liver temperature readings were recorded by the author. Observations ended at 32 hours after death at which time environmental temperature and humidity readings and rectal and liver temperature readings were recorded for a final time.

In addition, the minimum and maximum ambient temperature and humidity over the length of time each body was being monitored, was recorded for 12 of the 16 dogs.

After disconnecting all probes and cleanup, the body was immersed in a container filled to the rim with water. Displaced water was collected in a containment pool under the container and measured to determine body volume. Data from the data logger were downloaded onto a laptop computer for analysis.

Hair samples collected from each dog were weighed using a Fisher Scientific analytical balance (Suwanee, Georgia, USA) calibrated to the nearest 0.001 gram. General hair coat mass was determined by dividing the weight in grams of the hair by the area in cm^2. Body mass was calculated by dividing body weight by body volume measurements.

Data analyses were performed using JMP 6.0.0 statistical software by SAS Institute Inc. (Cary, NC, USA) at the 5% significance level unless noted. Descriptive statistics were generated using the "Distribution" analysis command in JMP, which analyzes data via univariate analysis. The significance level of specific variables on mean temperature decrease after death at each measurement site was generated using the "Fit Y by X" analysis command in JMP, which analyzes data via bivariate analysis or simple linear regression in which the distribution of one continuous variable is compared to another continuous variable. Analysis of VH was completed by the "Matched Pairs" analysis command in JMP, which analyzes the data by comparing the means of two responses. All graphs were generated by the "Overlay Plot" command in JMP.

CHAPTER III

RESULTS:

Descriptive Statistics for Dogs Used in Study

Descriptive statistics on the dogs used in the study are displayed in Figures 1-4. Of the 16 dogs, eight were male and eight were female with an average weight of 16.29 kg (median = 14.79 kg, range 8.53 kg - 26.17 kg, standard deviation (SD) = 6.05 kg) . Body volume had a mean of 19.19 L with a median of 16.85 L, range 9.30 - 38.62 L and a SD of 8.46 L. Mean body mass was 0.88 kg/l with median 0.90 kg/l, range 0.66 kg/l - 1.02 kg/l, and a SD of 0.12 kg/l. Hair coat density had a mean of 0.03 g/cm^2, a median value of 0.03 g/cm^2, and ranged from 0.01 g/cm^2 to 0.06 g/cm^2. The SD was 0.1 g/cm^2.

Body Temperature Data

Mean Temperatures at TOD Using OTC Thermometer N=16

Mean rectal temperature as measured by the OTC digital thermometer at TOD was 38.5 ^{o}C with a range of 37.4 ^{o}C - 39.9 ^{o}C and a SD of 0.59 ^{o}C. The mean aural temperature as measured by the OTC digital thermometer at TOD was 38.0 ^{o}C with range 37.2 ^{o}C - 39.3 ^{o}C and a SD of 0.46 ^{o}C.   All sixteen dogs from this study were compared to determine at what time after death the rectal and aural temperature fell below 32°C (90°F), the thermometer's lower temperature recording limit. Rectal temperature for all dogs fell below 32°C by 520 minutes (8.67 hours) after death and had a mean of 341.88 ± 98.32 minutes after death with range 185 to 520 minutes after death. Aural temperature for all dogs fell below 32°C by 275 minutes (4.58 hours) after death and had a mean of
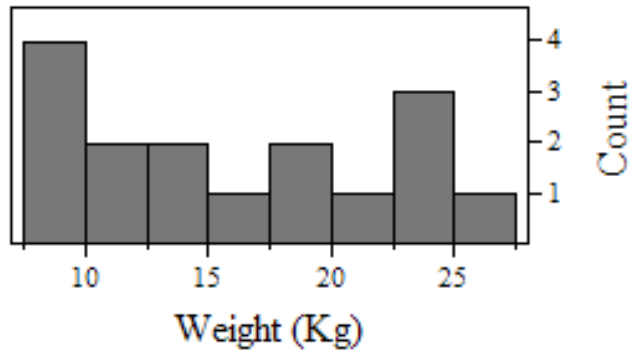
Figure 1:
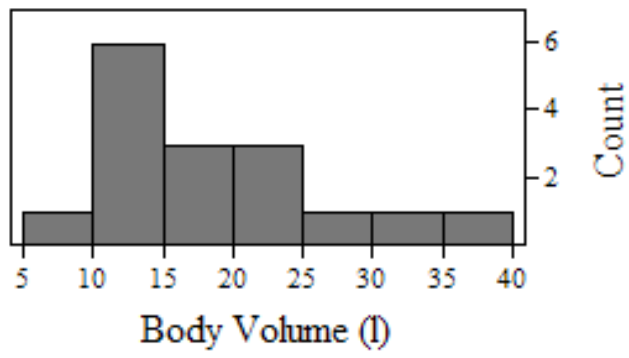**Weight Distribution for all Dogs in Kilograms (kg) N=16**

Figure 2:
Body Volume Distribution for all Dogs in Liters (l) N=16
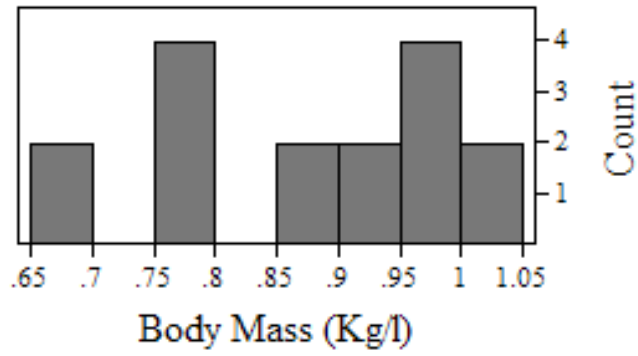
Figure 3:
Body Mass Distribution for all Dogs in Kilograms/Liter (kg/l) N=16
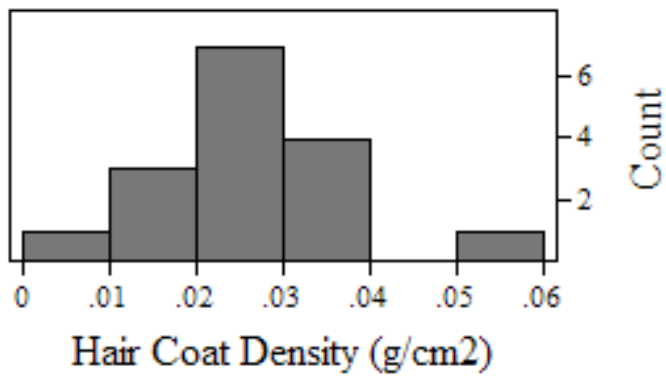
Figure 4:
Hair Coat Density Distribution for all Dogs in Grams/Centimeter^2 (g/cm^2) N=16

150.94 ± 58.72 minutes after death with range 75 to 275 minutes after death.

Placement Time of Data Logger Temperature Probes:

The rectal probe for the temperature data logger was placed at the shelter and relatively quickly after death was confirmed - mean of 6.0 minutes after death with a range of 3.0 – 11.0 minutes and a standard deviation of 2.17 minutes. The placement of the other probes was delayed due to transport to the study site. This was necessary because a more stable setting (e.g., no movement and relatively constant ambient temperature and humidity) was desired prior to surgical access to the liver and brain and placement of those probes. The liver probe was placed within a range 46.0 – 59.0 minutes after death with a mean of 50.8 and a standard deviation of 3.83 minutes. The mean placement time for the brain probe was 55.6 minutes after death with a range of 49.0 – 64.0 minutes and a standard deviation of 5.2 minutes. Finally, the aural probe was placed within a mean of 57.2 minutes after death with a range of 50.0 – 65.0 minutes and a standard deviation of 5.0 minutes.

Data Logger Descriptive Statistics and Probe Results for All Dogs:

A detailed display of the data logger ambient and probe temperature recordings for each dog are available in the appendix (Appendices 2-1 to 2-6). Using mean temperature observations on each dog, data logger temperature results for each probe were plotted over time to show graphically the decline of temperature (Figures 5-8). The mean temperatures by probe site for all dogs were combined and plotted (Figure 9) to display the visual relationship in temperature decline by probe site. Included in the appendices are overlay plots for all 16 dogs' mean temperature readings at each probe site (and ambient temperature) (Appendices 3-1 to 3-5).
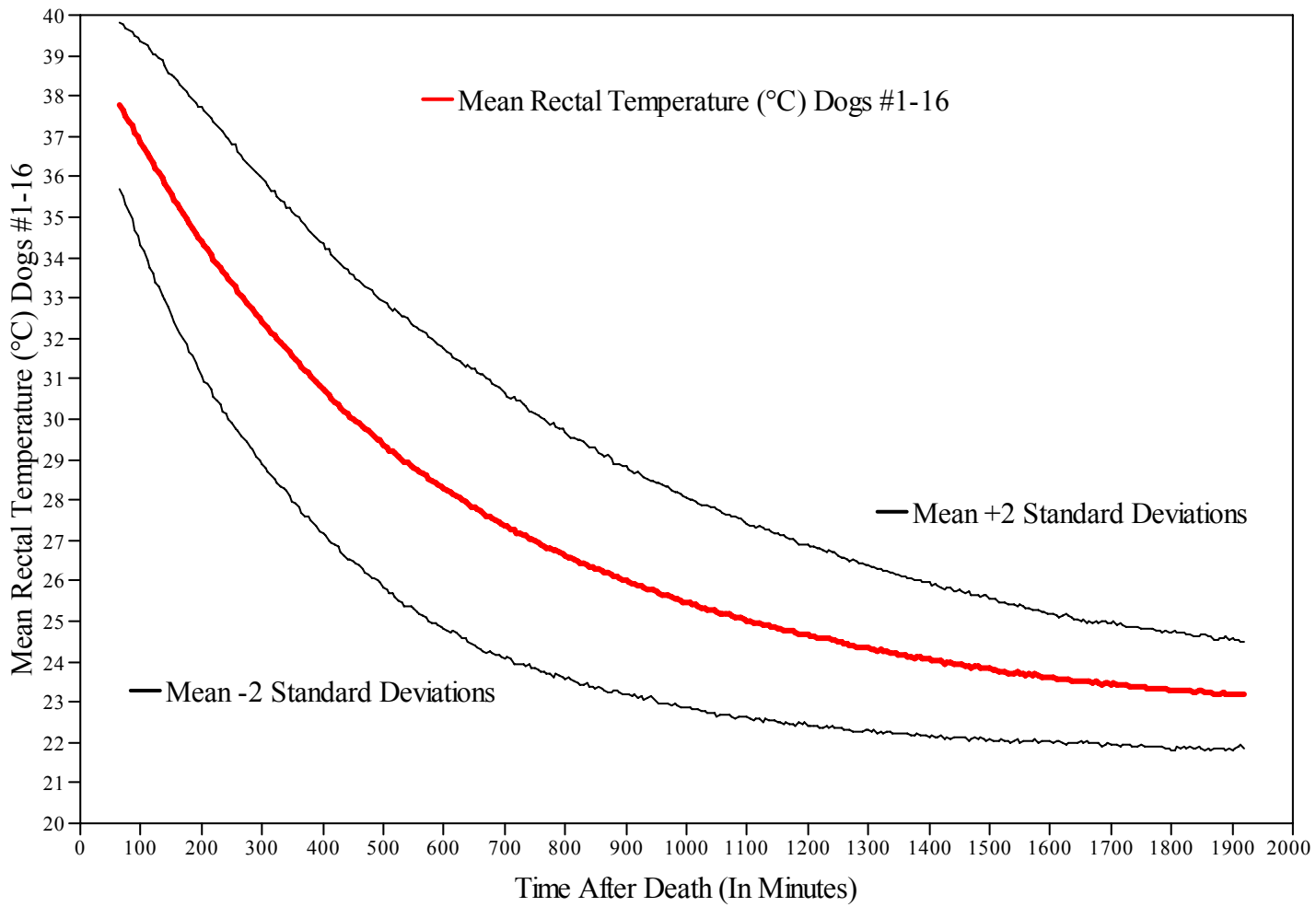
Figure 5:
Mean Rectal Temperature Readings (°C) for all Dogs every 5 minutes (N=16)
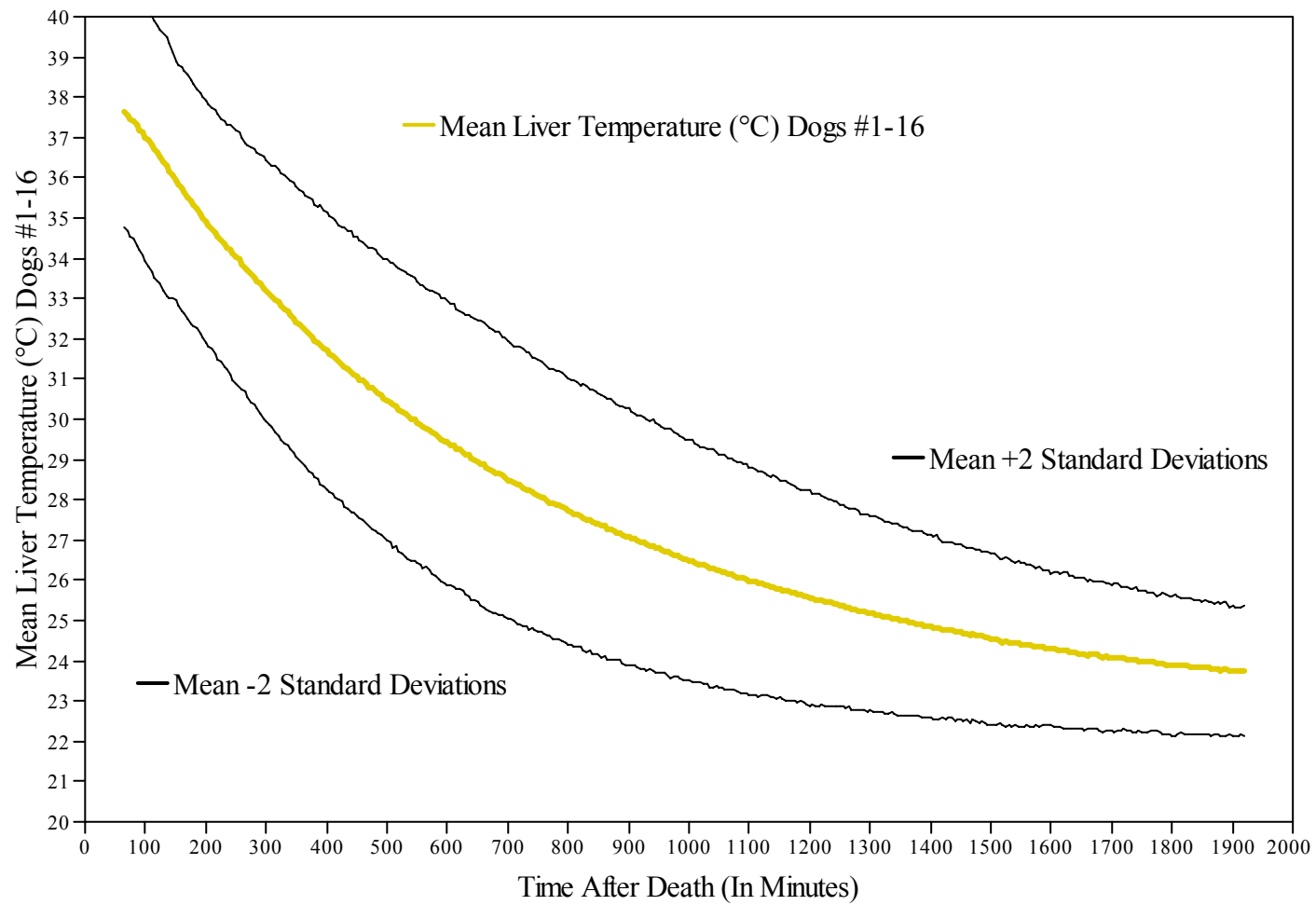
Figure 6:

Mean Liver Temperature Readings (^{o}C) for all Dogs every 5 minutes (N=16)

43
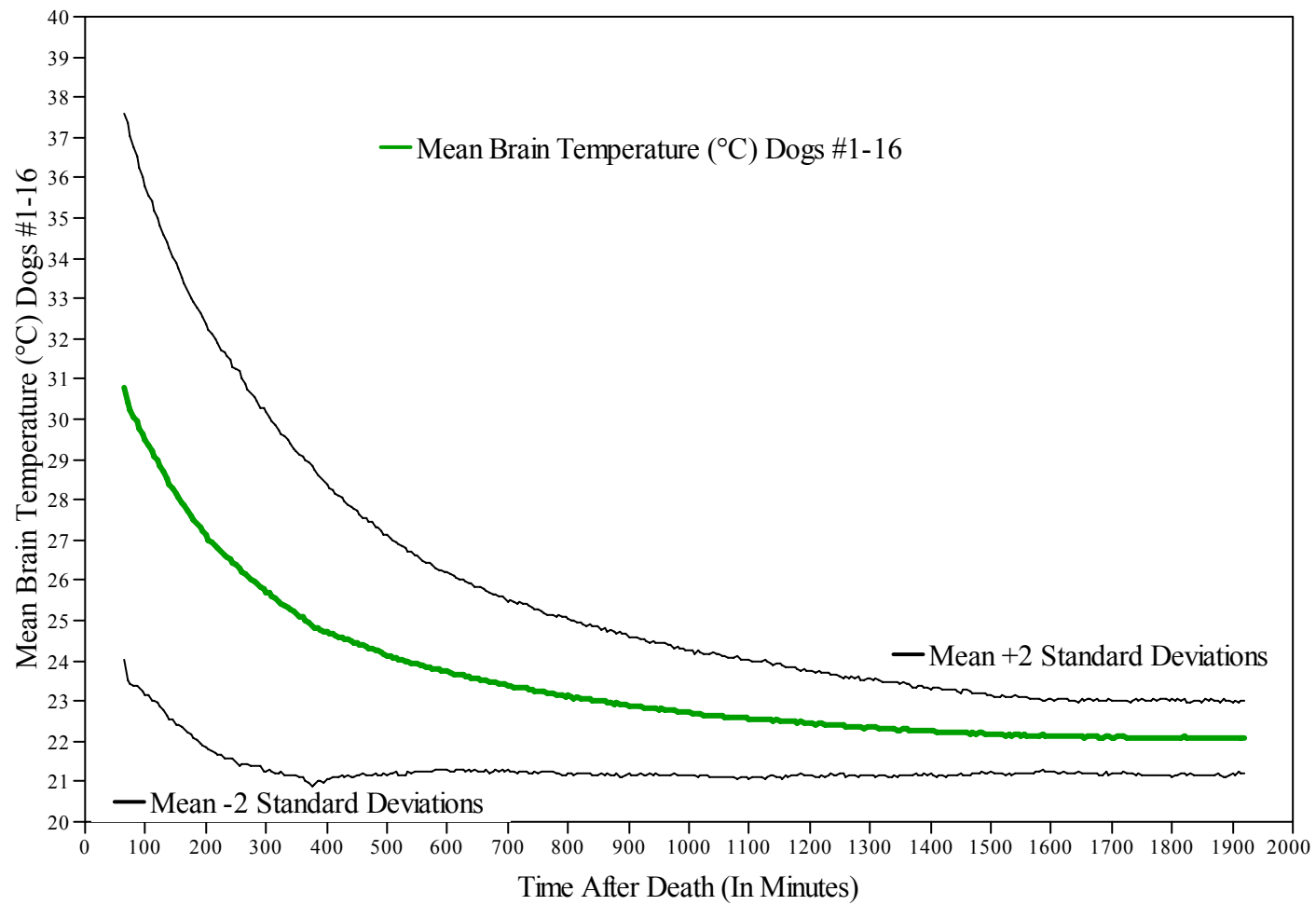
Figure 7:
Mean Brain Temperature Readings (^{o}C) for all Dogs every 5 minutes (N=16)

44
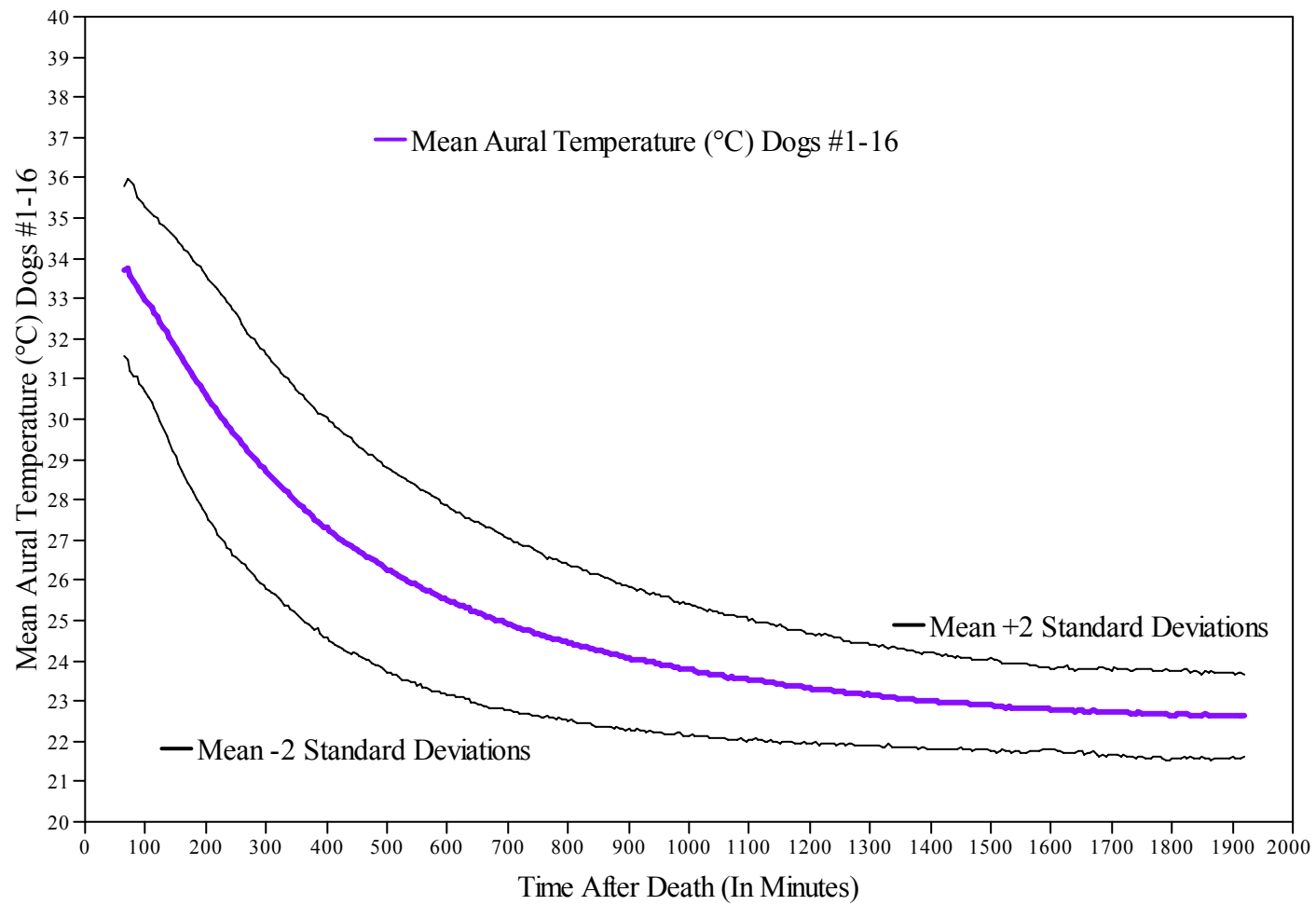
Figure 8:
Mean Aural Temperature Readings (^oC) for all Dogs every 5 minutes (N=16)
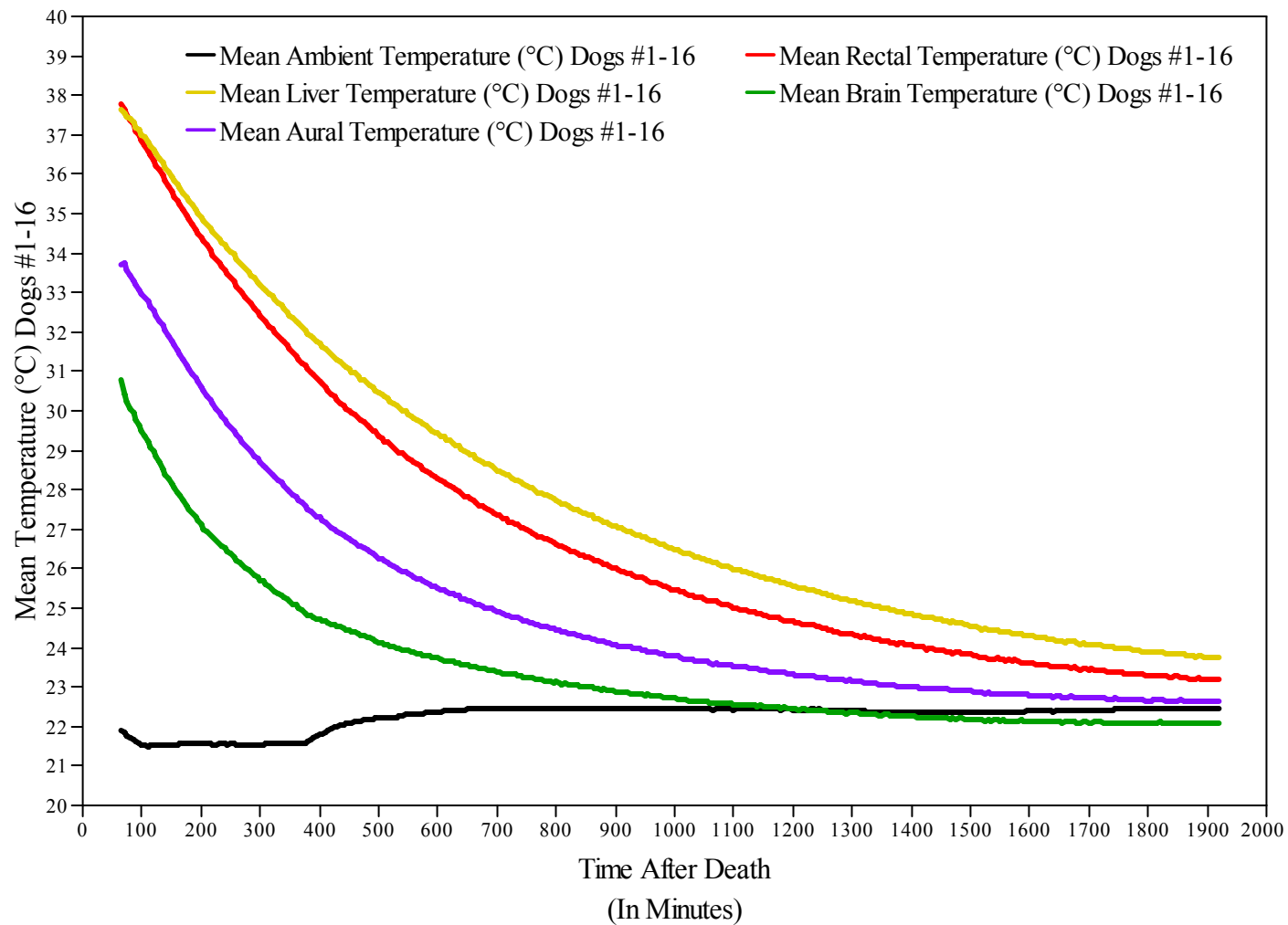
Figure 9:
Data Logger Mean Temperature Readings (°C) by Probe for all Dogs (N=16)

