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Determination of the time of death of a domestic cat by measuring the area of a wet spot

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Abstract. Animal cruelty is a criminal offence under the current legislation of Ukraine. When investigating criminal proceedings related to animal cruelty, including domestic cats, it is often necessary to establish the time of death. Therefore, the development of new methods for determining it and improving existing ones is extremely relevant. The purpose of the study was to establish the relationship between the obtained values of wet spot area indicators and the time of death. To achieve this goal, the authors propose a new method for determining the age of death of domestic cats based on the results of measuring the area of a wet spot obtained from skeletal muscle tissue samples of corpses (Shkundia method). To do this, samples of muscle tissue were taken from the corpses of cats, a wet spot preparation was obtained by pressing and its area was calculated to establish a correlation between the values of this indicator and the time that has passed since death. The study of the obtained indicators established that these values are constant, and the features of fluctuations in the values of this indicator with the time elapsed since the death of the animals were established and shown graphically. Using a number of statistical methods, the absence of dependence of wet spot area indicators on the muscles from which samples were taken for research and animal breeds was determined. The nature of changes in the values of the wet spot area indicator depending on time is established and the features of their fluctuations in different

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periods during 27 days from the moment of animal death were explained. Reference values of these indicators depending on the time elapsed since the death of animals are presented, which can be used by forensic veterinary experts in practical work to determine the time of the occurrence of death of domestic cats. Due to a number of advantages, this method can be widely implemented in the practice of forensic veterinary medicine

Keywords: forensic veterinary examination; postmortem interval; *Felis silvestris catus*; Shkundia method; muscle tissue

Introduction

One of the problems that hinders the development of a modern legal society is the cruelty to animals. Cruelty to animals is defined as the actions of certain persons towards animals that are committed intentionally and lead to their injury or death, as defined in Article 299 of the Criminal Code of Ukraine (Criminal Code of Ukraine, 2001). W. Huang *et al.* (2020), I. Yatsenko & O. Parilovsky (2022) report that quite often domestic cats (*Felis silvestris catus*) appear as the object of abuse, which are among the most common pets. There are known cases of killing cats as a result of the use of weapons, various tools or items, as noted by D. Doukas (2022). C.M. Romano *et al.* (2020) report that crimes committed by throwing cats from a height, strangling, poisoning, torturing, and targeting dogs on cats are not uncommon, as a result of which the animals die. In the vast majority of cases related to animal cruelty, it is the cases of their death that are considered and forensic veterinary examinations of corpses are appointed. In the process of investigating such crimes, there are cases when a body is discovered and sent for examination after a certain period of time after death, which raises a number of questions for the investigation. J. Viciano *et al.* (2022) considered one of the most common issues – determination of the time of animal death. The need to determine the time of death of cats may be related to the need to obtain information necessary for inquiry and investigation about the date of death of the animal, with clarification of the correspondence in time of the testimony of persons involved in the investigation and trial. Such data can also refute or confirm the alibi of one of the participating parties. In addition, even in cases not related to criminal proceedings, the determination of the time of death is necessary to confirm or deny the reliability of anamnestic data provided for conducting a pathologic and anatomical autopsy of an animal. The determination of the time of death, therefore, is one of the main issues to be resolved by a forensic veterinary expert based on the results obtained after performing a forensic veterinary autopsy, and during the inspection of the scene or the discovery of a corpse, if the expert participates in these activities. I. Yatsenko & O. Parilovsky (2022) note that such questions are often raised before a forensic veterinary expert in decisions on the appointment of forensic veterinary examinations.

To solve the issue of establishing the time of death of animals, in particular, domestic cats, it is of great importance to choose the method that allows this to be done. Methods for determining the time and duration of death are based on recording the regularities of the dynamics of cadaveric phenomena, the time of residual vital activity of various body tissues in the first hours after death, the nature of biochemical changes that develop in the tissues of the corpse, in particular, according to E.K. Hryhorian (2019).

Some of these methods help to determine the duration of death directly, some – indirectly, for example, by determining the time of stay of the corpse in certain conditions, for example, in water, soil, etc. To more accurately determine the time of death of animals, it is also necessary to consider natural and other factors that affect the condition of the corpse. S.J. Jeong *et al.* (2020), in particular, note that such factors can be climatic components, that is, temperature, humidity and air velocity, the impact of natural phenomena (rain, snowfall, etc.), the presence of a corpse in the ground, reservoirs, premises, under the rubble of buildings, death in fires and other man-made accidents and catastrophes. Damage to corpses by representatives of fauna and flora also has a certain impact. All these conditions can accelerate or slow down the development of cadaveric phenomena, that is, the criteria by which the time of death is determined. Of great importance is also the time interval from the moment of death, during which each of the methods can provide information that can be correlated with the time of its occurrence. Most methods are suitable for use only for short periods of time after death.

The purpose of the study was to correlate the obtained values of the wet spot area indicator with the time of death of a domestic cat (*F. silvestris catus*). To achieve the goal, the following tasks were set: to take material from the corpses of cats whose time of death is known; to examine tissue samples of cat corpses using the wet spot method; to conduct statistical processing of the obtained data, with the help of which to prove their reliability; to find out the patterns of changes in the studied indicators relative to the time that has passed since the onset of death and the presence of correlation.

Literature Review

To date, quite a few methods have been developed for determining the time of death. Yu.V. Sarkisova (2021), I.G. Savka *et al.* (2021), V.K. Sokol (2022) note that the vast majority of these methods were invented by scientists in the field of forensic medicine. These methods are primarily intended for the study of objects such as human corpses. E.K. Hryhorian (2019), Yu.V. Sarkisova & S.M. Malanchuk (2020), M. Merck & D. Miller (2013) note that only some of these methods are used by veterinary doctors in forensic veterinary practice in the case of examination of animal corpses. The most common methods in use are classical methods. These include, for example, the visual and palpation method, which involves assessing the state of development of cadaveric changes by examination and palpation. Thermometry is widely used, as noted by S.J. Jeong *et al.* (2020), the essence of which is to measure the temperature of the corpse at certain points. H. Muggenthaler *et al.* (2012) emphasise the need to correlate the obtained values with tabular data reflecting the dynamics of postmortem cooling for animals of a certain species, weight, etc. A variation of this method is to measure the temperature of the place of death of an animal or person. Of the biochemical methods used, as reported by G. Piegari *et al.* (2023), studies of protein concentrations in cadaveric tissues; a number of authors, such as M.T. Ave *et al.* (2021), J. Garland *et al.* (2020), and M. Nioi *et al.* (2021), propose to investigate changes in the vitreous body in the eyeballs; some other methods have also been developed. There are a number of methods that are based on image analysis. E. Watson & J.Kr. Baucom (2020) suggest analysing data obtained using computed tomography, and M. Zhang (2022) – magnetic resonance imaging. There is also an elastographic method. J. Byrd & L. Sutton (2021) recommend the widespread use of forensic entomology.

