Research Square

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Assessing the analytical reliability of platelet counts and related indices in dogs and cats: a correlation matrix analysis comparing K2-EDTA and MgSO4 anticoagulation

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Abstract

The standard anticoagulant, ethylene diamine tetra-acetic acid (EDTA), often produces inaccurate platelet results (platelet count (PLT), plateletcrit (PCT), mean platelet volume (MPV), and platelet distribution width (PDW) in dog and cat blood due to its tendency to induce aggregation. This research, therefore, proposes magnesium sulfate (MgSO4) as the superior alternative because it actively prevents this clumping. The research examined blood samples from 31 feline and 32 canine individuals, totaling 122 blood tubes. Two types of tubes were used: k2-EDTA and MgSO4. Clinically healthy dogs and cats were used to obtain the samples, and PLT levels and related indices, such as MPV, PCT, and PDW, were measured using the Celltac α VET MEK-6550 hematology analyzer. In dogs, platelet parameters in MgSO4 and EDTA showed strong agreement ($r \geq 0.90$ for MPV, PLT, PCT, and PDW), with stability in MPV but a decrease in mean PCT in MgSO4 (0.30 vs. 0.56 in K2-EDTA). Feline results confirmed a strong correlation between PLT and PCT ($r = 0.99$), but analytical agreement between K2-EDTA and MgSO4 was poor for MPV ($r = -0.11$) and PDW ($r = 0.21$). MgSO4 is effective as a replacement for K2-EDTA in dogs. MgSO4 in cats is critical because it helps prevent platelet clumping, thereby maintaining normal PLT and PCT levels. The beneficial compound in feline blood analysis requires caution in use. There are no favorable MgSO4 values for K2-EDTA with respect to the size parameters MPV and PDW. It suggests such parameters are not consistently accurate, irrespective of the anticoagulant used.

Introduction

Accurately measuring platelet parameters, such as platelet count (PLT), mean platelet volume (MPV), plateletcrit (PCT), and platelet distribution width (PDW), is essential for evaluating coagulation, inflammation, and thrombotic risk in veterinary medicine. It is commonly accepted that in hematology laboratories, ethylene diamine tetra-acetic acid (EDTA) becomes the preferred anticoagulant due to its ability to chelate calcium and, consequently, inhibit coagulation development [2, 14]. However, the use of EDTA can also lead to pre-analytical issues that invalidate test results. In humans, a rare side effect of EDTA is pseudothrombocytopenia (PTCP), in which the platelet count appears low because platelets clump together in the test tube. It occurs in about 0.07–0.27% of EDTA samples and is most often observed when samples are kept at room temperature [9]. Platelet aggregation is also a characteristic feature of canine and feline blood samples that can lead to misleading diagnoses of thrombocytopenia and to the inappropriate completion of diagnostic profiles or the supposed treatment process [11, 23]. Cold agglutinin antibodies, autoantibody precipitation upon addition of EDTA, or low anticoagulant levels or platelet activation can result in platelet aggregation in a venous blood sample [20, 22]. The development and progression of this process can differ among different species. It is less observed in dogs than in cats. Aggregation by platelets would be more common in felines, and the process could be found out easily due to the unique properties of feline platelets, such as their osmotic influence of EDTA and relatively smaller size [12, 19]. This clumping may lead to inaccurate platelet counts, slowing the diagnostic test for thrombocytopenia. EDTA may also alter the cell's osmotic pressure, causing swelling that can make platelet volume measurements, such as MPV, appear higher than they really are [9]. Some studies in humans have shown that EDTA-PTCP is associated with IgG autoantibodies targeting a hidden