46

Correlation of Data Logger Mean Rectal and Mean Liver Curves:

The data logger mean rectal probe temperature readings were compared with the data logger mean liver probe temperature readings using the "fit Y to X" in JMP to determine the correlation between the two temperature collection sites. The RSquare value for the correlation between the to temperature collection sites was calculated to be 0.996 indicating a virtually perfect fit.

Manual Chemical Thermometer Temperature Readings:

Rectal temperatures of all dogs, as measured by chemical thermometers declined from 39.9 ^{o}C to 22.4 ^{o}C with a mean of 31.6 ^{o}C and a SD of 5.43 ^{o}C. Liver temperatures of all dogs, as measured by chemical thermometers, ranged from 22.6 ^{o}C to 39.3 ^{o}C with a mean of 32.5 ^{o}C and a SD of 5.41 ^{o}C.

Comparing the Data Logger to Manual Chemical Thermometers:

The rectal and liver data logger temperature results were compared with manual rectal and liver chemical temperature results made at corresponding times and matched favorably (Figures 10-11). Using the "fit Y to X" command to compare the mean chemical thermometer readings at 8 points during the observation period with the data logger probe readings at the same points in time gave an RSquare value of 0.999 indicating a virtually perfect fit. Using the "fit Y to X" command to compare the mean chemical thermometer readings at 8 points during the observation period with the data logger probe readings at the same points in time gave an RSquare value of 0.999 indicating a virtually perfect fit.

─ Mean Datalogger Rectal Temperature (°C) Dogs #1-16
▪ Mean Chemical Rectal Temperature (°C) Dogs #1-16
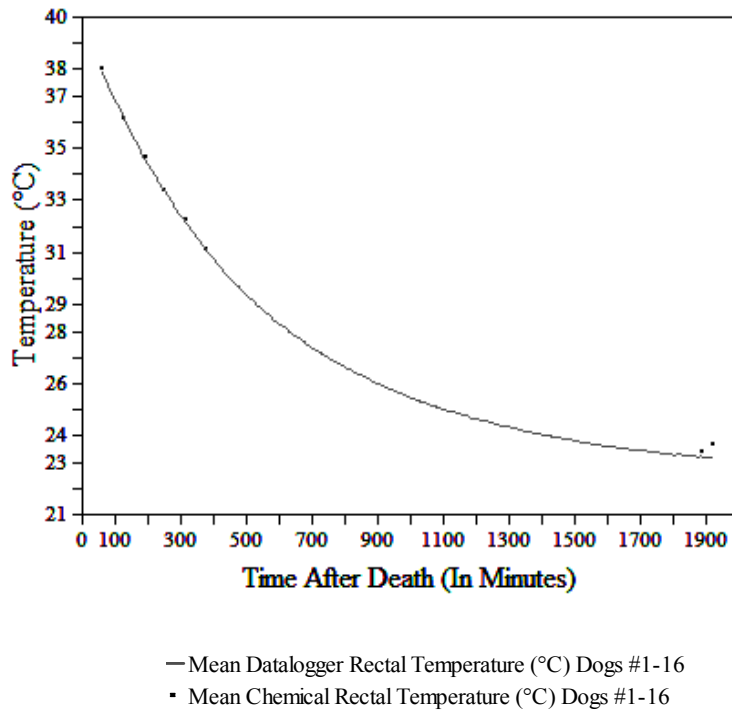
Figure 10:

Mean Rectal Data Logger Temperature Curve Compared with Mean Rectal Chemical Temperature Readings (°C) Over Whole Study for all Dogs

─ Mean Datalogger Liver Temperature (°C) Dogs #1-16
▪ Mean Chemical Liver Temperature (°C) Dogs #1-16
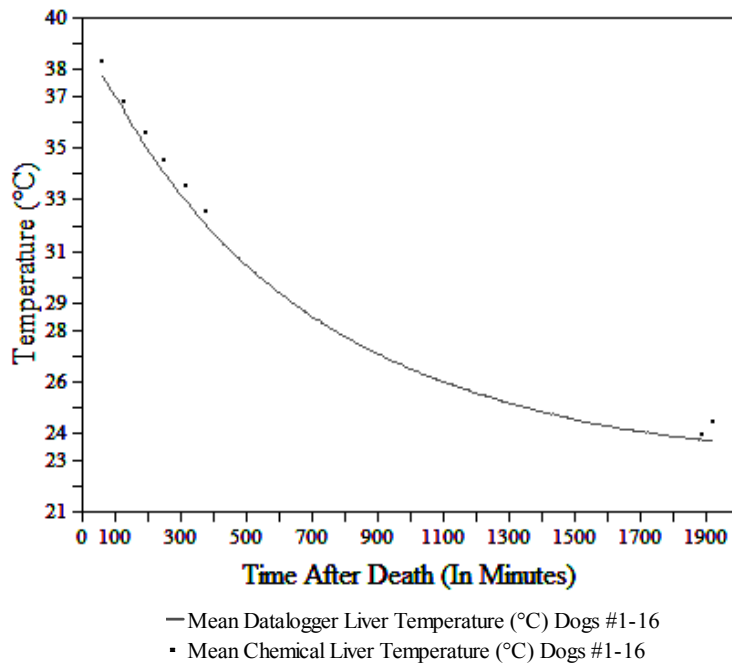
Figure 11:

Mean Liver Data Logger Temperature Curve Compared with Mean Liver Chemical Temperature Readings (°C) Over Whole Study for all Dogs

Ambient Humidity and Ambient Temperature Manual Readings:

The ambient temperature over the 32 hour observation period for each dog had a mean of 21.5 $^{\circ}$C and range of 20.7 $^{\circ}$C - 22.7 $^{\circ}$C and a SD of 0.55 $^{\circ}$C. The ambient humidity for the same period had a mean of 41.5% and range 29.0% - 59.0 % and a SD of 11.0%.

Comparison of Mean Temperatures and Descriptive Variables of Dogs:

Sex was not associated with temperature decrease after death for any of the data logger probe sites (Table 1), while weight was statistically significantly associated with every probe site except for the rectal temperature probe (Table 2). The heavier the dog, the more slowly the liver, brain and external ear canal cooled. The association remained significant when dogs with the four lowest weights were compared to those with the four highest weights (Tables 3-5). Body volume was found to be statistically significantly associated with temperature decrease after death for all of the data logger probe sites (Table 2). The more volume a dog had, the more slowly the rectal, liver, brain and external ear canal cooled. The association remained significant when dogs with the four lowest weights were compared to those with the four highest weights (Tables 6-9). Body mass, however, was found to only be statistically significantly associated with the aural temperature probe site (Table 2). The more mass the dog had, the more quickly the external ear canal cooled. The association remained significant when dogs with the four lowest mass estimates were compared to those with the four highest mass estimates (Table 10). Hair coat density was not statistically significantly associated with temperature decrease after death for any of the data logger probe sites (Table 2).

Table 1:
Comparison of Gender and Mean Temperatures at Each Measurement Site:

Gender:	Mean Temperature (^{o}C)			
	Rectal Mean Temperature	Liver Mean Temperature	Brain Mean Temperature	Aural Mean Temperature
Males (N=8)	27.10°C ± 1.28	27.64°C ± 1.45	23.50°C ± 1.10	25.08°C ± 1.01
Females (N=8)	26.88°C ± 1.29	28.01°C ± 1.26	23.54°C ± 1.05	24.91°C ± 0.74
p-value:	*p = 0.718*	*p = 0.570*	*p = 0.935*	*p = 0.675*

Table 2:
Comparison of Mean Temperatures of Rectal, Liver, Brain, and Aural Probe Sites with Descriptive Variables:

	Mean Temperature (^{o}C)			
	Rectal 26.99°C ± 1.25	Liver 27.83°C ± 1.33	Brain 23.52°C ± 1.04	Aural 25.00°C ± 0.86
Mean Body Weight (kg): 16.29 kg ± 6.05	*p = 0.070*	*p = 0.008** *(Table 3)*	*p = 0.001*** *(Table 4)*	*p = 0.002** *(Table 5)*
Mean Body Volume (l): 19.18 l ± 8.46	*p = 0.025** *(Table 6)*	*p = 0.007** *(Table 7)*	*p = 0.003** *(Table 8)*	*p < 0.001*** *(Table 9)*
Mean Body Mass (kg/l): 0.87 kg/l ± 0.12	*p = 0.079*	*p = 0.263*	*p = 0.623*	*p = 0.041** *(Table 10)*
Mean Hair Coat Density (g/cm^{2}): 0.03 g/cm^{2} ± 0.01	*p = 0.137*	*p = 0.271*	*p = 0.176*	*p = 0.978*

* Significant at $p \leq 0.05$ **Significant at $p \leq 0.001$

Table 3:
The Effect of Weight (Kg) on Liver Temperature (^{o}C) Decline:

	The Effect of Weight (Kg) on Liver Temperature (^{o}C) Decline			
	All Dogs	**Dogs with Lowest Weight**	**Dogs with Highest Weight**	**Comparison Lowest to Highest**
Number of Dogs	16	4	4	
Mean Liver Temperature	27.83$^{°}$C ± 1.33	26.37$^{°}$C ± 1.24	28.77$^{°}$C ± 0.75	
Level of Significance	*p = 0.008*			*p = 0.014*
Note: As weight increased, speed of liver temperature decline decreased. The association was significant when all dogs were used as well as when dogs with the four lowest weights were compared to those with the four highest weights.				

Table 4:
The Effect of Weight (Kg) on Brain Temperature (^{o}C) Decline:

	The Effect of Weight (Kg) on Brain Temperature (^{o}C) Decline			
	All Dogs	**Dogs with Lowest Weight**	**Dogs with Highest Weight**	**Comparison Lowest to Highest**
Number of Dogs	16	4	4	
Mean Brain Temperature	23.52$^{°}$C ± 1.04	22.70$^{°}$C ± 0.55	24.62$^{°}$C ± 0.57	
Level of Significance	*p = 0.001*			*p = 0.002*
Note: As weight increased, speed of brain temperature decline decreased. The association was significant when all dogs were used as well as when dogs with the four lowest weights were compared to those with the four highest weights.				