According to S. Matuszewski (2021), this method consists of estimating the time after death, which can often coincide with the time of

colonisation of cadaver tissue by entomofauna. Such a study is based on determining the activity and number of insects that contaminate the corpse, and determination of the age and stage of development of these insects. M. Heba El-Sayed *et al.* (2023) propose to apply DNA studies in the aspect of assessing the degree of its degradation over the time that has passed since the onset of death, and to identify the species affiliation of the remains of an animal's corpse. A number of methods consist in studying the microstructure of cadaver tissues using light, luminescent, and electron microscopy. A method for determining the time of death based on the results of microscopic examination, as reported by V.K. Sokol (2022), is based on visual and morphometric assessment of tissue-level changes caused by tertiary cadaveric changes, namely autolysis and putrefaction. The development of destructive changes directly depends on the time of death. In the field of forensic medical examination today, in addition to histological examination, some of its varieties are used. Thus, Yu.V. Sarkisova & S.M. Malanchuk (2020) suggest using the spectropolarimetric method, the essence of which is to establish the time of death in a long-term interval by analysing data obtained by spectral-selective laser-induced fluorescence microscopy of tissue sections, and O.Iu. Lytvynenko (2022) – reconstruction of the polycrystalline structure of tissue sections, which consists in determining the relationships between changes of values that characterise the distribution of values of the degree of crystallisation of tissue sections of various organs and the time of death.

D.Yu. Shkundia *et al*. (2023) proposed a method for determining the time of death by measuring the area of a wet spot obtained from cadaver tissue samples. In turn, the literature, both Ukrainian and foreign, devoted to the problem of determining the time of death, does not reflect the features of such studies of cadaveric material, especially in the aspects of studying corpses of different types and breeds of animals; their gender and age categories.

Materials and Methods

The study was conducted in 2022-2023 at the Academician Volodymyr Kasyanenko Department of Animal Anatomy, Histology and Pathomorphology of the National University of Life and Environmental Sciences of Ukraine, Kyiv. The selection of cadaveric material from the animals used in the experiment was carried out in accordance with the ethical norms of international and Ukrainian legislation (Law of Ukraine No. 3447-IV, 2006; European convention…., 1986).

The material was taken from the corpses of 4 domestic cats (Felis silvestris catus), of which two animals belonged to the Burmese breed, the other two – to the Maine Coon breed. The animals involved in the experiment were male, aged 2.5-3.5 years, of average fatness. Their corpses were received by the department from various veterinary clinics in Kyiv after euthanasia on the recommendations of veterinarians, subject to the consent of the animal owners, and were used by the authors to establish the time of death. After that, pathologic and anatomical autopsy of cat corpses was performed according to generally accepted methods (without evisceration). In the future, samples of skeletal muscle tissue weighing 3 g were taken from animal corpses from the shoulder and thigh area, provided that there were no visible pathologic and anatomical changes. Samples were taken once a day, for 27 days from the moment of arrival of the corpse. Corpses were stored in sterile conditions at a stable temperature of 20°C during the material selection period. After that, the sampling was stopped.

The selected samples were examined using the Shkundia method (Shkundia *et al.*, 2023). To do this, each sample was placed in the middle of a separate sheet of 10×10 cm plastic film,

and then placed between two 10×10 cm glass plates. A sheet of decontaminated filter paper was previously placed on the bottom plate. The polyethylene film with the sample was placed so that the surface of the sample was in contact with the filter paper. After placing the sample between two glass plates in the described manner, the plates were placed under a 1 kg press for 15 minutes. Next, the top plate and plastic film were removed, the contours of the wet spot and the contours of the pressed sample were outlined with a simple pencil, the pressed sample was removed, and then the filter paper was dried.

The wet spot area was measured using a polar planimeter PP-2k and calculated using the equation:

$$S = \frac{x_1+x_2}{2} \text{ x } P - \frac{y_1+y_2}{2} \text{ x } P, \qquad (1)$$

where S – area of the wet spot, cm^2; x_1, x_2 – indicators of planimeter divisions when measuring the external contour of a wet spot; y_1, y_2 – indicators of planimeter divisions when measuring the internal contour of a wet spot; P – price of dividing the planimeter in units of measurement (cm^2).

Statistical processing of the obtained results was carried out in the following sequence. The normality of the distribution was determined by the Shapiro-Wilk test (González-Estrada *et al.*, 2022; Rodrigues de Souza *et al.*, 2023). The equality of variances was determined by the Levene test (Wang *et al.*, 2022). The T-test (for two independent samples) was used to compare samples between animals of the same breed (Novak, 2022). ANOVA (univariate analysis of variance) was used to compare samples between animals of different breeds (Emerson, 2022). The calculations were performed using the Real Statistics Resource Pack 2007, which is an add-on to Excel-2010 software suite.

Results and Discussion

Based on the results of calculating the wet spot area obtained from samples of skeletal muscle tissue of cat corpses, their values for various muscle groups in the animals under study were obtained (Table 1).