region of the IIb unit of the GPIIbIIIa receptor (also known as CD41/CD61), which is revealed only in the presence of EDTA [2]. Researchers use three main ways to reduce the effects of PTCP in dogs and cats. The first is to prewarm refrigerated samples before analysis. The second is to add PLT antagonists, such as prostaglandin E1 (PGE1), citrate, theophylline, adenosine, dipyridamole (CTAD), and Iloprost, to collection tubes before drawing blood [19, 21]. The third intervention involves pretreating EDTA tubes with amikacin, which neutralizes the fibrinogen receptor GPIIbIIIa and reduces its fibrinogen binding [20]. To reduce these pre-analytical problems, especially in preparations that are likely to exhibit platelet aggregation, other anticoagulants, including magnesium sulfate ($MgSO_4$), are also added. $MgSO_4$ works as an anticoagulant because magnesium ions compete with calcium in coagulation reactions and help inhibit clotting [4]. More importantly, $MgSO_4$ is also recognized as an antiplatelet aggregation agent; magnesium, being a calcium antagonist, can inhibit platelet activation and prevent clumping [3]. Studies in people have shown that $MgSO_4$ improves PLT function in those with PTCP. It means $MgSO_4$ can give more true results [3, 16]. Right now, there is no other choice like $MgSO_4$ for EDTA-PTCP that can deliver better or more accurate results for dogs and cats. Given the species-specific challenges and the use of the specific hematology analyzer, Celtac-Alpha (Nihon Kohden), in our study, the main objective of this research is to compare the discrepancies and statistical correlations of PLT and related platelet indices between K2-EDTA and $MgSO_4$ anticoagulants in dog and cat blood samples.

Material and methods

Studied animals and sample collection

We conducted a cross-sectional study utilizing dogs and cats that presented for routine health check-ups at a private veterinary hospital in Tehran, Iran. A total of 61 client-owned dogs and cats (30 dogs and 31 cats) were ultimately selected for this study. All animals included in the study were clinically healthy, with no history of coagulation disorders, underlying platelet diseases, or use of anticoagulants or nonsteroidal anti-inflammatory drugs (NSAIDs). For each dog and cat included in the study, essential demographic data such as age, sex, breed, and the individual's name were carefully recorded along with the blood sample. No animals were euthanized or sacrificed for this study. Blood samples were collected from live animals as non-invasive clinical specimens, with no anesthesia required; the animals remained fully conscious throughout the collection process.

Anticoagulants used

For this study, we collected paired, simultaneous blood samples from 31 cats and 30 dogs (a total of 122 tubes) immediately after venipuncture. We used two types of tubes: K2-EDTA (S-Monovette K2-EDTA; fill volume 2.6 mL) as the routine standard for hematology, and $MgSO_4$ (S-Monovette ThromboExact; fill volume 2.7 mL) [10]. The $MgSO_4$ collection tubes were particularly selected as an anticoagulant method alternative based on research work that proposes it assists in combating PTCP, which stands for the phenomenon wherein the clotting agent EDTA causes the platelet cell masses to aggregate, thus producing an inflated cell count [3, 16].

Pre-analytical phase

To prevent platelet preactivation, the blood was fully extended after the tourniquet was released, gently mixed with the anticoagulant, and immediately brought to the laboratory for further processing [10]. Platelet-related measurements, particularly sample storage, may result in volume changes in K2-EDTA. Aggregation, a property seen with feline platelets, would be enhanced [19]. To prevent unwanted changes, all hematological analyses were performed within 30 minutes of blood collection [9].

Measurement of platelet parameters

PLT and platelet indices, including MPV, PCT, and PDW, were measured using the Celltac α VET MEK-6550 hematology analyzer (Nihon Kohden). This device operates using the impedance method and can accurately measure these parameters in canine and feline blood [9]. PCT was calculated as a function of PLT and MPV.