Table 5:
The Effect of Weight (Kg) on Aural Temperature (^{o}C) Decline:

	The Effect of Weight (Kg) on Aural Temperature (^{o}C) Decline			
	All Dogs	**Dogs with Lowest Weight**	**Dogs with Highest Weight**	**Comparison Lowest to Highest**
Number of Dogs	16	4	4	
Mean Aural Temperature	25.00$^°$C ± 0.86	24.39$^°$C ± 0.44	25.94$^°$C ± 0.73	
Level of Significance	*p = 0.002*			*p = 0.005*
Note: As weight increased, speed of aural temperature decline decreased. The association was significant when all dogs were used as well as when dogs with the four lowest weights were compared to those with the four highest weights.				

Table 6:
The Effect of Body Volume (liters) on Rectal Temperature (^{o}C) Decline:

	The Effect of Body Volume (liters) on Rectal Temperature (^{o}C) Decline			
	All Dogs	**Dogs with Lowest Volume**	**Dogs with Highest Volume**	**Comparison Lowest to Highest**
Number of Dogs	16	4	4	
Mean Rectal Temperature	26.99$^°$C ± 1.25	25.45$^°$C ± 0.59	27.51$^°$C ± 1.06	
Level of Significance	*p = 0.025*			*p = 0.001*
Note: As body volume increased, speed of rectal temperature decline decreased. The association was significant when all dogs were used as well as when dogs with the four lowest volumes were compared to those with the four highest volumes.				

Table 7:
The Effect of Body Volume (liters) on Liver Temperature (^{o}C) Decline:

	The Effect of Body Volume (liters) on Liver Temperature (^{o}C) Decline			
	All Dogs	**Dogs with Lowest Volume**	**Dogs with Highest Volume**	**Comparison Lowest to Highest**
Number of Dogs	16	4	4	
Mean Liver Temperature	27.83$^{°}$C ± 1.33	25.90$^{°}$C ± 0.35	28.58$^{°}$C ± 0.71	
Level of Significance	*p = 0.007*			*p < 0.001*
Note: As body volume increased, speed of liver temperature decline decreased. The association was significant when all dogs were used as well as when dogs with the four lowest volumes were compared to those with the four highest volumes.				

Table 8:
The Effect of Body Volume (liters) on Brain Temperature (^{o}C) Decline:

	The Effect of Body Volume (liters) on Brain Temperature (^{o}C) Decline			
	All Dogs	**Dogs with Lowest Volume**	**Dogs with Highest Volume**	**Comparison Lowest to Highest**
Number of Dogs	16	4	4	
Mean Brain Temperature	23.52$^{°}$C ± 1.04	22.35$^{°}$C ± 0.46	24.33$^{°}$C ± 0.81	
Level of Significance	*p = 0.003*			*p < 0.001*
Note: As body volume increased, speed of brain temperature decline decreased. The association was significant when all dogs were used as well as when dogs with the four lowest volumes were compared to those with the four highest volumes.				

Table 9:
The Effect of Body Volume (liters) on Aural Temperature (^{o}C) Decline:

	The Effect of Body Volume (liters) on Aural Temperature (^{o}C) Decline			
	All Dogs	**Dogs with Lowest Volume**	**Dogs with Highest Volume**	**Comparison Lowest to Highest**
Number of Dogs	16	4	4	
Mean Aural Temperature	25.00°C ± 0.86	24.10°C ± 0.25	26.03°C ± 0.69	
Level of Significance	*p < 0.001*			*p < 0.001*

Note: As body volume increased, speed of aural temperature decline decreased. The association was significant when all dogs were used as well as when dogs with the four lowest volumes were compared to those with the four highest volumes.

Table 10:
The Effect of Body Mass (kg/l) on Aural Temperature (^{o}C) Decline:

	The Effect of Body Mass (Kg/l) on Aural Temperature (^{o}C) Decline			
	All Dogs	**Dogs with Lowest Mass**	**Dogs with Highest Mass**	**Comparison Lowest to Highest**
Number of Dogs	16	5	5	
Mean Aural Temperature	25.00°C ± 0.86	25.51°C ± 1.00	24.35°C ± 0.55	
Level of Significance	*p = 0.041*			*p = 0.031*

Note: As body mass increased, speed of aural temperature decline increased. The association was significant when all dogs were used as well as when dogs with the five lowest masses were compared to those with the five highest masses.

Potassium Concentrations in the Vitreous Humor

Vitreous Potassium Concentration Distributions (First Collection):

The mean potassium concentration for the right eye of all 16 dogs was 5.8 meq/l with range 4.3 meq/l - 7.2 meq/l (Figure 12). For the left eye, the mean K+ concentration for 14 of the 16 dogs (due to the inability to collect sufficient VH from 2 of the dogs) was 6.2 meq/l with range 5.1 meq/l - 7.3 meq/l (Figure 13). For both eyes, the mean K+ concentration for 14 of the 16 dogs was 6.0 meq/l with range 4.7 meq/l - 7.2 meq/l (Figure 14).

Vitreous Potassium Concentration (Second Collection):

The mean K+ concentration for the right eye of 15 of the 16 dogs was 8.9 meq/l with range 6.5 meq/l - 10.2 meq/l (Figure 15). An insufficient quantity of VH was collected from one of the dogs. For the left eye, the mean K+ concentration for 14 of the 16 dogs (due to collection difficulties) was 8.7 meq/l with range 6.3 meq/l - 10.0 meq/l (Figure 16). The mean K+ concentration for both eyes for 14 of the 16 dogs was 8.8 meq/l with range 7.4 meq/l - 10.1 meq/l (Figure 17).

Comparison of Vitreous Potassium Concentration by Collection:

No significant difference was found between the first collection mean K+ concentration of the left and right eyes (p-value = 0.11) or between the second collection mean K+ concentration of the left and right eyes (p-value = 0.34). However there was a statistically significant increase in K+ concentrations between the 1st and 2nd collections when eyes were compared to themselves (e.g., left to left) or to the opposite eye (e.g., left to right) (p-value < 0.001) (Table 11).
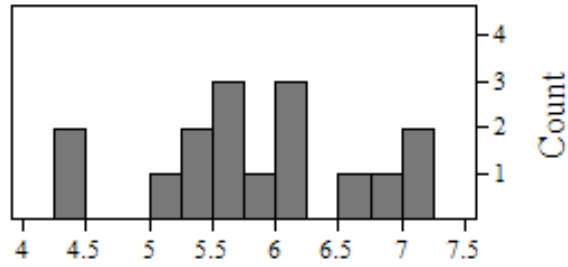
Figure 12:

(First Collection) Right Eye Vitreous K+ Concentration for all Dogs in meq/l Distribution N=16
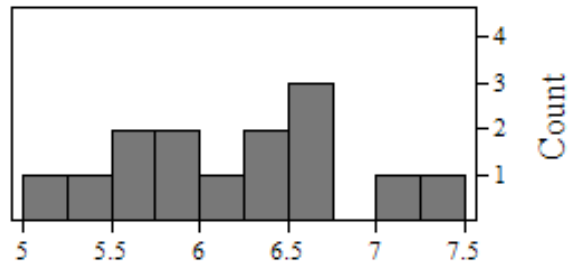
Figure 13:

(First Collection) Left Eye Vitreous K+ Concentration for all Dogs in meq/l Distribution N=14
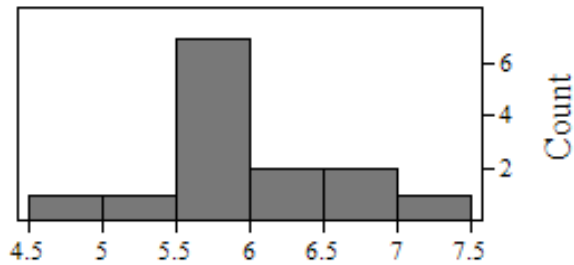
Figure 14:

(First Collection) Mean Vitreous K+ Concentration in both Eyes for all Dogs in meq/l Distribution N=14
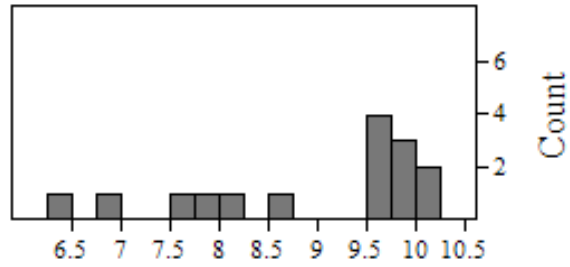
VH K+ Concentration (meq/l)

Figure 15:
(Second Collection) Right Eye Vitreous K+ Concentration for all Dogs in meq/l Distribution N=15
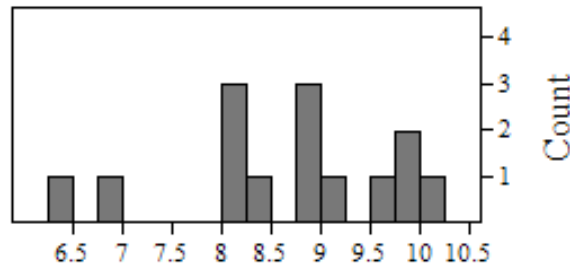
VH K+ Concentration (meq/l)

Figure 16:
(Second Collection) Left Eye Vitreous K+ Concentration for all Dogs in meq/l Distribution N=14
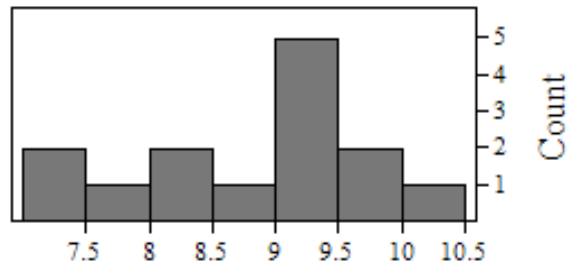
VH K+ Concentration (meq/l)

Figure 17:
(Second Collection) Mean Vitreous K+ Concentration in both Eyes for all Dogs in meq/l Distribution N=14

Table 11:
Comparison of Means of Vitreous Potassium Concentration by Eye and Collection:

Matched Pairs (Comparison of Means)			Significance Level of Difference (p-value)
	1st Collection		
	Left Eye	Right Eye	
Mean Vitreous K+	6.17	5.79	0.11
	2nd Collection		
	Left Eye	Right Eye	
Mean Vitreous K+	8.62	8.92	0.34
	Left Eye		
	1st Collection	2nd Collection	
Mean Vitreous K+	6.17	8.62	**< 0.001
	Right Eye		
	1st Collection	2nd Collection	
Mean Vitreous K+	5.79	8.92	**< 0.001
	Left Eye	Right Eye	
	1st Collection	2nd Collection	
Mean Vitreous K+	6.17	8.92	**< 0.001
	Right Eye	Left Eye	
	1st Collection	2nd Collection	
Mean Vitreous K+	5.79	8.62	**< 0.001

*Significant at $p \leq 0.05$ **Significant at $p \leq 0.001$*

CHAPTER IV

DISCUSSION:

The main objectives of this study were to take selected TOD measures and apply them for the purpose of estimating the TOD of dogs. Further, the intent was to aid animal cruelty/abuse investigations by providing a practical and inexpensive quantitative methodology of estimating TOD, maximize the probability that investigators with limited experience will collect useful data, and aid in teaching animal cruelty/abuse investigators and others proper forensic techniques in handling and collecting data in the field. All of these objectives were addressed directly or indirectly through the data collected and the results generated during the course of this study. Information obtained from the data was organized based on the following categories: temperature data, vitreous potassium concentration, and other factors.

TEMPERATURE-BASED DATA:

The decline of core body temperature is important in estimating TOD. For the purposes of this study, the external ear canal, brain, liver, and rectum were chosen as sites to record core body temperature declines. The thigh and nasal cavity were not chosen due to a limited number of thermocouples and because current scientific literature suggests the other sites are better for determining core body temperature changes.

Comparison of data collected with the data logger and the chemical thermometer addresses a main objective of the study. The data logger device gave accurate temperature recordings from the rectum, liver, brain, and ear canal and provided a "gold

standard" for comparison with the chemical thermometer. The chemical thermometer proved to be as accurate as the data logger with the added benefit of being much easier to use and less expensive. No computer equipment or special software was needed to obtain temperature readings with the chemical thermometers. The data logger must be attached to probes and needs to be connected to a computer with specific software in order to record and store data. Whereas, the data logger is useful in a research setting, the chemical thermometer is much more feasible for use under field conditions. The data logger and temperature probes can cost up to or over $1000, while the chemical thermometers cost less than $100.00 each.