Table 1. Value of the wet spot area obtained from the muscles of experimental animals at different time intervals from the moment of death, n=4

Days after death	Wet spot area, shoulder muscles, Maine Coon, cm^2	Wet spot area, thigh muscles, Maine Coon, cm^2	Wet spot area, shoulder muscles, Burmese, cm^2	Wet spot area, thigh muscles, Burmese, cm^2
0	3.42	4.01	2.36	3.39
1	4.36	2.34	3.67	2.55
2	2.07	5.62	2.88	4.92
3	9.36	6.53	7.00	6.31
4	5.08	4.45	5.28	7.66
5	3.78	10.67	4.70	9.09
6	8.73	8.05	7.03	7.18
7	6.88	5.22	6.00	5.89
8	9.00	8.10	8.50	8.24
9	7.10	6.58	7.36	6.60
10	6.70	7.89	6.83	7.05
11	10.02	7.24	9.10	8.19
12	5.49	5.04	5.66	5.44
13	7.29	4.45	7.00	5.05
14	6.75	3.06	5.24	3.39

Table 1. Continued

Days after death	Wet spot area, shoulder muscles, Maine Coon, cm²	Wet spot area, thigh muscles, Maine Coon, cm²	Wet spot area, shoulder muscles, Burmese, cm²	Wet spot area, thigh muscles, Burmese, cm²
15	3.11	4.20	3.72	3.48
16	5.00	4.54	4.49	4.01
17	2.25	5.49	3.42	3.65
18	6.34	2.16	5.93	3.92
19	4.05	4.46	4.71	4.09
20	6.35	2.62	6.19	3.62
21	4.05	3.75	5.88	3.83
22	6.17	3.91	5.24	4.00
23	4.23	5.68	5.94	5.86
24	10.58	6.84	8.09	6.51
25	8.73	7.12	9.12	7.00
26	10.35	8.03	9.86	8.29
27	10.83	9.52	10.59	9.72
Average	6.36	5.63	6.14	5.68
Standard deviation	2.59	2.17	2.10	2.01
Sample variance	6.70	4.70	4.40	4.02

***Source**: developed by the author*

Changes in the wet spot area index depending on the time elapsed since the animal's death in samples taken from the shoulder muscles of a Maine Coon cat are graphically shown in Figure 1.

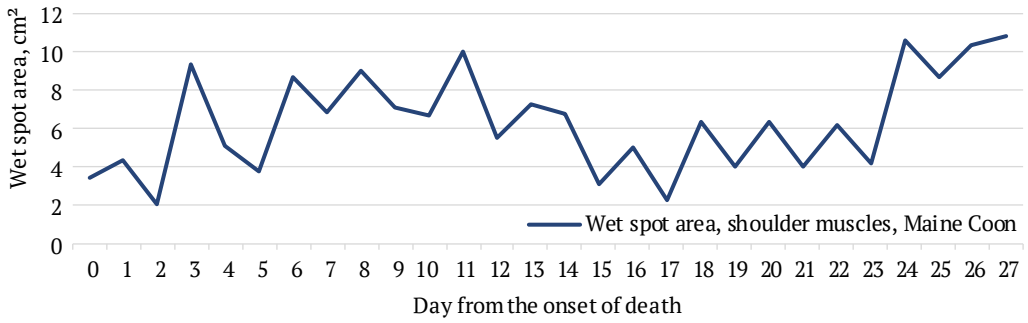

Figure 1. Diagram of changes in the wet spot area indicator depending on the time since death, Maine Coon, shoulder muscle

***Source**: developed by the authors*

From days 1 to 3 after the death of the animal, the indicator of the wet spot area first slightly decreased, and then sharply increased, to 9.36 cm² on day 3. From days 3 to 5, a decrease was again observed, and from days 6 to 12 – a stable increase to values in the range of 8-10 cm². From days 12 to 17, a gradual decrease in the wet spot area indicator to values in the range of 2-4 cm² was detected, from days 17 to 23, the indicator was consistently in the range of 4-6 cm², after which it grew abruptly to values of 10-11 cm² and remained at this level in the future.

Changes in the wet spot area depending on the time elapsed since the animal's death in samples taken from the thigh muscles of a Maine Coon are graphically shown in Figure 2.

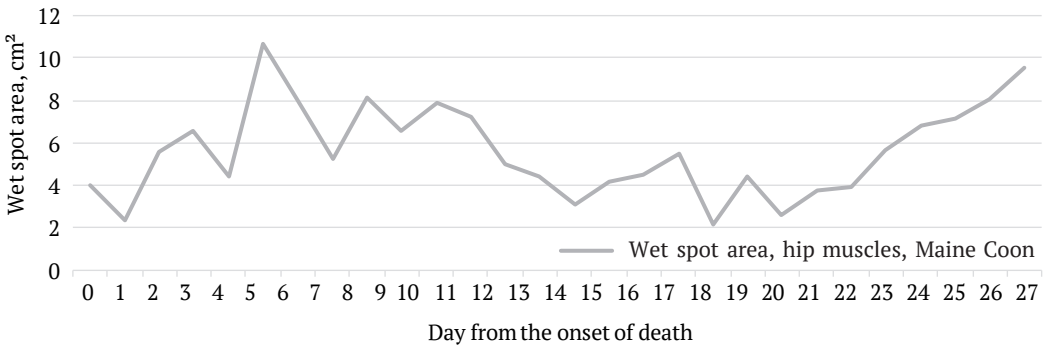

Figure 2. Graph of changes in the wet spot area indicator depending on the time since death, Maine Coon, thigh muscles

***Source**: developed by the authors*

From days 1 to 3 after the death of the animal, the indicator of the area of the wet spot first slightly decreased, and then increased, to 6.53 cm² on day 3. From days 4 to 5, a sharp increase to 10.67 cm² was observed, and from day 6 to 14 – a stable decrease to a value of 3.06 cm². From days 15 to 17, a gradual increase in the value of the wet spot area in the range of 4-6 cm² was detected, and from days 18 to 20, this indicator steadily decreased and was in the range of 2-4.5 cm², after which it gradually increased to a value of 9.52 cm². Changes in the size of the wet spot area depending on the time elapsed since the animal's death in samples taken from the shoulder muscles of a Burmese cat are graphically shown in Figure 3.