Data analysis

Measurements for each parameter were recorded in paired sets using both the K2-EDTA and MgSO4 anticoagulants to enable direct comparison. Initially, the Shapiro-Wilk test was performed on each parameter to assess its data distribution and normality. Since the majority of parameters (PLT, MPV, and PCT) did not exhibit a normal distribution in either dog or cat samples, the Wilcoxon Signed-Rank Test, a non-parametric method, was strategically employed to compare the effects of K2-EDTA versus MgSO4 on these values. Conversely, because the PDW parameter did follow a normal distribution, its mean values were compared using the Paired Samples t-Test, a parametric test. To evaluate the level of agreement between the two anticoagulants and to determine the relationships among all measured parameters, a Pearson's Correlation Coefficient matrix was calculated. A strong correlation, close to 1, was considered representative of strong agreement between variables, implying the possibility of interchangeability. Data analysis and graphics processing were carried out in R-Studio (version 4.4.2). A p-value less than 0.05 was considered statistically significant.

Results

The platelet parameters, such as PLT, MPV, PCT, and PDW, were evaluated in whole-blood samples from 30 dogs and 31 cats using two anticoagulants: K2-EDTA (E) and MgSO4 (S). The exact statistical distribution, minimum, Q1, median, Q3, maximum, and mean, is given for each parameter in Tables 1 and 2.

Comparison of PLT between K2-EDTA and MgSO4 anticoagulants in dogs and cats

In the PLT range in K2-EDTA, from 177 to 956, the mean was 404.43, with a median of 394.0; in MgSO4, the range was similar, from 183 to 967, but the mean was slightly higher, at 418.63, with a median of 386.0. Such an increase in mean could indicate reduced platelet aggregation in MgSO4 (Table 1 and Fig. 1a). This parameter varied more in cats: in K2-EDTA, it ranged from 139 to 1232 (mean 485.16, median

369.00), whereas in MgSO4, the mean increased to 545.52 (range 53 to 1250, median 445.00). This difference of about 12% may be important for improving diagnostic accuracy in cats, where PLT is generally variable due to aggregation (Table 2 and Fig. 1b).

Comparison of MPV between K2-EDTA and MgSO4 anticoagulants in dogs and cats

In dogs, MPV was found to be highly stable, with a range of 5.60–15.10 in K2-EDTA (mean: 7.69, median: 7.30) and a comparably stable range in MgSO4 (mean: 7.64, median: 7.40), thus highlighting the MgSO4 anticoagulant's strong capacity for maintaining platelet morphology (Table 1, Fig. 2a). In the case of cats, MPV was higher, with a range of 8.00–12.00 in K2-EDTA (mean: 9.78, median: 9.70) and 7.60 to 12.20 in MgSO4 (mean 9.50, median 9.30). This slight difference could indicate species-specific effects of anticoagulants on platelet volume, underscoring the importance of adjusting laboratory protocols (Table 2 and Fig. 2b).

Comparison of PCT between K2-EDTA and MgSO4 anticoagulants in dogs and cats

In dogs, PCT ranged from 0.14 to 0.73 in K2-EDTA with a mean of 0.56 and a median of 0.28, while in MgSO4, the mean decreased to 0.30 (range 0.11 to 0.72, median 0.27). This significant difference might reflect differences in the overall effect on platelet volume (Table 1 and Fig. 3a). In cats, this parameter increased significantly more in MgSO4; in K2-EDTA, the range was 0.12 to 1.27, with an average of 0.48 and a median of 0.37, while in MgSO4, the values ranged from 0.05 to 1.31, with an average value of 0.56 and a median of 0.42. An increase of 17% may represent one of the main discoveries for improved diagnostic performance in cats, underscoring the potential of MgSO4 to reduce variability (Table 2 and Fig. 3b).

Comparison of PDW between K2-EDTA and MgSO4 anticoagulants in dogs and cats

PDW in dogs was very similar, with a range of 14.10 to 17.30 in both anticoagulants (mean approximately 15.8 in both; median around 15.7), strongly emphasizing the reliability of $MgSO_4$ in measuring platelet size distribution (Table 1 and Fig. 4a). In cats, there was a larger spread: the range in EDTA was from 10.70 to 17.40 (mean 14.39, median 14.40), while in $MgSO_4$, it ranged from 8.90 to 21.60 (mean 15.49, median 15.70). The approximately 8% increase in mean platelet size suggests greater variation in the platelet size distribution under $MgSO_4$ (Table 2 and Fig. 4b). This could be an important marker for evaluating platelet heterogeneity in sensitive species such as cats.