Economy as well as accuracy is a major concern for agencies involved in forensic investigations. Forensic investigations can be costly and responsible agencies are often limited in the supplies and equipment they can purchase and utilize. It is reasonable to assume that this is especially true of animal control agencies and humane societies which are usually tasked with animal abuse/cruelty investigations. These limitations can severely hamper a criminal investigation. Evidence that could be vital to the prosecution of a case may not be collected due to lack of funds or unavailable equipment. Any equipment that is affordable, accurate, and efficient can be a major asset for forensic investigative agencies. Furthermore, equipment that is easy to use, requiring little training is certainly advantageous. Chemical thermometers are accurate, relatively inexpensive, and easy to use, therefore, forensic investigative agencies may want to consider including chemical thermometers with other standard forensic equipment.

Comparison of temperature data recorded from each of the four sites with reports of other investigators in the published literature represents an expansion of knowledge of

60

this method that is specific for canines. While the brain and liver are considered to be the better sites for determining core body temperature than the rectum, rectal temperature is the most widely used measure [2-4, 7]. This is likely due to the fact that the liver and brain are more difficult to access under field conditions [2-3]. In previous TOD studies that focused on humans, it has been reported that after death the rectal temperature will decrease faster than any of the other sites, followed by the liver, and finally the brain temperature (aural temperature was not compared in this way) [2-4, 7]. Brain is thought to be the best site, in humans, for determining core body temperature, because it is thought to be less susceptible to outside factors such as environmental conditions that may affect temperature [3]. The brain is insulated by CSF which plays a role in protecting it from environmental temperature effects [3]. In humans, clothing and hair play little role in brain temperature changes [3]. The head is globular in shape and relatively uniform in humans with few variations in the size [3]. Due to these factors, the brain should not be affected as quickly by changes in environmental conditions as other sites and should decrease at a slow and steady rate.

Liver temperature should decrease slightly faster in the early PMI due to factors such as putrefaction, environmental conditions, and clothing, while the rectal temperature after death should be affected most by external factors decreasing sharply in the early PMI [2-4, 7]. The rectum is positioned in the body such that it is more exposed to factors outside of the body, and contains material and bacteria which make it more susceptible to putrefaction processes [2-4, 7].

This study obtained different results. As determined by the mean temperature results obtained by the data logger, brain temperature appeared to decrease the fastest,

followed by aural, rectal, and finally liver temperature. Also, rectal temperatures in humans have been shown to plateau within the first 6 hours after death, while this was not found in the present study [7].

The first temperature measures for each site showed that the ear and brain temperatures start out at a lower temperature than either rectal or liver. These discrepancies may at least be due in part to differences between species. Factors specific to species such as post-mortem metabolism, and pre-mortem muscular activity or the use of clothing versus hair coat probably have an effect and should be studied further [7]. The shape of the head in canines varies greatly among dogs and even among breeds. Canine brains are much smaller than humans and may not be as well insulated. Brain size varies among dogs and smaller dogs may be susceptible to more rapid changes in temperature than larger dogs.

Based on the data from the present study, the brain and aural temperature sites are likely more susceptible to environmental factors and the rectum and liver are less susceptible. In addition, the rectum and liver temperature collection sites were determined to be correlated, suggesting that the liver site could be used in place of the rectum for temperature data collection. Accordingly, the liver may be the preferred site for collecting core body temperature readings followed by the rectum, ear, and finally brain.

Other specific findings involved the data recorded for aural temperature. Much like another study, aural temperature at TOD differed greatly from the rectal and liver temperature at TOD [47]. Thus the external ear canal temperature may be more variable due to environmental conditions [7, 40-41, 47].

62

The atmospheric effects of rain, sun, and wind, as well as physical effects such as time of day, emotional state, and age on external ear canal temperature were not determined by the present study. Neither were the effects of blood in the ear (i.e., due to trauma), CSF, water, otitis externa (ear infections), and wax on ear temperature. No comparisons were made to determine which of the ears is more appropriate to record temperature. Variations due to these factors are limitations of this study, but measuring the effects of each of these was beyond the scope of this investigation

The depth that a thermometer is inserted into the ear canal was controlled since this was indicated as a possible confounding factor in at least one other study [47]. The problems associated with inserting the temperature probe too deep included damaging the tympanic membrane and possibly causing bleeding into the canal, but this was not encountered [47]. None of the external ear canal temperatures reached ambient temperature within the 32 hour observation period of this study [Figure 9]. Consequently, external ear canal temperature may be a usable measure for estimating TOD for periods longer than 15 hours. This contradicts another published report in which it was determined that the external ear canal temperature should reach ambient temperature by 15 hours post-mortem, thereby rendering it useless for bodies that have been dead for more than 15 hours [7].

Use of OTC digital thermometers for temperature data collection was considered initially. In the course of this investigation, OTC digital thermometers, produced for use in humans (and thus readily available), were found to be limited by the temperature range they were designed to record. The lower temperature limit was 32°C (90°F) which was inadequate for this study, since expectations were to be able to measure body temperature

decline until it equilibrated with ambient temperature. In addition, the OTC thermometer was only suitable for use rectally or aurally. Nevertheless, rectal and aural temperatures were recorded in each dog using OTC digital thermometers at the animal shelter just after death in order to determine an initial body temperature. Temperature data generated from OTC thermometers were not utilized in this study, but such instruments may be useful to future studies as a starting point but their usefulness will quickly become limited in determining a dog's temperature as an estimate of the TOD. They may also be used initially if other, more versatile thermometers are not immediately available. The use of OTC thermometers may also be important in cases in which the temperature of a dog falls below the lower limit of the OTC ($<90^{o}C$) thermometer, in which case, it can be assumed that the body temperature is less than $90^{o}C$.

VITREOUS POTASSIUM CONCENTRATION:

The methods used to collect VH in humans, canines, or other animals are fairly consistent among published studies. However, variations occur in the method of analysis and sample preparation. Depending on the study, one of three methods for determining the K+ concentration in VH is used; flame photometry, ion specific electrode analysis, and capillary zone electrophoresis. Depending on the study, some samples were centrifuged and/or diluted and/or filtered prior to testing, and may have been stored frozen or refrigerated prior to analysis as well [1, 9-10, 14-15, 22-24, 26-27, 30-32, 34-35, 37-38, 41, 43, 48, 51-52, 55]. In the present study, VH samples were collected and refrigerated until analyzed via ion specific electrode analysis Samples did not need to be centrifuged, diluted, or filtered prior to analysis.

In previously published studies, it has been reported that canines, prior to death, have a vitreous K+ concentration mean of 5.92 to 6.58 meq/l [1, 22, 36]. This compares favorably with the current study in which, the mean vitreous K+ concentration, 1st collection, for both eyes in all 16 dogs was determined to be 6.0 meq/l with a range of 4.7 meq/l - 7.2 meq/l.

Discrepancies have been reported in some studies over whether vitreous K+ is statistically significantly different between the left and right eye at one point in time [1, 9-10, 14-15, 22-24, 26-27, 30-32, 34-35, 37-38, 41, 43, 48, 51-52, 55]. Some studies report a statistically significant difference in the vitreous K+ concentration between the right and left eyes collected at the same time prior to death [9, 31, 42]. Those studies reporting a difference in vitreous K+ concentration between eyes used flame photometry to analyze the VH [9, 31, 42]. Of those studies that found no difference between the vitreous K+ concentration of each eye, used the ion specific electrode analysis or capillary zone electrophoresis for their analysis [1, 14, 38-39, 51].

The present study did not find a statistically significant difference in the vitreous K+ between left and right eyes sampled at the same time. The difference in the type of instrumentation is most likely the reason. Flame photometry which was used in several earlier studies is not as sensitive as ion specific electrode analysis used in this study or capillary zone electrophoresis used in other studies.

OTHER FACTORS:

Selected other factors that could possibly affect temperature declines were examined. Weight was associated with brain, liver, and aural, temperature declines in that the greater the weight the more slowly the body temperature declined. Weight showed a

trend in the same direction for decline of body temperature as measured in the rectum (p = 0.07). The association was also significant when dogs with the four lowest weights were compared to those with the four highest weights.

Body volume was statistically significantly associated with rate of temperature decline as measured at all sites. The larger the body volume the more slowly the body temperature declined. The association was also significant when dogs with the four lowest weights were compared to those with the four highest weights.

Body mass was not associated with body temperature decline as measured in the brain and liver (p = 0.263 and p = 0.623, respectively). Aural temperature decline was statistically significantly associated with body mass in that aural temperature declined more quickly as mass increased (p = 0.041). A trend in the same direction was observed for rectal temperature (p = 0.079). The association was also significant when dogs with the four lowest mass estimates were compared to those with the four highest mass estimates. Factors that might explain this difference include surface area and amount of body fat. It is reasonable to assume that each of these would influence core body temperature after death. Using mass as an indirect measure of body surface, the greater the mass, the more quickly the body would cool. No attempt was made to measure body fat, so the role of this factor is unknown.

Neither gender nor hair coat density were significantly associated with temperature decrease after death for any of the data logger probe sites. One study looked at the effect of color and density of the hair coat on the dogs' temperature at the skin level only and it found no association between color or density and skin temperature [12]. However, this study dealt with only one breed of dog, and was conducted on live dogs.

This is the only study found that looked at a possible influence of hair coat on temperature.

STUDY LIMITATIONS:

While useful data were generated by this study, it also has limitations. Monetary and logistical practicalities limited the ability to vary certain factors that may affect the rate of temperature decline after death. The exothermic processes involved in decay were not controlled. Variable environmental conditions such as ambient temperature, wind and moisture were not evaluated. Instead, efforts were taken to control some environmental conditions. Ambient temperature was kept relatively constant and varied only by a range of 2°C. Ambient humidity was recorded as being more variable with a range of 30%. Data were collected only on body of dogs that were kept indoors.

Canines used in the study were not selected based on any characteristics other than availability. Therefore, physical characteristics such as weight, mass, volume, hair coat density and color, or gender were not specifically controlled. The equal number of female and male dogs was a result of chance.

As was done in other studies, effort was made to limit sampling errors, sample handling and transport, sample storage, and technical errors [1, 14-15, 23-25, 27, 31-32, 35-36, 38-39, 43, 48, 51-52, 55]. Equipment and methods of sample collection and analysis were standardized. Modalities of death and ante-mortem pathologies were not considered in this study.

There were problems associated with collecting enough VH from each dog in the study and a sample was not collected from each eye at each collection time. VH is very

viscous and depending upon eye size, can be difficult to obtain. Through experience and a standardized methodology, this problem was minimized.

Collection of hair for the purpose of estimating hair coat density, while not difficult to collect, was limited as a measure of density. Samples were taken from only one site on the body. While this site is likely the site of densest hair growth, hair coat is unevenly distributed across the body, and this one site is not a true representative sample of hair coat density.

The length of the observation period for each dog was limited because of practical restrictions imposed by the putrefaction process of the carcasses and limited space. For this reason, the study was conducted for a duration of only 32 hours after death. After this point, odor and bloating was so obvious that continued data collection was impractical in the selected setting (e.g., adjacent rooms were potentially affected). A relatively cool ambient temperature was maintained and likely delayed these changes by an unknown magnitude.

Placement time of data logger temperature probes may have been a limitation of this study. The animal shelter where the dogs were obtained was approximately 45 minutes from the room at the UTVTH where continuous data were recorded. All probes were placed by approximately 1 hour 5 minutes after death, therefore this time was chosen as the starting point for the study. Some animals with lower beginning temperatures may have resulted from this delay.

Some studies have offered equations and computer programs to assist in the estimation of TOD. These have been compiled in appendix 4-1 through 4-11. Data were not generated in this study that would specifically match the variables needed for these

formulas and programs. Consequently, they are offered as a summary resource for future investigators.

FUTURE RESEARCH:

Future studies should be conducted to address the limitations inherent in this study or to expand the subject of TOD estimation in canines. Future studies must take in to account more specific environmental factors, such as variations in ambient temperature and humidity. One way to do this is to repeat elements of this study on bodies that are maintained outside and subject to environmental effects. Entomological studies (e.g. blow fly egg and larvae development) have been found to be useful in forensic investigations of human deaths. Repeating such studies with dogs would also be useful.

Some elements of the research presented here that could be repeated or expanded are the validation of the temperature curves as they may be generated in realistic settings such as in a veterinary practice or animal shelter when an animal dies or is euthanized, and an exact TOD is known. Temperatures at specific points in time could then be plotted on one of the graphs generated by this study (Figures 11-14) to see how close the true TOD can be estimated.

