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# Postmortem interval estimation in cats using vitreous potassium: Development of a regression model#

**F. Almas[1], M. Pradeep[1*], A. J. George[1], B. D. Krishna[2], S. S. Devi[3], R. Rajasekhar[4] and N.K. Mansoor[5]**

[1]Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pookode, Wayanad- 673 576,[2]Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Mannuthy, Thrissur- 608 651,[3]Bioscience Research and Training Centre, Thonnakkal, Thiruvananthapuram- 695 317,[4]Department of Veterinary Microbiology, College of Veterinary and Animal Sciences, Pookode, Wayanad- 673 576., Kerala Veterinary and Animal Sciences University, Kerala, India, [5]Department of Statistics, Sir Syed college Taliparamba, Kannur- 670 142, Kannur University, Kerala, India

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**Abstract**

*Accurate postmortem interval (PMI) estimation is critical in forensic investigations and in vetero-legal procedures. Even though vitreous humour potassium concentration has been studied previously in humans and dogs to estimate PMI, studies on cats are scanty. The present study investigated vitreous potassium concentrations in cat carcasses at different PMI and its potential for estimating PMI in cats. The cats with known PMI brought for post-mortem examination at the Department of Veterinary Pathology, College of Veterinary and Animal Sciences, Pookode, Wayanad formed the study material. Vitreous humour was collected from 12 cat carcasses with PMIs ranging from one to fifty-two hours. Data including the age and sex of the cats and ambient temperature at the time of collection were recorded. Potassium concentration was quantified from clear supernatant collected after centrifugation of vitreous humour using the ion-selective electrode method. Statistical analysis revealed a strong positive correlation between potassium concentration and PMI (r= 0.919, $p < 0.01$) with no significant influence of age, sex or ambient temperature. Regression analyses demonstrated that potassium concentration explained 84.5% of the variance in PMI with the power regression model demonstrating the best predictive accuracy ($R^2 = 0.978$). This study confirms that vitreous potassium concentration is a robust biomarker for PMI estimation in cats in early periods after death and provides a reliable regression model for forensic applications.*

**Keywords:** *Post-mortem interval, vitreous humour, potassium concentration, cat, forensic veterinary medicine, regression model*

The period between the death and discovery of a carcass is known as the post-mortem interval (PMI). Estimating PMI is a critical aspect of forensic investigation, as it provides vital information regarding the time of death. Various methodologies for estimating PMI have been studied, but their accuracy varies significantly due to several influencing factors. Research on humans has shown that potassium levels in the vitreous humour increase in a time-dependent manner after death, primarily due to the loss of cellular integrity and the passive diffusion of potassium ions

*Corresponding author: pradeep@kvasu.ac.in, Ph. 9447945170

from intracellular compartments (Zilg *et al*., 2015). The vitreous potassium concentration demonstrates minimal susceptibility to post-mortem changes and environmental factors, giving it strong potential for PMI estimation (Ahi and Garg, 2011). Although there are studies on humans and dogs (Devika *etal*., 2024), the potential of this marker for estimating PMI in cats has been scarcely studied, and species-specific studies are highly warranted in veterinary forensics.

The present investigation focusses on understanding how vitreous potassium concentration relates to PMI in cats. By quantitatively analysing potassium levels in vitreous samples from cat carcasses, the study aims to understand the potential of vitreous humour as a reliable biomarker for PMI estimation.

## Materials and methods

Twelve cat carcasses, presented at the Department of Veterinary Pathology, College of Veterinary and Animal Science, Pookode, Wayanad with a known time of death were utilised for the study. The animals had no pre-existing ocular abnormalities, ensuring sample integrity.