Correlation Matrix Analysis

Correlation matrix illustrating the bivariate correlation coefficients (r) between PLT, PCT, MPV, and PDW measured in canine and feline blood using two anticoagulants: K2-EDTA and MgSO4 (Fig. 5a and Fig. 5b). The most striking results involve the analytical consistency between the two anticoagulants (EDTA-'E' and MgSO4-' S'): the correlation between MPV-E and MPV-S was perfect (r = 1.0). The agreement was also nearly flawless, showing excellent reliability for the PLT (PLT-E and PLT-S at r = 0.97), the PCT (PCT-E and PCT-S at r = 0.92), and the PDW (PDW-E and PDW-S also at r = 0.92). Moving to the physiological

relationships, the link between PLT and total mass was powerful, especially within the MgSO4 samples (PLT-S and PCT-S at *r* = 0.92), and remained strong in the K2-EDTA samples (PLT-E and PCT-E at *r* = 0.88). Furthermore, the parameters related to platelet size showed a robust association: the correlation between MPV and PDW was uniformly strong for both methods (MPV-E and PDW-E at *r* = 0.85; MPV-S and PDW-S at *r* = 0.85). Finally, the relationship between the PLT and the MPV was notably the weakest, registering as moderate: PLT-E and MPV-E correlated at r=0.44, while PLT-S and MPV-S showed a slightly lower *r* = 0.39 (Fig. 5a).

Analysis of the correlation matrix for feline platelet indices reveals a complex structure, including both weak and negative correlations. The strongest positive relationships, as expected, exist between the PLT and PCT. This link was near-perfect for the K2-EDTA sample (PLT-E and PCT-E at *r* = 0.99) and remained very strong for the MgSO4 sample (PLT-S and PCT-S at *r* = 0.94). However, the analytical consistency between the two anticoagulants (E vs. S) was variable: PLT showed strong positive agreement (*r* = 0.75), while PCT showed moderate to strong agreement (*r* = 0.63). In contrast, the MPV showed inferior agreement, with a weak negative correlation (MPV-E and MPV-S at *r* = -0.11), and the PDW showed very weak agreement (*r* = 0.21). Looking at the size metrics, the correlation between PDW-E and MPV-E was moderate and negative (*r* = -0.35). Similarly, relationships between PLT and MPV were moderate (PLT-E and MPV-E at *r* = 0.43) or very weak (PLT-E and MPV-S at *r* = 0.15). Finally, the relationship between PCT-E and PDW-E was practically zero (*r* = −0.048; Fig 5b).

Discussion

Historically, MgSO4 was recognized as a highly suitable anticoagulant for blood analysis, but it later fell out of favor for routine use following the widespread adoption of EDTA [1, 13]. Today, due to renewed scientific interest, MgSO4 is being successfully reestablished as a reliable alternative, particularly for resolving EDTA-induced PTCP and improving the accuracy of automated PLT [5, 9, 15]. EDTA anticoagulant is routinely used for hematological evaluations in veterinary medicine [2]. However, because of challenges with EDTA in sensitive species, the primary objective of this study was to introduce MgSO4 as a potential anticoagulant for the first time and to evaluate its efficacy for assessing PLT, MPV, PCT, and PDW in dogs and cats. Our results demonstrated species-specific critical differences, with significant implications for laboratory protocols. Our primary finding was a significant increase in mean PLT in feline blood samples collected with MgSO4 (about a 12% increase) compared to those with K2-EDTA. This result has direct clinical relevance in feline hematology. Because of their unique cellular characteristics, feline platelets are prone to severe aggregation in the presence of EDTA [17]. This aggregation leads to systematic errors in automated counters and to the misdiagnosis of PTCP, thereby compromising diagnostic accuracy [16]. The observed increase in PLT with MgSO4 use indicates that MgSO4 reduces or inhibits platelet aggregation, thereby providing more accurate and reliable PLT measurements. Human studies show that MgSO4 can serve as an alternative for estimating platelet counts in cases of EDTA-induced [3, 16]. Stability assessments have also shown that tube agitation has an acceptable impact on PLT results when using MgSO4, enhancing its potential for routine use [4].