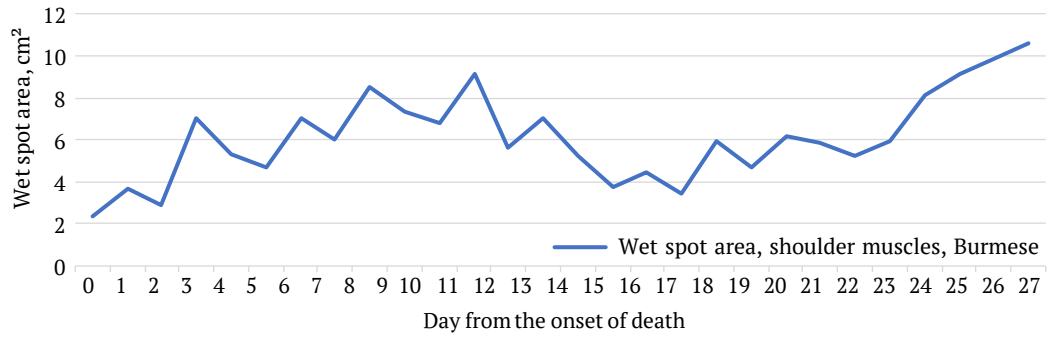

Figure 3. Diagram of changes in wet spot area indicator depending on the time since death, Burmese, shoulder muscles

***Source**: developed by the authors*

From days 1 to 2 after the death of the animal, the indicator of the wet spot area first slightly decreased, and then sharply increased, to 7 cm² on day 3. From days 4 to 5, a gradual decrease in its value to 4.7 cm² was observed, and from days 6 to 11, an abrupt increase in values in the range of 6-9 cm² was observed. From days 12 to 17, first a sharp and then gradual

decrease in the wet spot area to values in the range of 3-4 cm² were detected. From days 18 to 24, the parameters of this indicator increased and were in the range of 5-6 cm², after which they sharply increased to 10.59 cm².

Changes in the wet spot area indicator depending on the time elapsed since the animal's death in samples taken from the thigh muscles of a Burmese cat are graphically shown in Figure 4.

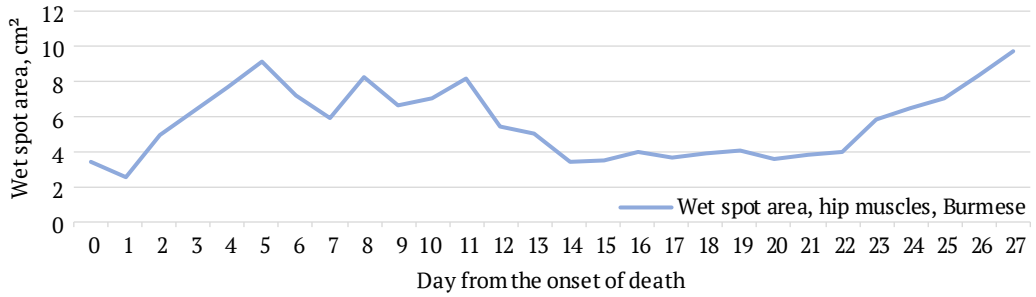

Figure 4. Graph of changes in the wet spot area indicator depending on the time since death, Burmese, thigh muscle

***Source**: developed by the authors*

From day 1 to 5 after the death of the animal, the indicator of the area of the wet spot initially increased very sharply to a value of 9.09 cm², and from days 6 to 11 this indicator remained in the range of values of 6-8 cm². From days 12 to 14, a sharp decrease in its value to 5.66 cm² was observed, and then from days 15 to 22 – the values almost did not change and were in the range of 3-4 cm². From days 23 to 27, the indicator increased sharply to a value of 9.72 cm². Changes in wet spot area indicators as a function of the time elapsed since animal death in samples taken from the shoulder and thigh muscles of Maine Coon and Burmese cats are graphically shown in Figure 5.

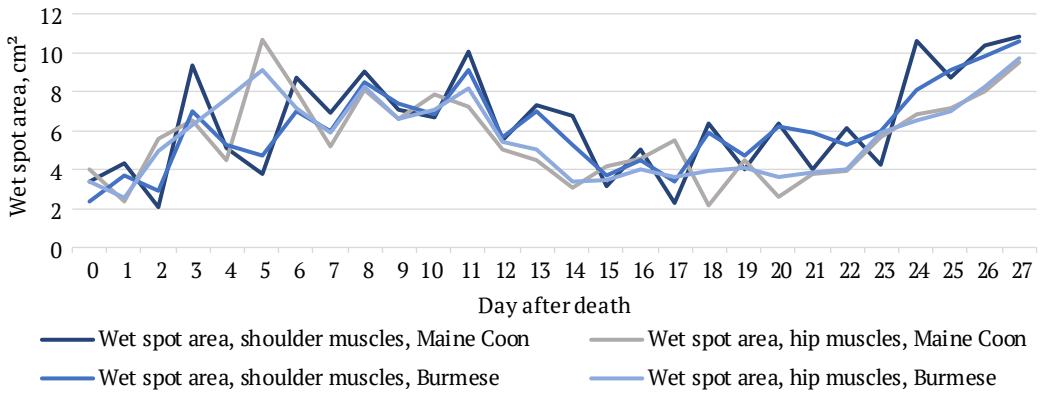

Figure 5. Diagram of changes in wet spot area indicators depending on the time since death of all animals under study

***Source**: developed by the authors*

When assessing the results obtained from the animals corpses, it is possible to identify general patterns of changes in the wet spot area obtained from samples of their skeletal muscles depending on the time elapsed since their death. From the onset of death to days 1-2, the indicator was in the range of 2-4 cm^2, sometimes even having a tendency to decrease. Starting from days 1-2, an abrupt increase in the values of this indicator was observed, which reached peak values at days 3-5, in the range of 8-11 cm^2. In the future, their values with small fluctuations corresponded to the range of 6-10 cm^2, and starting from day 11, a sharp decrease in the value of these indicators was observed, which corresponded to the range of 2-4 cm^2. The values were in the range of 2-6 cm^2 up to days 22-23. Next, there was a sharp abrupt increase in the size of the wet spot area obtained from skeletal muscle samples of cats, depending on the time that has passed since the death of animals, with a subsequent tendency to increase.

For mathematical processing of data as a null hypothesis, the statement was taken as a basis that according to the results of measuring the area of a wet spot obtained from samples of muscle tissue of cat corpses, there should be no significant difference between the indicators taken from animals of different breeds, and the difference between the indicators obtained during the study of samples taken from different skeletal muscles. Thus, the values obtained at certain time intervals, regardless of the breed of cats and the muscle group from which the samples were taken, should correlate with the time that has passed since the onset of death, and these indicators can be used to calculate the time of death of animals, using the data obtained by the authors as a reference. Using the Shapiro-Wilk test, the distribution normality was established in all the data samples under study (Table 2).