Clinical history, sex and age of the animals, ambient temperature at the time of collection were recorded. Vitreous humour (VH) was collected from the vitreous chamber of both eyes using a 2 mL syringe and 21-gauge needle. The samples were pooled from both eyes. The procedure involved performing a scleral puncture with the needle tip positioned centrally within the globe, allowing for the extraction of approximately 1-2 mL of vitreous humour. Only clear and transparent vitreous humour, free of any tissue debris was included in the analysis. Uniform sample collection and handling protocols were ensured. The samples were centrifuged at 13,000 rotations per minute for 15 minutes (Chavhan *et al*., 2014) to obtain clear supernatant and stored at -20 °C until analysis. Potassium was quantified from the thawed vitreous humour samples using the ion-selective electrode (ISE) method with an Epsilyte electrolyte analyser (Epsilon Diagnostics, India). The vitreous potassium concentration of cats was compared with that of dogs, as reported by Devika *et al*. (2024) and tested the predictability of the regression formula developed by them for dogs. Statistical analyses, including Pearson's correlation and regression modelling, were performed using SPSS (Statistical Package for the Social Sciences), version 24.0. Various regression models were tested to determine the best-fit equation for PMI prediction. The significance of age, sex, and ambient temperature was also evaluated.

## Results and discussion

Vitreous humour samples of suitable quality were obtained from 12 cat carcasses covering a PMI range of 1 to 52 hours. In later periods the quality of the vitreous humour was found to be poor and unsuitable for the analysis. The details of PMI and vitreous humour potassium concentration in the collected samples are provided in Table 1.

Potassium concentration in the vitreous humour samples showed a time-dependent variation. The minimum concentration of 4.31 mmol/L was recorded at around one-hour PMI while the highest concentration of 22.87 mmol/L reached at 52 hours PMI. A steady increase in potassium concentration was observed as the PMI increased.

### *Correlation of vitreous potassium concentration and PMI*

The Pearson correlation coefficient between PMI and potassium concentration in the vitreous humour was 0.919, which is statistically significant at the 0.01 level confirming a strong positive correlation between PMI and potassium concentration.

### *The correlation of ambient temperature, age, and sex of the animal with potassium concentration*

**Table 1.** Vitreous potassium concentration of cat carcasses with PMI, ambient temperature, sex and age

| Sl. No. | PMI (hour) | Potassium concentration (mmol/L) | Ambient temperature (°C) | Sex Male (M)/ Female(F) | Age (month) |
|---|---|---|---|---|---|
| 1 | 1 | 4.5 | 26.6 | M | 12 |
| 2 | 1.15 | 4.31 | 25.1 | F | 30 |
| 3 | 4 | 8.93 | 25.8 | M | 42 |
| 4 | 10 | 11.48 | 22.5 | M | 18 |
| 5 | 11 | 12.25 | 23.7 | M | 36 |
| 6 | 12 | 11.52 | 22.9 | M | 18 |
| 7 | 14 | 12.88 | 24.6 | M | 24 |
| 8 | 18 | 13.23 | 24.8 | F | 11 |
| 9 | 19 | 17.07 | 24.2 | M | 6 |
| 10 | 20 | 15.73 | 24.8 | F | 48 |
| 11 | 22 | 17.32 | 23 | F | 1 |
| 12 | 52 | 22.87 | 24 | F | 2.5 |

The correlation between potassium concentration and ambient temperature (r= -0.493, p = 0.103), and the correlation with age (r=0.387, p = 0.214), indicated no statistically significant relationships with either factor.

In the t-test for equality of means, to compare the significance of sex and vitreous potassium concentration, the assumptions of independence, normality, and equality of population variances were satisfied, as indicated by p-values greater than 0.05. The test (p = 0.213) confirmed that sex has no statistically significant influence on the vitreous potassium concentration.

***Regression analysis***

Regression analysis was conducted to examine the relationship between the PMI and vitreous potassium concentration, with PMI as the dependent variable and potassium concentration as the independent variable. Various regression models were tested to determine the best-fit equation for estimating PMI. The coefficient of determination ($R^2$), which explains the proportion of variation in PMI that can be attributed to potassium concentration, was used as a key criterion for model selection. The results of different regression models are summarised in Table 2.