Mechanistically, the action of EDTA is the chelation of divalent ions, while MgSO4 provides Mg^{2+} ions. Subsequent studies in dogs also found that the addition of magnesium chloride ($MgCl_2$) can overcome the effects of EDTA, which interferes with hormone measurements [14]. Indeed, Mg^{2+} ions play a crucial role in protecting blood components from EDTA's chelating effects. This aspect has also been considered in studies combining EDTA and MgSO4 [16]. The MPV parameter in dogs demonstrated high stability with both anticoagulants (very similar means), confirming MgSO4's ability to preserve platelet morphology and volume in this species, comparable to EDTA [9]. PDW also yielded nearly identical results in dogs, indicating the high reliability of MgSO4 in measuring platelet size distribution. In cats, although MPV showed a slight difference, PDW with MgSO4 exhibited an 8% increase in the mean and a broader range. This increase in dispersion better reflects the true heterogeneity of platelets in cats, which might be overlooked with EDTA due to platelet aggregation or swelling phenomena [9]. The importance of PDW as a marker for assessing platelet production dynamics in the bone marrow is well established, and the ability of MgSO4 to capture a broader size spectrum could make it a critical diagnostic marker in sensitive felids, a species in which platelet measurement has long been challenging [19]. This change in PCT, which is a value calculated from PLT and MPV, provides an indicator for the mass of the platelets, which is particularly relevant for feline species. However, there was a mean increase in PCT of up to 17% with MgSO4 administration. This increase is likely attributable to the elevated PLT and indicates enhanced diagnostic utility, as PCT offers a more accurate assessment of circulating platelet mass in cats. In dogs, a profound decrease in PCT was observed with MgSO4 compared with EDTA. Such a difference may be due to a complex anticoagulant effect, in which the correlation between platelet volume and concentration is involved. Future studies must evaluate MgSO4 alongside other therapeutic variables, such as its analgesic effect in dogs, to provide a well-rounded perspective on its biological effects [6].

The correlation analysis successfully validated the essential relationship between PLT and PCT (total platelet mass) in feline blood. This link was nearly perfect in K2-EDTA samples (r = 0.99) and remained exceptionally strong in MgSO4 samples (r = 0.94). The use of MgSO4 as an alternative anticoagulant is warranted because it is effective in preventing spontaneous in vitro platelet agglutination in anticoagulant-induced PTCP [10]. The correlation is significant and provides a reliable basis for assessing the reliability of other platelet size measures (MPV and PDW). The results are consistent with other veterinary studies aimed at improving the precision of platelet measurement in cats [12]. While PLT showed a strong positive agreement (r = 0.75), PCT showed moderate-to-strong agreement (r = 0.63). The result indicates that although the PLT can be determined consistently regardless of the anticoagulant used, the PCT calculated is less consistent, which suggests that PCT is more sensitive to the differential effects of anticoagulants, a long-recognized problem of measurement disparity in hematology [7, 8, 18]. Additionally, the weak negative correlation between MPV in K2-EDTA and MgSO4 samples (r = -0.11) suggests that these anticoagulants do not yield consistent MPV measurements. It is in line with earlier findings where platelet aggregation, common in feline blood, interfered with MPV readings [7]. Moreover, the moderate negative correlation (r = -0.35) between PDW and MPV in K2-EDTA samples suggests a compensatory relationship, which could explain variability in platelet size and

distribution indices in cats [8]. The very weak correlation between PCT and PDW (r = -0.048) in K2-EDTA samples indicates that these two indices are mainly independent. It further supports the complexity of interpreting platelet indices in feline blood, as similar findings have been reported in other studies exploring platelet aggregation and the effects of different anticoagulants [8, 12].