Table 2. Normality of the distribution of samples of wet spot area values obtained from the muscles of experimental animals at different time intervals from the moment of death, n=4

Shapiro-Wilk test	**Wet spot area, shoulder muscles, Maine Coon**	**Wet spot area, thigh muscles, Maine Coon**	**Wet spot area, shoulder muscles, Burmese**	**Wet spot area, thigh muscles, Burmese**
Distribution normality indicator (W-stat)	0.957271813	0.969241318	0.979122624	0.940969325
Significance level (p-value)	0.299880488	0.560395402	0.828890163	0.11699154
Threshold significance level (alpha)	0.05	0.05	0.05	0.05
Normality	Yes	Yes	Yes	Yes

***Source**: developed by the author*

The Shapiro-Wilk test (Table 2) shows that the distribution is normal in all the data samples under study, i.e., no sample has values that differ significantly from all the others. Using the Levene test, equality of variances was established in all the data samples under study (Table 3).

Table 3. Equality of sample dispersions of wet spot area values obtained from the muscles of experimental animals at different time intervals from the moment of death, n=4

Levene test	Variance between wet spot area samples of shoulder and thigh muscles, Maine Coon	Variance between wet spot area samples of shoulder and thigh muscles, Burmese	Variance between wet spot area samples of Maine Coon and Burmese cats
Average values (means)	0.325441	0.798616	0.492355
Threshold significance level (alpha)	0.05	0.05	0.05

Source: *developed by the author*

The Levene test (Table 3) determined the equality of variances between the values of the wet spot area of the shoulder and thigh muscles in animals of the same breed and between the animals of different breeds. The average values of variances exceeded the threshold level of significance. Since the difference in variances in the cases was not statistically significant, the variances can be considered homoscedastic. Thus, with the normality of the distribution and equality of variances, the null hypothesis is confirmed. Using the T-test (for two independent samples with equal variances in the Student's distribution), the absence of differences between the samples of these values of the wet spot area of the shoulder and thigh muscles in animals of each breed was established (Table 4).

Table 4. Value of the t-criterion for samples of wet spot area indicators obtained from the muscles of experimental animals at different time intervals from the moment of death, n=4

Cat breed	Resulting value of the Student's criterion (t-stat)	Critical value of the Student's criterion (t-crit)	Significance level (p-value)
Maine Coon	1.1474	2.0048	0.2562
Burmese	0.8375	2.0048	0.4060

Source: *developed by the author*

Using the T-test, it was found that in animals of each studied breed there was no difference between the samples of data on the area of the wet spot of the shoulder and thigh muscles, since the obtained values of the Student's criterion for the data samples of each breed compared with each other were less than the corresponding critical values of this variable. Therefore, the values of the wet spot area indicator do not significantly depend on which group of skeletal muscles (shoulder or thigh) to take samples for research using this method.

As a result of using univariate analysis of variance (ANOVA) with the calculation of the Fischer criterion, there was no difference between the samples of these values of the wet spot area of the shoulder and thigh muscles in cats of different breeds (Table 5).

Table 5. Value of the F-criterion for samples of wet spot area indicators obtained from the muscles of experimental animals at different time intervals from the moment of death, n=4

Source of variation	Resulting value of the Fischer criterion (F)	Critical value of the Fischer criterion (F-crit)	Significance level (p-value)
Between groups, Maine Coon and Burmese breeds	0.7179	2.6887	0.5433

***Source**: developed by the author*

Based on the results of univariate variance analysis, it was found that there is no difference between the value of the wet spot area of the shoulder and thigh muscles in cats of different breeds, since the obtained values of the Fischer criterion for comparable data samples obtained from cats of different breeds were less than the corresponding critical values of this variable. Thus, the breed of cats whose corpses were used for research by this method did not significantly affect the value of the wet spot area indicator. Thus, the null hypothesis, according to the calculations performed on the Shapiro-Wilk, Levene, t-test, and univariate analysis of variance (Wang *et al*; 2022, Emerson, 2022; Rodrigues de Souza *et al.*, 2023), it is confirmed that the values obtained at certain time intervals, regardless of the breed of cats and the muscle group from which the samples were taken, correlate with the time elapsed since the onset of death.

The idea of this study was based on the assumption that changes in wet spot area indicators over time would represent uniform growth in the form of a linear relationship. However, the data obtained had a slightly different trend. The wet spot area increased on day 3-5 and remained relatively stable for days 5-11, slightly decreased from day 11 to day 15, stabilised again between days 15 and 22-23, and began to increase sharply thereafter. The increase in the area of the wet spot on days 3-5 can be explained by the release of water due to the collapse of the structures of intermuscular connective tissue, and the result of the diffusion of the liquid part of the blood after death from the vessels that supply blood to the muscles into the intermuscular space. At this time, water remained in the muscle fibres, and their structure was preserved in this period of time from the moment of death. The relative stability of the indicators during days 5-11 after the death of the animals can be explained by the limited amount of water in the intermuscular space, as muscle fibres are not yet destroyed during this period. The decrease in the area of the wet spot during days 11-15 from the moment of death is explained by the loss of a certain amount of water through diffusion to other parts of the corpse and through its surface. The stability of indicators from days 15 to 22-23 may be associated with the beginning of the breakdown of muscle fibres, which compensates for the diffusion of water to other parts of the corpse and through its surface. An abrupt increase in the area of a wet spot, starting from days 22-23, can be the result of the breakdown of a large number of muscle fibres, and the destruction of macromolecules of proteins and lipids, as a result of which liquid is released. However, in all data samples under study, the described trend was clearly observed, so the data obtained can serve as a reference against which to compare the data from the corpses of cats obtained by the Shkundia method (Shkundia *et al.*, 2023), whose time of death must be established for forensic examinations.

Based on the above, it is proposed to calculate the time of death in cats based on the wet spot area obtained from skeletal muscle samples of corpses, using the reference values given in Table 6.