If the scenario for this experiment was a veterinary clinic, then rectal temperatures would probably be available so that a set point at the TOD could be established. Such a setting would have the added benefit of generating data on the potential effects of disease or sickness on temperature at TOD. For example if an animal is moribund due to illness, the temperature curve may start out at a lower/higher point. The mental state of the animal at time of death is also an important factor that needs to be considered. Temperature at TOD may be elevated due to agitation brought about by the handling and

restraint needed for euthanasia. In contrast, a dog that dies slowly of disease or injury (e.g., situations that result in coma before death) might result in a lower temperature at the TOD. None of these factors were addressed by this study but would be important elements for inclusion in future studies.

The use of the temperature and vitreous K+ concentration data for purpose of generating a computer model to estimate the early PMI may be beneficial to forensic investigators.

Based on findings of this study and other reports in the literature, using one and perhaps two measures of body temperature in combination with a determination of vitreous K+ concentration promises to be the most defensible measures in estimating TOD [3-4, 7, 13, 37, 41, 56]. However, the correlation of temperature measures and vitreous K+ concentration needs to be validated. One study approached this validation by looking at the correlation of external ear canal temperature, vitreous K+, blood and cerebral spinal fluid electrolytes, and rectal temperature with core body temperature decline after death [7]. However this study was conducted on humans and has not been evaluated in canines. In addition, this study and others indicate that the rectum may not be the best site for determining body temperature after death.

CONCLUSION:

This study can serve as a foundation for future research aimed at determining more accurate methods for estimating TOD in canines. Although more extensive investigation is needed, valuable information was produced in this study. The objectives of this study were to: aid animal cruelty/abuse investigations, maximize the probability that investigators with limited experience will collect useful data, and aid in teaching

70

animal cruelty/abuse investigators and others proper forensic techniques in handling and collecting data in the field. Each of these objectives was addressed in some form.

The techniques and equipment used in this study are relatively inexpensive, easy to learn, and are simple to perform in the field. The information generated from this study, in particular, the temperature data collected from the rectum, liver, brain, and external ear canal as well as the vitreous K+ concentration data, may one day prove important to the forensic community as a reference material, teaching resource, or possibly practical application in the field.

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APPENDICES

APPENDIX 1: EQUIPMENT

Appendix 1-1:
Digital Thermometer with Accu-Beep[TM] feature (Becton Dickinson (BD), Franklin Lakes, New Jersey, USA) "15cm (6in) ruler included for scale":
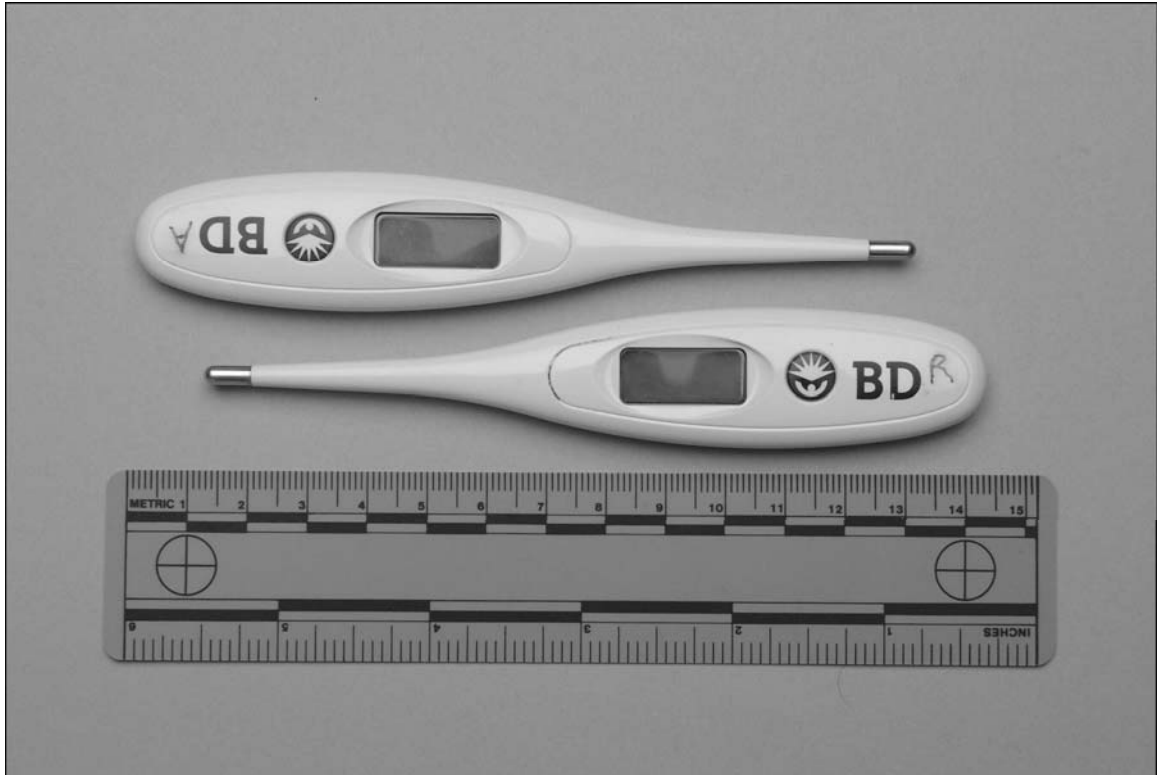
Appendix 1-2:

Model OM-CP-QUADTEMP 4-channel continuous temperature measurement data logger and VIP-T series probes (Omega Engineering, Inc. Stamford Connecticut, USA) "15cm (6in) ruler included for scale":
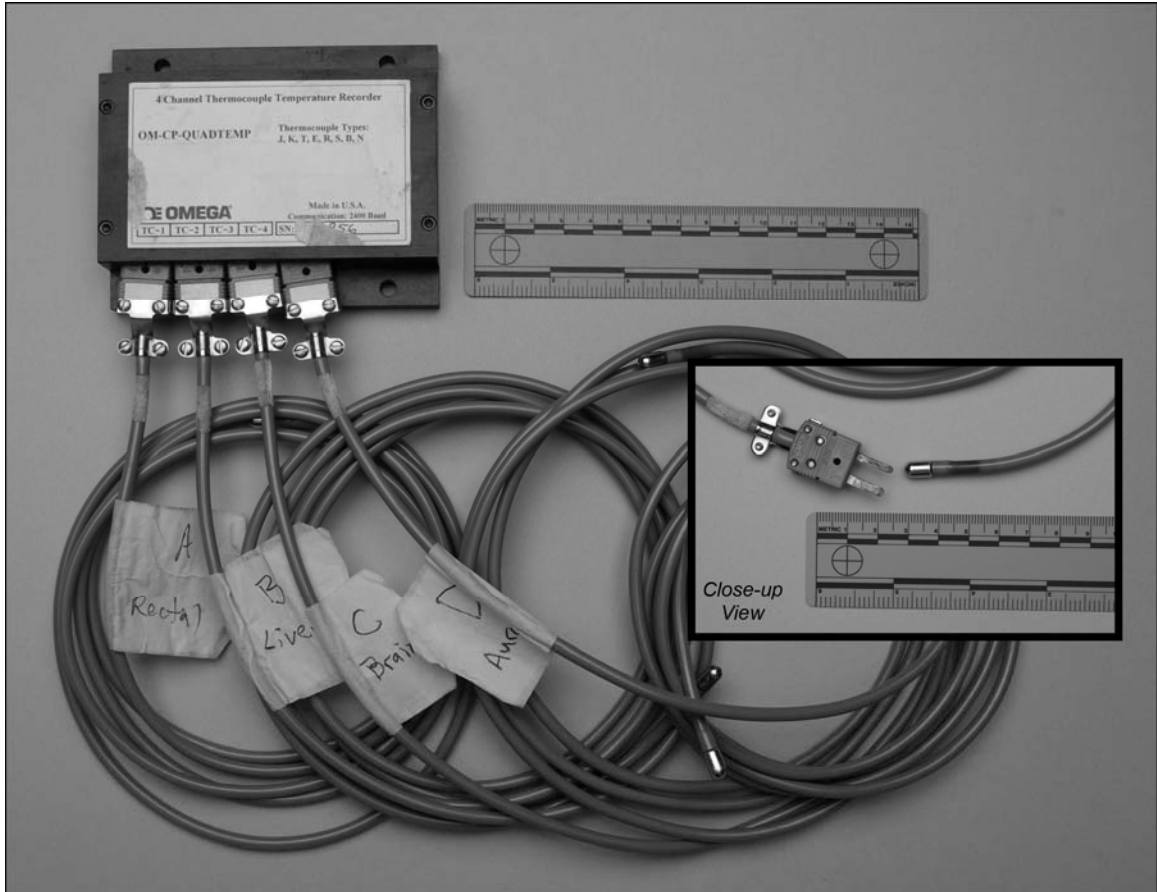
Appendix 1-3:
Checktemp Digital Chemical Thermometers (Celsius Model) (Hanna Instruments, Woonsocket, Rhode Island, USA) "15cm (6in) ruler included for scale":
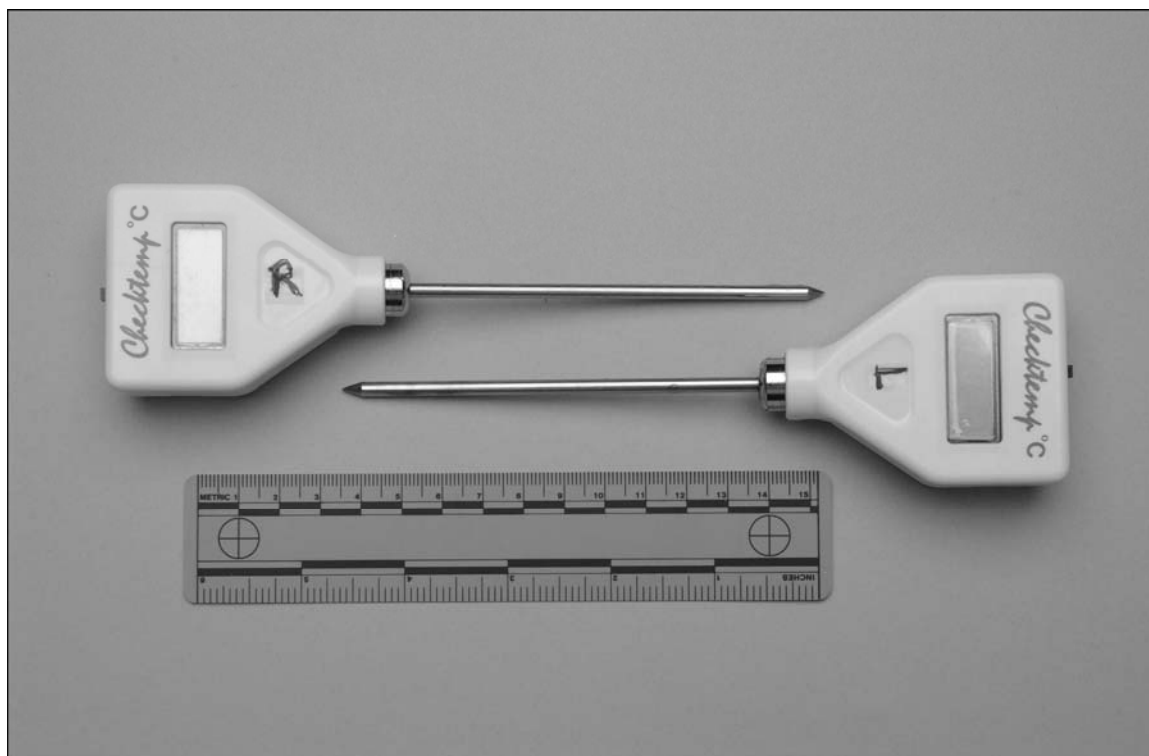
Appendix 1-4:
Surgical Trephine with 0.8 cm diameter (Adler Instrument Company, Norcross, Georgia, USA) "15cm (6in) ruler included for scale":

Appendix 1-5:

Temperature-Hygrometer (Fisher Scientific, Suwanee, Georgia, USA) "15cm (6in) ruler included for scale":

APPENDIX 2: DATA LOGGER TEMPERATURE READINGS

Appendix 2-1:
Data Logger Ambient Temperature Readings (ºC) by Dog

Dog Number	Mean (From 1hr 5 min After Death to 32 hrs After Death)	Range (From 1hr 5 min After Death to 32 hrs After Death)		
		Minimum	Maximum	Max-Min
1	23.57	22.90	26.30	3.40
2	21.81	18.70	23.10	4.40
3	21.70	20.20	23.20	3.00
4	21.95	19.00	22.60	3.60
5	21.86	18.20	22.70	4.50
6	22.19	18.20	22.70	4.50
7	22.53	16.30	23.40	7.10
8	21.71	19.60	22.60	3.00
9	22.18	16.30	23.00	6.70
10	22.39	21.40	23.00	1.60
11	22.09	20.20	22.60	2.40
12	22.28	20.40	22.90	2.50
13	22.90	22.60	24.40	1.80
14	22.29	21.60	26.10	4.50
15	22.29	21.70	27.70	6.00
16	21.83	21.20	26.50	5.30
All Dogs ±SD	22.22 ± 0.47	16.30 ± 2.12	27.70 ± 1.89	11.40 ± 2.41