**Table 2.** Comparison of different regression models

| Model | $R^2$ | F-value | p-value |
|---|---|---|---|
| Linear | 0.845 | 54.651 | 0.000 |
| Logarithmic | 0.648 | 18.388 | 0.002 |
| Inverse | 0.459 | 8.480 | 0.016 |
| Quadratic | 0.957 | 101.048 | 0.000 |
| Cubic | 0.973 | 97.864 | 0.000 |
| Power | 0.978 | 440.934 | 0.000 |
| Exponential | 0.907 | 97.040 | 0.000 |

Among the models tested, the power regression model, cubic model and quadratic model demonstrated strong predictive ability with the power regression model achieving the highest coefficient of determination ($R^2$ = 0.978), indicating the best fit for the data. Although the linear model was significant, it demonstrated a lower $R^2$ value (0.845) in comparison to the nonlinear models. Curve estimation analysis further confirmed that nonlinear models, especially the power model, closely matched the observed data, while the linear model deviated at higher potassium levels. Therefore, the power model was the most suitable for estimating PMI from vitreous potassium concentration. The derived regression equation is:

PMI (hours) = 0.034 × ((Potassium concentration)$^{2.314}$

This equation suggests that PMI increases exponentially with potassium concentration, reinforcing the strong correlation observed between these variables.

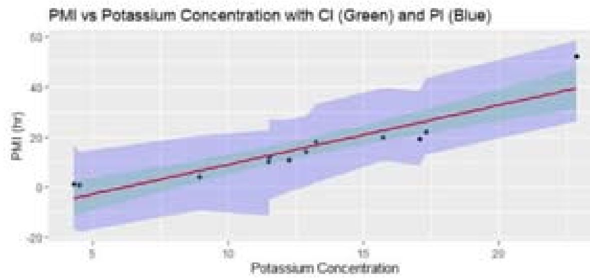


**Fig.1.** PMI with confidence interval and prediction interval

The model was highly significant (F= 440.934, p = 0.000), confirming that potassium concentration serves as a reliable predictor of PMI.

The PMI with confidence interval (CI) and prediction interval (PI) are shown in figure 1.

In this graph, with PMI on the Y-axis and potassium concentration on the X-axis, the narrow green confidence interval (CI) indicates a reliable estimate of the average PMI for a given potassium concentration. The majority of data points falling within this interval suggest a strong and precise relationship between potassium concentration and PMI, reflecting low uncertainty in predicting mean values. Conversely, the wide blue prediction interval (PI) demonstrates that while the average trend is clear, individual PMIs can show a tendency to vary for the same potassium concentration. The CI indicates confidence in the average relationship, while the PI indicates uncertainty in individual predictions.

The vitreous potassium concentration in cats was not comparable to that in dogs, and the regression formula developed for dogs by Devika *et al*. (2024) did not fit cats, indicating the necessity of species-specific data for regression model development.

In this study, vitreous humour was selected for PMI estimation due to its relative stability and minimal susceptibility to post-mortem changes when compared to other body fluids such as blood and cerebrospinal fluid (Coe *et al*., 1993). Its anatomical isolation reduces the likelihood of contamination and degradation, making it particularly valuable in forensic analysis (Camba *et al*., 2014).

The significant positive correlation between potassium concentration and PMI in cats is in line with previous findings in other species such as humans (Ahi and Garg, 2011), bovine (George and Ajayi, 2016) and dogs (Devika *et al*., 2024) and found to be not influenced by the ambient temperature, age and sex of the animal as reported previously in other species (Jashnani *et al*., 2010).

**Conclusion**

In the present study, we evaluated vitreous potassium concentration over a range of 1 to 52-hour PMI. This study demonstrated a robust and statistically significant positive correlation between vitreous potassium concentration and postmortem interval in cats, establishing its utility as a reliable biomarker for PMI estimation. The mathematical model developed herein exhibits high predictive accuracy. The regression model's strong explanatory power ($R^2 = 0.978$) underscores its potential for practical application in forensic veterinary medicine.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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