Table 6. Reference values of the wet spot area indicator obtained from skeletal muscle samples from cat corpses, M±m

Days after death	Reference values, cm^2	Days after death	Reference values, cm^2	Days after death	Reference values, cm^2
0	3.30±0.33	10	7.120.27	20	4.70±1.13
1	3.23±0.56	11	8.64±0.66	21	4.38±0.54
2	3.87±1.01	12	5.41±0.13	22	4.83±0.63
3	7.30±0.74	13	5.95±0.86	23	5.43±0.43
4	5.62±0.73	14	4.61±1.01	24	8.01±0.96
5	7.06±2.04	15	3.63±0.24	25	7.99±0.67
6	7.75±0.46	16	4.51±0.18	26	9.13±0.71
7	6.00±0.32	17	3.70±0.64	27	10.17±0.39
8	8.46±0.20	18	4.59±1.11		
9	6.91±0.23	19	4.33±0.18		

***Source**: developed by the author*

A specialist who performs a forensic veterinary examination, if representatives of the investigative authorities are tasked with establishing the time of the occurrence of death in a cat, can, after performing a study using the Shkundia method, compare the obtained indicator with the reference value, and establish the time that has passed since the death of the animal, with an accuracy of one day. The data obtained can be compared with data obtained using other methods for determining the time of death, in order to exclude possible errors. Thus, the method of determining the area of a wet spot is informative and can be implemented in forensic veterinary practice. Compared to other methods of determining the time of death, the advantage of such studies is a long time interval – about 1 month from the moment of death, while thermometry is suitable in the time interval of 1-3 days, which is indicated, for example, by S.J. Jeong *et al*. (2020), microscopic examination – 1-18 days, and a method based on determining the state of entomofauna of the corpse, according to J. Byrd & I. Sutton (2021) and S. Matussewski (2021) – no more than 10-15 days. In addition, the equipment required for working with the wet spot method is not difficult to use and expensive, as, for example, for studies using computer or magnetic resonance imaging, which are proposed by E. Watson & J.Kr. Baucom (2020) and M. Zhang (2022).

J. Serdioucov *et al.* (2023) note that one of the most common and accurate methods for determining the time of death – histological examination of postmortem changes in various tissues and organs of the corpse – provides valid results only during the first 18 days after death. It is characteristic that the results presented by J. Serdioucov *et al.* (2023) are obtained specifically on the corpses of domestic cats. The wet spot method proposed by the authors has a number of advantages over the histological method. The postmortem interval, which can be determined by the Shkundia method, is significantly longer than that that can be determined histologically – 27 days and 18 days, respectively. The time required to make a wet spot preparation is approximately 1 hour, while a histological preparation, depending on the latest equipment used by the researcher, takes from 2 to 14 days. Although, according to most researchers, for example, V.K. Sokol (2022), histological examination is a widely available diagnostic method, but the amount of equipment required for histology (automatic filling of material and staining of histoses, microtoms,

microscopes, photo nozzles, etc.) is several times greater than that required for the wet spot method (glass plates, planimeter). In addition, the wet spot method does not require the use of chemical reagents. Wet spot preparations are better preserved than histopreparations. Quantitative data, as noted by D. Shkundia *et al.* (2023), also easier to get by the wet spot method. However, histopreparations have much greater visibility than wet spot preparations. In addition to classic micrographs, as reported by Yu.V. Sarkisova & S.M. Malanchuk (2020), microscopy of histopreparations can produce spectropolarimetric images and reconstruction of their polycrystalline structure, as noted by O.Iu. Lytvynenko (2022).

Measurement of cadaveric cooling rate by thermometry, by definition of C. Henßge & B. Madea (2004), is the most commonly used method for determining the time of death of humans and animals. The features of postmortem cooling in domestic cats have not yet been practically studied. The wet spot method proposed by the authors has a number of advantages over the thermometric method. The most important of these is that the time interval for the onset of death, which can be determined by the Skundia method, is much longer than that which, in particular, according to S.J. Jeong *et al.* (2020), can be recorded by thermometry – 27 days and 1-3 days, respectively. Domestic cats are small animals and therefore a complete equalisation of the temperature of the corpse with the ambient temperature occurs at most during the 2nd day after death. In addition, as noted by S.J. Jeong *et al.* (2020), the dependence of the accuracy of determining the time of death by the thermometric method on climatic conditions is very significant, which affects the measurement results, often not allowing to establish a correlation between the obtained and tabular data. The results of the thermometric study are particularly negatively affected by the air temperature both in open areas and in various rooms, the humidity level and speed of air movement, weather conditions (rain, snow, etc.). The data obtained by the wet spot method are much less affected by these factors, especially when preparations are obtained from cadaveric skeletal muscle, as recommended by D. Shkundia *et al.* (2023). In addition, there is a dependence of the validity of indicators obtained using thermometry on the causes of death, especially diseases, the symptoms of which, in particular, are characterised by a pronounced increase or decrease in body temperature. The authors are not aware of any such influence on the results obtained by the wet spot method. In addition, the wet spot preparation can be used as physical evidence, while the reliability of temperature measurements, especially in the field, sometimes raises doubts among law enforcement officials. Thermometry also has a number of advantages over the wet spot method. This is significantly less time, as noted by C. Henßge & B. Madea (2004), which takes from 1 to 5 minutes depending on the design features of the thermometers, while the production time of the wet spot preparation is approximately 1 hour; simple portable equipment that allows on-site measurements, etc.

Entomological method, according to J. Byrd & L. Sutton (2021), is extremely common in forensic and veterinary medicine. When comparing it with the wet spot method, the obvious advantage of the latter is one thing: an even greater dependence on climatic conditions and, even, the time of year than with the thermometric method, which is associated with the seasonality of the insect life cycle, as noted by S. Matuszewski (2021). For these two methods, the measurement range of the postmortem interval and the time spent on conducting research are approximately the same. The advantage of the entomological method is also the low need for equipment.

This study presents a proprietary method. It is based on determining the moisture-retaining ability of animal body tissues, which changes after the death of an animal, since liquid is released during the breakdown of cellular structures and macromolecules. When comparing the wet spot method with other widely used methods for determining the time of death, a number of advantages over each one are revealed, however, as well as some disadvantages. Therefore, the authors consider it appropriate to use it widely, in particular, in the practice of forensic veterinary examination.