Appendix 2-2:
Data Logger Rectal Temperature Readings (^{o}C) by Dog

Dog Number	Mean (From 1hr 5 min After Death to 32 hrs After Death)	Range (From 1hr 5 min After Death to 32 hrs After Death)		
		Minimum	Maximum	Max-Min
1	29.31	24.22	39.62	15.40
2	28.66	23.94	39.62	15.68
3	26.38	22.43	38.73	16.30
4	24.95	22.00	38.76	16.76
5	28.38	23.93	40.74	16.81
6	28.45	23.72	39.65	15.93
7	29.01	24.13	39.15	15.02
8	27.48	23.26	39.09	15.83
9	25.92	22.70	38.70	16.00
10	27.60	23.17	38.47	15.30
11	27.17	22.72	39.26	16.54
12	26.12	22.43	39.35	16.92
13	26.99	22.90	41.41	18.51
14	27.36	22.87	38.56	15.69
15	26.13	22.83	37.31	14.48
16	26.92	22.70	37.97	15.27
All Dogs ±SD	27.30 ± 1.19	22.00 ± 0.71	41.41 ± 1.10	19.41 ± 1.24

Appendix 2-3:
Data Logger Liver Temperature Readings (^{o}C) by Dog

Dog Number	Mean (From 1hr 5 min After Death to 32 hrs After Death)	Range (From 1hr 5 min After Death to 32 hrs After Death)		
		Minimum	Maximum	Max-Min
1	29.43	25.10	38.83	13.73
2	29.20	24.70	37.64	12.94
3	27.50	23.07	38.27	15.20
4	25.62	22.17	46.90	24.73
5	29.27	24.47	39.18	14.71
6	28.28	24.13	38.26	14.13
7	29.49	24.67	38.83	14.16
8	27.74	23.79	35.49	11.70
9	25.96	23.20	33.74	10.54
10	28.89	24.10	38.30	14.20
11	27.76	23.19	38.82	15.63
12	26.38	22.63	37.73	15.10
13	28.89	23.77	42.68	18.91
14	27.48	23.27	38.29	15.02
15	25.95	22.94	36.66	13.72
16	28.45	23.90	38.24	14.34
All Dogs ±SD	27.89 ± 1.28	22.17 ± 0.87	46.90 ± 3.44	24.73 ± 3.89

Appendix 2-4:
Data Logger Brain Temperature Readings (^{o}C) by Dog

Dog Number	Mean (From 1hr 5 min After Death to 32 hrs After Death)	Range (From 1hr 5 min After Death to 32 hrs After Death)		
		Minimum	Maximum	Max-Min
1	24.84	22.88	33.12	10.24
2	24.62	22.06	34.96	12.90
3	24.09	21.70	35.63	13.93
4	22.09	21.06	28.49	7.43
5	23.57	21.94	32.10	10.16
6	23.35	22.24	33.13	10.89
7	22.46	21.78	27.23	5.45
8	25.24	22.50	36.74	14.24
9	23.00	21.91	29.95	8.04
10	23.88	22.10	30.09	7.99
11	23.11	21.90	30.64	8.74
12	21.96	21.07	27.50	6.43
13	23.42	21.71	29.85	8.14
14	25.18	22.46	36.42	13.96
15	22.48	21.64	31.35	9.71
16	23.50	21.87	30.72	8.85
All Dogs ±SD	23.55 ± 1.01	21.06 ± 0.50	36.74 ± 3.17	15.68 ± 3.01

Appendix 2-5:
Data Logger Aural Temperature Readings (°C) by Dog

| Dog Number	Mean (From 1hr 5 min After Death to 32 hrs After Death)	Range (From 1hr 5 min After Death to 32 hrs After Death)		
		Minimum	**Maximum**	**Max-Min**
1	27.02	23.93	36.15	12.22
2	25.53	22.64	33.56	10.92
3	24.22	21.87	34.01	12.14
4	24.05	21.84	33.44	11.60
5	25.29	22.93	33.42	10.49
6	25.03	23.13	33.26	10.13
7	25.49	22.83	34.47	11.64
8	25.45	22.67	34.83	12.16
9	24.21	22.30	32.33	10.03
10	25.86	22.57	34.56	11.99
11	24.29	22.24	32.98	10.74
12	23.87	21.81	33.34	11.53
13	24.54	22.24	33.46	11.22
14	25.24	22.57	34.37	11.80
15	24.40	22.47	33.62	11.15
16	25.94	22.74	35.98	13.24
All Dogs ±SD	25.03 ± 0.83	21.81 ± 0.55	36.15 ± 1.13	14.34 ± 1.08

Appendix 2-6:
Mean and (Standard Deviation) Temperature Readings for Each Probe Site Over the Period of 1hr 5 min to 32 hrs after Death for Each Dog:

Dog Number	Ambient Means	Rectal Means	Liver Means	Brain Means	Aural Means
1	23.57	29.31	29.43	24.84	27.02
2	21.81	28.66	29.20	24.62	25.53
3	21.70	26.38	27.50	24.09	24.22
4	21.95	24.95	25.62	22.09	24.05
5	21.86	28.38	29.27	23.57	25.29
6	22.19	28.45	28.28	23.35	25.03
7	22.53	29.01	29.49	22.46	25.49
8	21.71	27.48	27.74	25.24	25.45
9	22.18	25.92	25.96	23.00	24.21
10	22.39	27.60	28.89	23.88	25.86
11	22.09	27.17	27.76	23.11	24.29
12	22.28	26.12	26.38	21.96	23.87
13	22.90	26.99	28.89	23.42	24.54
14	22.29	27.36	27.48	25.18	25.24
15	22.29	26.13	25.95	22.48	24.40
16	21.83	26.92	28.45	23.50	25.94
Overall Mean ± SD:	22.22 ± 0.48	27.30 ± 1.23	27.89 ± 1.32	23.55 ± 1.04	25.03 ± 0.86

APPENDIX 3: DATA LOGGER OVERLAY PLOTS
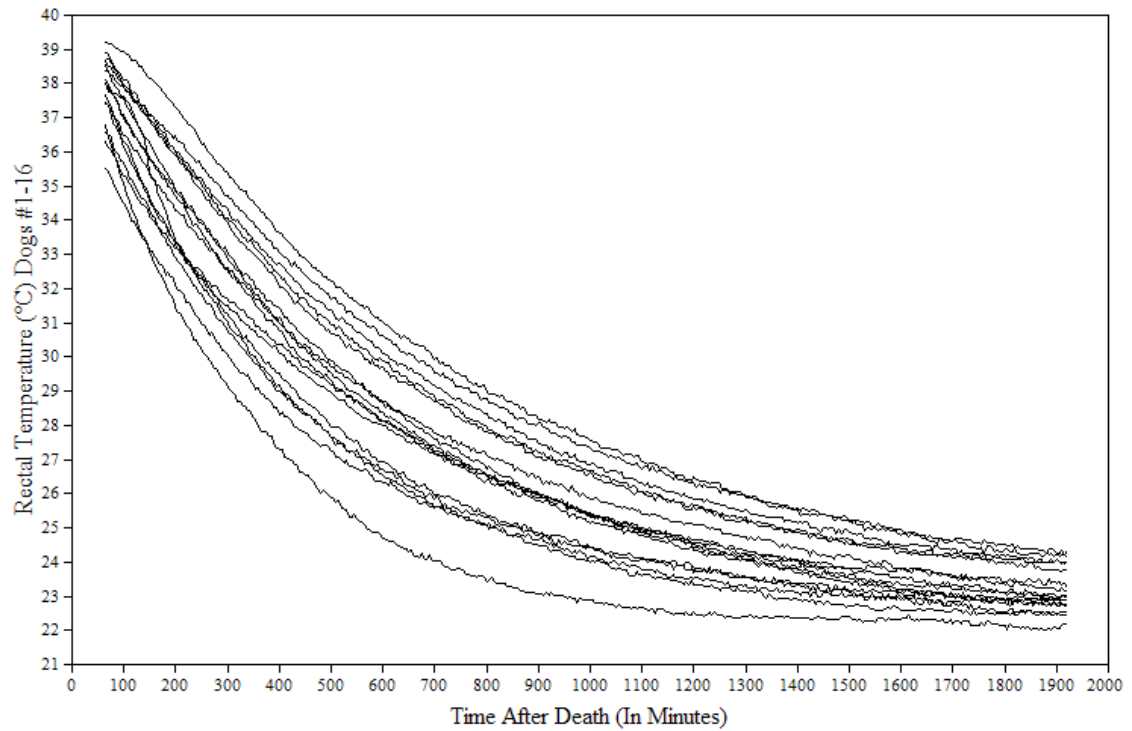
Appendix 3-1:
Data Logger Ambient Temperature (°C) Overlay Plot for each individual Dog (N=16)
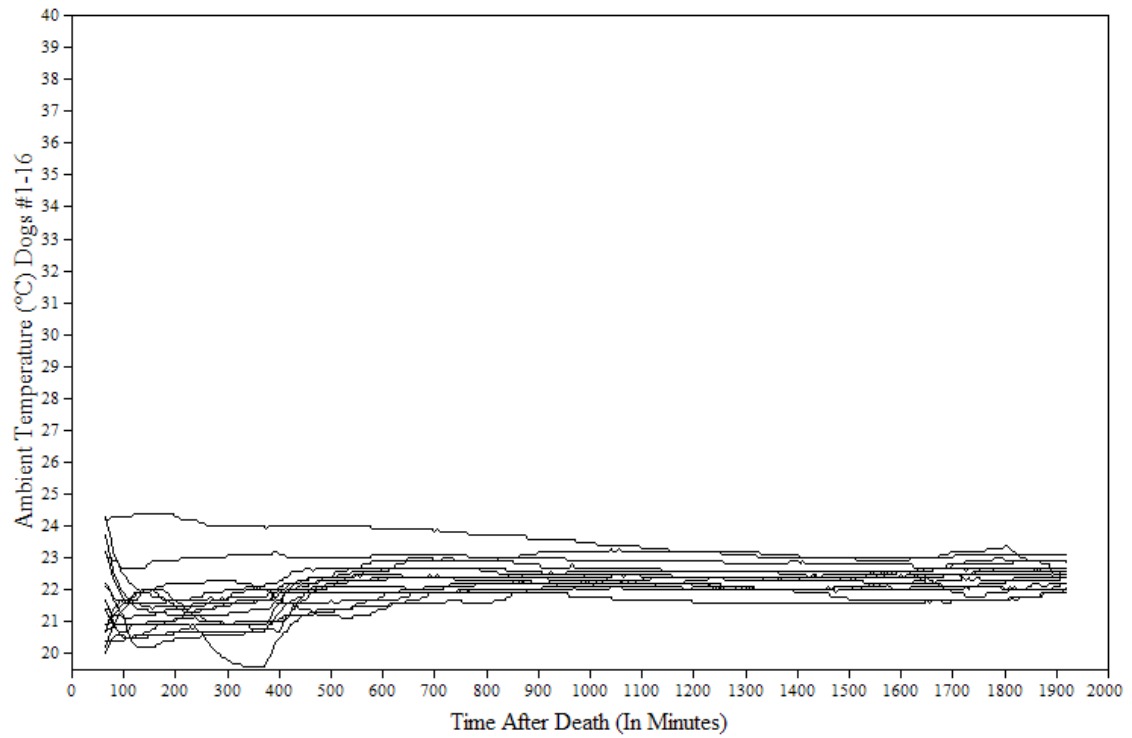
Note: Each line represents an individual dog's mean temperature readings over the 32 hour study period.