Conclusions

The proposed method for determining the time of death by measuring the area of a wet spot obtained from skeletal muscle tissue samples from domestic cat corpses is informative and has a number of advantages over other methods for determining the time of death. The most important advantage is the long time interval in which results can be obtained that correlate with the time of death. In addition, the validity of this method is much less dependent on climatic conditions, such as temperature and humidity, weather conditions in the place where the corpse of the animal was found, being in the room, packing corpses in plastic bags, wrapping in blankets, clothing elements, etc. Wet spot preparations obtained when working with fabrics using this method can be stored for a long time and serve as material evidence in the investigation and judicial review of criminal cases. The method provides quantitative data that allows determining the time of death much more accurately than, for example, visual and palpation, and is subjected to statistical processing.

An explanation is found for fluctuations in the values of the wet spot area indicator depending on the time that has passed since death. Statistical studies have shown that the data obtained by measuring the area of a wet spot correlate with the time of death. Reference values of the wet spot area obtained from samples of skeletal muscle of cat corpses have been developed, which forensic veterinarians can use in their practical activities to compare with the data obtained by this method of examining material from cat corpses in specific cases that become the basis for investigating criminal proceedings.

The disadvantages of the method for determining the time of death by measuring the area of a wet spot obtained from skeletal muscle tissue samples from cat corpses are its specificity for certain animal species and certain tissues. In addition, to perform research using this method, it is necessary to have a device (planimeter), which is not widely used equipment. The influence of climatic and other conditions in which the animal's corpse is located on the reliability of the results obtained by applying this method remains unclear. The prospects for further research are to determine the time of death by measuring the area of the wet spot from the material of various organs, species, breeds, sex and age categories of animals, developing amendments to reference values that take into account climatic conditions and the environment in which the corpse can be located, etc.

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Conflict of Interest

None.

References

[1] Ave, M.T., Ordonez-Mayan, L., Camina, M., Febrero-Bande, M., & Munoz-Barus, J.I. (2021). Estimation of thepost-mortem interval: Effect of storage conditions on the determination of vitreous humour [K+]. *Science & Justice*, 61(5), 597-602. doi: 10.1016/j.scijus.2021.07.005.

[2] Byrd, J., & Sutton, L. (2020). Forensic entomology for the investigator. *WIREs Forensic Science*, 2(4), article number e1370. doi: 10.1002/wfs2.1370.

[3] Criminal Code of Ukraine. (2001, September). Retrieved from https://zakon.rada.gov.ua/laws/show/2341-14#Text.

[4] Doukas, D. (2022). Non-accidental injuries in dogs and cats: Review of post-mortem forensic evaluations and the social significance of small animal practice. *Journal of the Hellenic Veterinary Medical Society*, 73(1), 3543-3552. doi: 10.12681/jhvms.23296.

[5] Emerson, R.W. (2022). ANOVA Assumptions. *Journal of Visual Impairment & Blindness*, 116(4), article number 0145482X2211241. doi: 10.1177/0145482X221124183.

[6] European convention for the protection of vertebrate animals used for research and other scientific purposes. (1986). Retrieved from https://zakon.rada.gov.ua/laws/show/994_137#Text.

[7] Garland, J., Olds, K., Rousseau, G., Palmiere, C., Ondruschka, B., & Kesha, K., Glenn, C., & Morrow, P. (2020). Using vitreous humour and cerebrospinal fluid electrolytes in estimating post-mortem interval – an exploratory study. *Australian Journal of Forensic Sciences*, 52(6), 626-33. doi: 10.1080/00450618.2019.1642956.

[8] González-Estrada, El., Villaseñor, J.A., & Acosta-Pech, R. (2022). Shapiro-Wilk test for multivariate skew-normality. *Computational Statistics*, 37(3), 1985-2001. doi: 10.1007/s00180-021-01188-y.

[9] Heba El-Sayed, M., El-Shafei, D.A., Abouhashem, S.N., & Alaa El-Din, E.A. (2023). Could skeletal muscle changes provide a reliable method for estimating the time since death: A histological, biochemical, and DNA study. *Australian Journal of Forensic Sciences*, 55(1), 46-58. doi: 10.1080/00450618.2021.1921272.

[10] Henßge, C., & Madea, B. (2004) Estimation of the time since death in the early post-mortem period. *Forensic Science International*, 144(2-3), 167-175. doi: 10.1016/j.forsciint.2004.04.051.

[11] Hryhorian, E.K. (2019). Content analysis of the forensic medical assessment of postmortem interval. *Ukrainian Journal of Medicine, Biology and Sports*, 4, 6(22), 265-270. doi: 10.26693/jmbs04.06.265.

[12] Huang, W.-Hs., Kuo, Ch.-Ch., Hu, Hs.-Y., Pan, Ch.-Hs., Taiching, L.A., & Liu, Ch.-Hs. (2020). Manual strangulation of a stray cat: Linking pathologic findings with the crime. *Journal of Veterinary Forensic Sciences*, 1(2), 11-14. doi: 10.32473/jvfs.v1i2.128634.

[13] Jeong, S.J., Park, S.H., Park, J.E., Park, S.H., Moon, T.Y., Shin, S.E., & Lee, J.W. (2020). Extended model for estimation of ambient temperature for postmortem interval (PMI) in Korea. *Forensic Science International*, 309, article number 110196. doi: 10.1016/j.forsciint.2020.110196.

[14] Law of Ukraine No. 3447-IV "On Protection of Animals from Cruelty". (2006, February). Retrieved from https://zakon.rada.gov.ua/laws/show/3447-15#Text.

[15] Lytvynenko, O.Iu. (2022). Method of reconstruction of polycrystalline structure of histological cuts in determination of the stature of formation of damages of internal bodies. *Forensic Sciences Research*, 1, 81-86. doi: 10.24061/2707-8728.1.2022.12.

[16] Matuszewski, S. (2021). Post-mortem interval estimation based on insect evidence: Current challenges. *Insects*, 12(4), article number 314. doi: 10.3390/insects12040314.

[17] Merck, M., & Miller, D. (2013). Postmortem changes and the postmortem interval. In *Veterinary forensics: Animal cruelty investigations* (pp. 255-271). New Jersey: John Wiley. doi: 10.1002/9781118704738.ch14.

[18] Muggenthaler, H., Sinicina, I., Hubig, M., & Mall, G. (2012). Database of post-mortem rectal cooling cases under strictly controlled conditions: A useful tool in death time estimation. *International Journal of Legal Medicine*, 126(1), 79-87. doi: 10.1007/s00414-011-0573-6.

[19] Nioi, M., Napoli, P.E., Demontis, R., Locci, E., Fossarello, M., & d'Aloja, E. (2021). Postmortem ocular findings in the optical coherence tomography era: A proof of concept study based on six forensic cases. *Diagnostics*, 11(3), article number 413. doi: 10.3390/diagnostics11030413.

[20] Novak, S.Y. (2022). On the T-test. *Statistics & Probability Letters*, 189, article number 109562.

[21] Piegari, G., De Pasquale, V., d'Aquino, I., De Biase, D., Caccia, G., Campobasso, P.C., Tafuri, S., Russo, V., & Paciello, O. (2023). Evaluation of muscle proteins for estimating the post-mortem interval in veterinary forensic pathology. *Animals*, 13(4), article number 563. doi: 10.3390/ani13040563.

[22] Rodrigues de Souza, R., Toebe, M., Mello, A. Ch., & Bittencourt, K.Ch. (2023). Sample size and Shapiro-Wilk test: An analysis for soybean grain yield. *European Journal of Agronomy*, 142, article number 126666. doi: 10.1016/j.eja.2022.126666.

[23] Romano, C.M., Dorman, C.D., & Gaskill, L.C. (2020). Postmortem veterinary toxicology: Animal death investigation and the veterinary diagnostic laboratory. *WIREs Forensic Science*, 2(5), article number e1375. doi: 10.1002/wfs2.1375.

[24] Sarkisova, Yu.V. (2021). Diagnostic possibilities of accurate determination of the time since death on the concentration of electrolytes in the vitreous body of the human eye. *Forensic Medical Examination*, 1, 79-85. doi: 10.24061/2707-8728.1.2021.10.

[25] Sarkisova, Yu.V., & Malanchuk, S.M. (2020). Spectral-selective laser-induced autofluorescent microscopy of polycrystalline fraction of the human vitreous body in diagnostics time since death. *Forensic Medical Examination*, 1, 61-69. doi: 10.24061/2707-8728.1.2020.8.

[26] Savka, I.G., Kozan N.M., Dunaev, O.V., & Oliynyk, I.Y. (2021). *Establishment of accurate criteria for diagnosis of the time since death in forensic medical practice.* Retrieved from http://dspace.bsmu.edu.ua/bitstream/123456789/17806/1/SME_2021_1_018.pdf.

[27] Serdioucov, J., Shkundia, D., & Kruchynenko, O. (2023). Identification of time of death of cats according to histological changes in some organs. *Regulatory Mechanisms in Biosystems*, 14(3), 399-406. doi: 10.15421/10.15421/022359.

[28] Shkundia, D.Yu., Serdioucov, J.K., Malyuk, M.O., & Bokotko, R.R. (2023). *The method of determining the time of death by measuring the area of the wet spot obtained from the tissue samples of cat corpses* (Patent of Ukraine No. 153614). Retrieved from https://iprop-ua.com/inv/h0zfzhbd/.

[29] Sokol, V.K. (2022). *Peculiarities of microscopic changes in the tissue of the human organism at different periods of death*. Retrieved from http://forensic.bsmu.edu.ua/article/view/262428/258860.

[30] Viciano, J., Lopez-Lazaro, S., & Tanga, C. (2022). Post-mortem dental profile as a powerful tool in animal forensic investigations – a review. *Animals*, 12(16), article number 2038. doi: 10.3390/ani12162038.

[31] Wang, Y., Tang, M., Wang, P., Liu, B., & Tian, R. (2022). The Levene test based-leakage assessment. *Integration*, 87, 182-193. doi: 10.1016/j.vlsi.2022.06.013.

[32] Watson, E., & Baucom, J.Kr. (2020). Two case studies in veterinary forensic imaging and a brief literature review. *Forensic Imaging*, 21(4), article number 200382. doi: 10.1016/j.fri.2020.200382.

[33] Yatsenko, I., & Parilovsky, O. (2022) Animal mutilation as a subject of forensic veterinary expertise. *Bulletin of the Black Sea Littoral*, 102-103, 71-86. doi: 10.37000/abbsl.2022.102.13.

[34] Zhang, M. (2022). Forensic imaging: A powerful tool in modern forensic investigation. *Forensic Sciences Research*, 7(3), 385-392. doi: 10.1080/20961790.2021.2008705.

Визначення давності настання смерті кота свійського методом вимірювання площі вологої плями

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Анотація. Жорстоке поводження з тваринами, відповідно до чинного законодавства України, є кримінальним злочином. Під час розслідування кримінальних проваджень, пов'язаних із жорстоким поводженням із тваринами, в тому числі котами свійськими, часто виникає необхідність встановлення давності настання смерті. Тому надзвичайно актуальними є розробка нових методів її визначення та вдосконалення вже існуючих. Метою дослідження було встановлення залежності між отриманими значеннями показників площі вологої плями та часом настання смерті. Для досягнення поставленої мети авторами запропонований новий метод визначення давності настання смерті котів свійських за результатами вимірювання площі вологої плями, отриманої зі зразків тканини скелетних м'язів трупів (метод Шкунді). Для цього відбирали від трупів котів зразки м'язової тканини, за допомогою пресування отримували препарат вологої плями та вираховували її площу, з метою встановлення кореляції значень цього показника з часом, який пройшов з настання смерті. Дослідженням отриманих показників встановлено, що ці значення є сталими, встановлено і показано графічно особливості коливань значень даного показника з часом, що пройшов з моменту смерті тварин. За допомогою низки статистичних методів визначено відсутність залежності показників площі вологої плями від м'язів, із яких відбирають зразки для дослідження, та порід тварин. Встановлено характер змін значень показника площі вологої плями залежно від часу і проаналізовано особливості їх коливань у різні періоди протягом 27 діб з моменту загибелі тварини. Представлено еталонні значення даних показників залежно від часу, що пройшов з моменту смерті тварин, які судово-ветеринарні експерти можуть використовувати в практичній роботі для встановлення давності настання смерті котів свійських. Завдяки низці переваг даний метод може бути широко впроваджений у практику судової ветеринарної медицини

Ключові слова: судово-ветеринарна експертиза; постмортальний інтервал; *Felis silvestris catus*; метод Шкунді; м'язова тканина