Appendix 3-2:
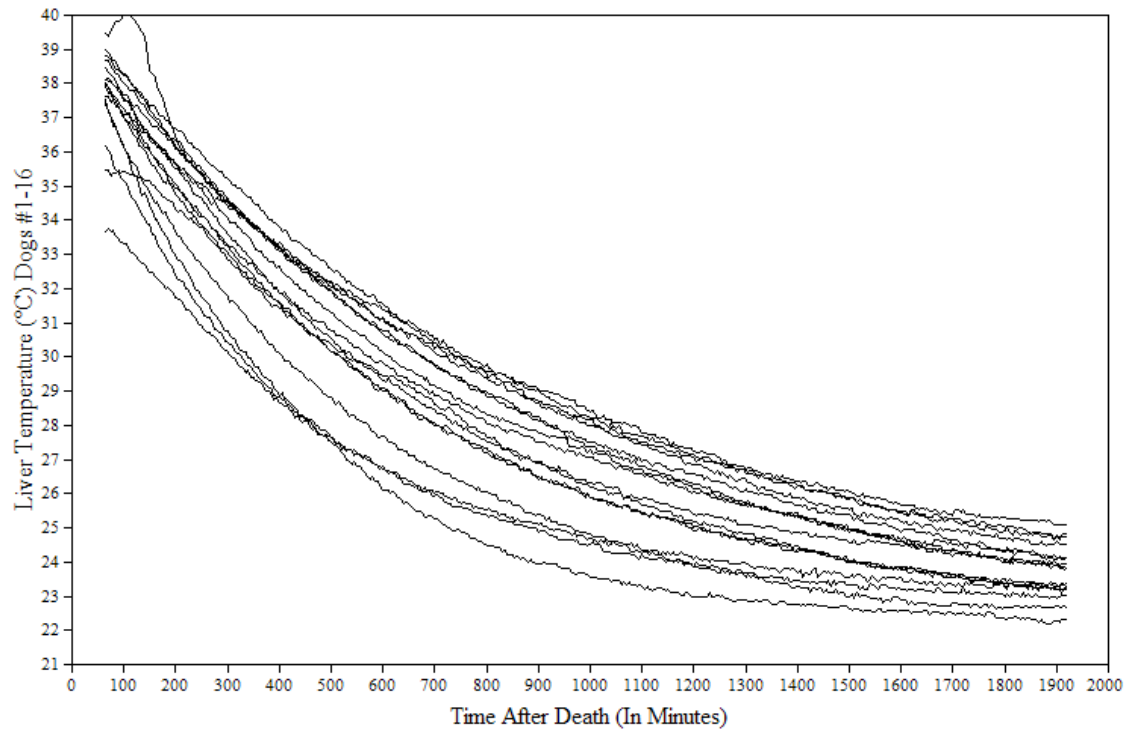**Data Logger Rectal Temperature (^{o}C) Overlay Plot for each individual Dog (N=16)**

Note: Each line represents an individual dog's mean temperature readings over the 32 hour study period.
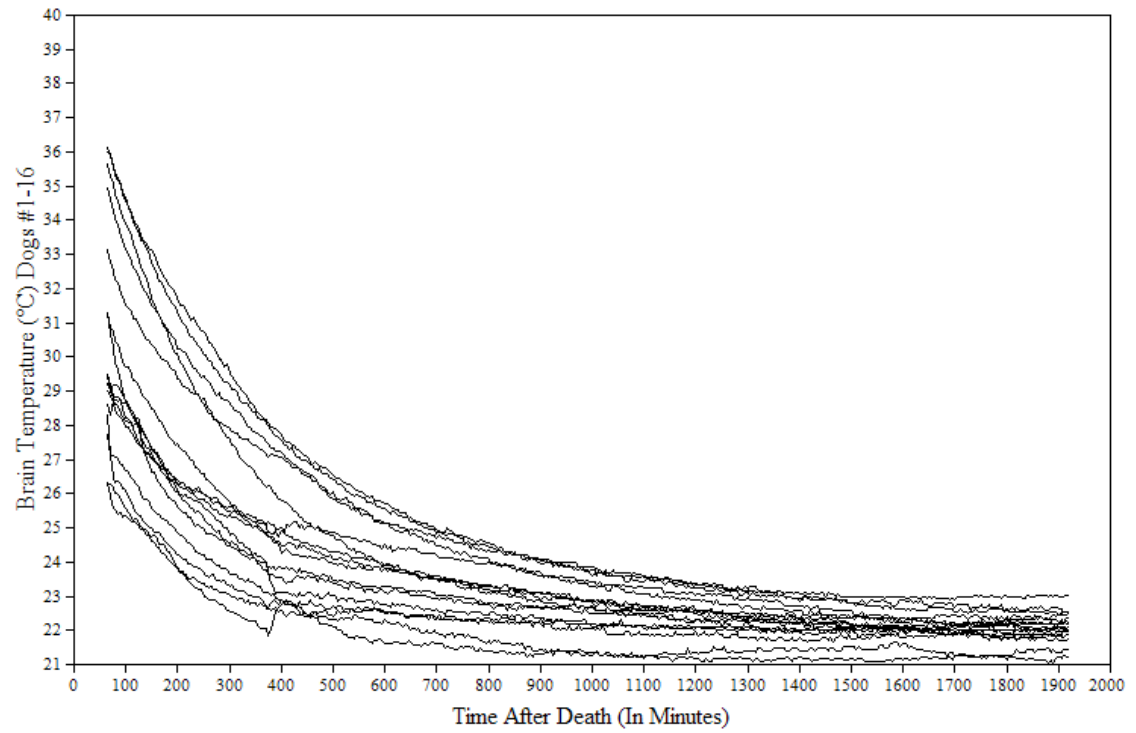
Appendix 3-3:
Data Logger Liver Temperature (°C) Overlay Plot for each individual Dog (N=16)

Note: Each line represents an individual dog's mean temperature readings over the 32 hour study period.

Appendix 3-4:
Data Logger Brain Temperature (^{o}C) Overlay Plot for each individual Dog (N=16)

Note: Each line represents an individual dog's mean temperature readings over the 32 hour study period.

Appendix 3-5:
Data Logger Aural Temperature (°C) Overlay Plot for each individual Dog (N=16)

Note: Each line represents an individual dog's mean temperature readings over the 32 hour study period.
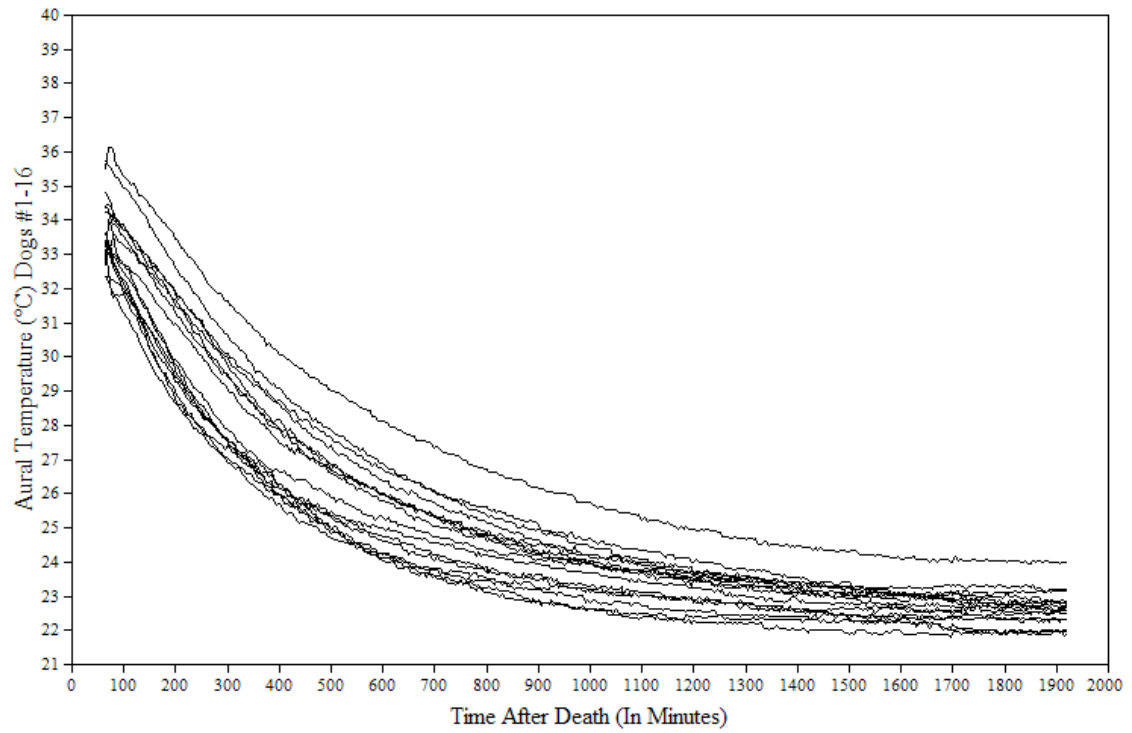
APPENDIX 4: FORMULAS AND EQUATIONS

Appendix 4-1:
Temperature Difference Ratio (Single Exponential Equation) from Reference [3]:

$$R = (T_{bt} - T_{et})/(T_{b0} - T_{et})$$

Where: T_{bt} = temperature at any body site measured at b given time t

T_{b0} = temperature of any body site at the moment of death

T_{et} = temperature of environment measured at time t

R = temperature difference ratio

Appendix 4-2:
Temperature Difference Ratio (Three Exponential Functions) from Reference [3]:

$$R = P1e^{P2t} + P3e^{P4t} + P5e^{P6t}$$

Where: P_1 to P_6 = parameters derived specifically by authors

t = time after death in hours

R = temperature difference ratio

Appendix 4-3:
Average Cooling Curve Formula from Reference [4]:

$$R_M = M_1 e^{M_2 tm} + M_3 e^{M_4 tm} + M_5 e^{M_6 tm}$$

Where: M_1 to M_6 = mean values for parameters P_1 to P_6 derived from reference no. [3]

tm = most probable PMI estimate

R_M = mean value of temperature difference ratio from reference no. [3]

Appendix 4-4:
Upper Limit Formula from Reference [4]:

$$R_U = U_1 e^{U_2 t u} + U_3 e^{U_4 t u} + U_5 e^{U_6 t u}$$

Where: U_1 to U_6 = upper limit values for parameters P_1 to P_6 derived from reference no. [3]

tu = upper limit PMI estimate

R_U = upper limit temperature difference ratio from reference no. [3]

Appendix 4-5:
Lower Limit Formula from Reference [4]:

$$R_L = L_1 e^{L2tL} + L_3 e^{L4tL} + L_5 e^{L6tL}$$

Where:
L_1 to L_6 = lower limit values for parameters P_1 to P_6 derived from reference no. [3]

tL = lower limit PMI estimate

R_L = lower limit temperature difference ratio from reference no. [3]

Appendix 4-6:
Rectal Body Cooling Model from Reference [22]:

$$Q=(T_r - T_a)/(T_0 - T_a) = A \ x \ exp \ (B \ x \ t) + (1 - A) \ x \ exp \ ((A \ x \ B)/(A - 1) \ x \ t)$$

Where: Q = standardized temperature

         T_r = rectal temperature at any time t

         T_0 = rectal temperature at death (t=0)

         T_a = ambient temperature

         A, B = constants

         t = death time

Appendix 4-7:
Rectal Body Cooling Model ($T_a \leq 23^{\circ}C$) from Reference [22]:

$$Q = (T_r - T_a)/(37.2 - T_a) = 1.25 \text{ x } exp \text{ } (B \text{ x } t) - 0.25 \text{ x } exp \text{ } (5 \text{ x } B \text{ x } t)$$

Where: Q = standardized temperature

        T_r = rectal temperature at any time t

        T_a = ambient temperature

        B = constant

        t = death time

Appendix 4-8:
Rectal Body Cooling Model ($T_a > 23^oC$) from Reference [22]:

$$Q=(T_r - T_a)/(37.2 - T_a) = 1.11 \times exp\ (B \times t) - 0.11 \times exp\ (10 \times B \times t)$$

Where: $Q =$ standardized temperature

             $T_r =$ rectal temperature at any time t

             $T_a =$ ambient temperature

             $B =$ constant

             $t =$ death time

Appendix 4-9:
Standard Cooling Curve from Reference [29]:

$$O_r = \left(\left((O_d - O_a) - (Z(O_d - O_a))/(Z-p) \right)e^{-Zt} \right) + \left((Z(O_d - O_a))/(Z - p) \right) e^{-pt} \right) + O_a$$

Where: O_r = rectal temperature

          O_a = ambient temperature

          O_d = rectal temperature at death

          Z, p = constants

          t = length of cooling period

Appendix 4-10:
Standard Cooling Curve (Linear Equation) from Reference [37]:

$$ln\ (O_n\ (t) - O_a) = -a_n t + k_n$$

Where: $O_n\ (t)$ = body temperature at site n

O_a = ambient temperature

$a,\ k$ = constants

t = time after death

Appendix 4-11:
PMI Estimate Equation from Reference [40]:

$$[((37.5 - A)/(B - A)) \times 60] + C$$

Where: A = closest temperature of a value less than 37.5°C

B = closest temperature of a value greater than 37.5°C

C = time at which A was recorded

VITA:

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