In conclusion, MgSO4 is effective as a replacement for K2-EDTA in dogs. MgSO4 in cats is critical because it helps prevent platelet clumping (PTCP), thereby maintaining normal PLT and PCT levels. The beneficial compound in feline blood analysis requires caution in use. There are no favorable MgSO4 values for K2-EDTA with respect to the size parameters MPV and PDW. It suggests such parameters are not consistently accurate, irrespective of the anticoagulant used. Taken together, our findings reveal that MgSO4 may be a reliable in vitro anticoagulant for blood cell counting, as even high agonist concentrations do not induce platelet aggregation in the presence of MgSO4.

Declarations

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Authors' contributions

All authors were involved in the conception and design of the study. M.A-h took the lead in designing and overseeing the research. A.H.T.S, H.G, and M.A-h prepared the materials. M.A-h and H.A carried out data collection and analysis. M.A-h wrote the first draft of the manuscript. All authors provided feedback on earlier drafts of the manuscript and read and approved the final version.

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Data availability

Raw data is available from the corresponding author upon request.

Ethics approval and consent to participate

This study received approval from the Semnan University Ethics Committee (IR.SU.REC.1403.24). The relevant ethical committee approved all clinical, experimental, and laboratory protocols. Methodological procedures were strictly complied with national and institutional guidelines and ethical standards. Informed consent was obtained from all pet owners prior to sample collection. Owners were fully informed about the purpose of the study, sampling procedures, potential risks, and benefits, and their right to withdraw at any time.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Tables

Table 1 Platelet Parameters (PLT, MPV, PCT, PDW) in Canine Blood Samples Anticoagulated with K2-EDTA and MgSO4, Featuring Minimum, Maximum, Mean, Median, and Quartile (25% and 75%) Values

Statistic	PLT (× 10^3/µL)		MPV (fL)		PCT (%)		PDW (%)	
	EDTA	$MgSO_4$	EDTA	$MgSO_4$	EDTA	$MgSO_4$	EDTA	$MgSO_4$
min	139.00	53.00	8.00	7.60	0.12	0.05	10.70	8.90
25%	295.00	183.00	9.10	8.70	0.28	0.16	13.30	12.50
Median	369.00	445.00	9.70	9.30	0.37	0.42	14.40	15.70
75%	643.00	952.00	10.40	10.5	0.66	0.93	15.20	17.40
max	1232.0	1250.0	12.00	12.20	1.27	1.31	17.40	21.60
Mean	485.16	545.52	9.78	9.50	0.48	0.56	14.39	15.49

Table 2 Platelet Parameters (PLT, MPV, PCT, PDW) in Feline Blood Samples Anticoagulated with K2-EDTA and MgSO4, Featuring Minimum, Maximum, Mean, Median, and Quartile (25% and 75%) Values

Statistic	PLT (× 10^3/µL)		MPV (fL)		PCT (%)		PDW (%)	
	EDTA	$MgSO_4$	EDTA	$MgSO_4$	EDTA	$MgSO_4$	EDTA	$MgSO_4$
min	177.00	183.00	5.60	5.80	0.14	0.11	14.10	14.10
25%	270.25	296.50	6.85	6.70	0.23	0.23	15.12	15.07
Median	394.0	386.0	7.30	7.40	0.28	0.27	15.70	15.65
75%	464.75	476.00	7.85	7.95	0.34	0.35	16.70	16.52
max	956.0	967.0	15.10	15.00	0.73	0.72	17.30	17.30
Mean	404.43	418.63	7.69	7.64	0.56	0.30	15.81	15.78

Figures

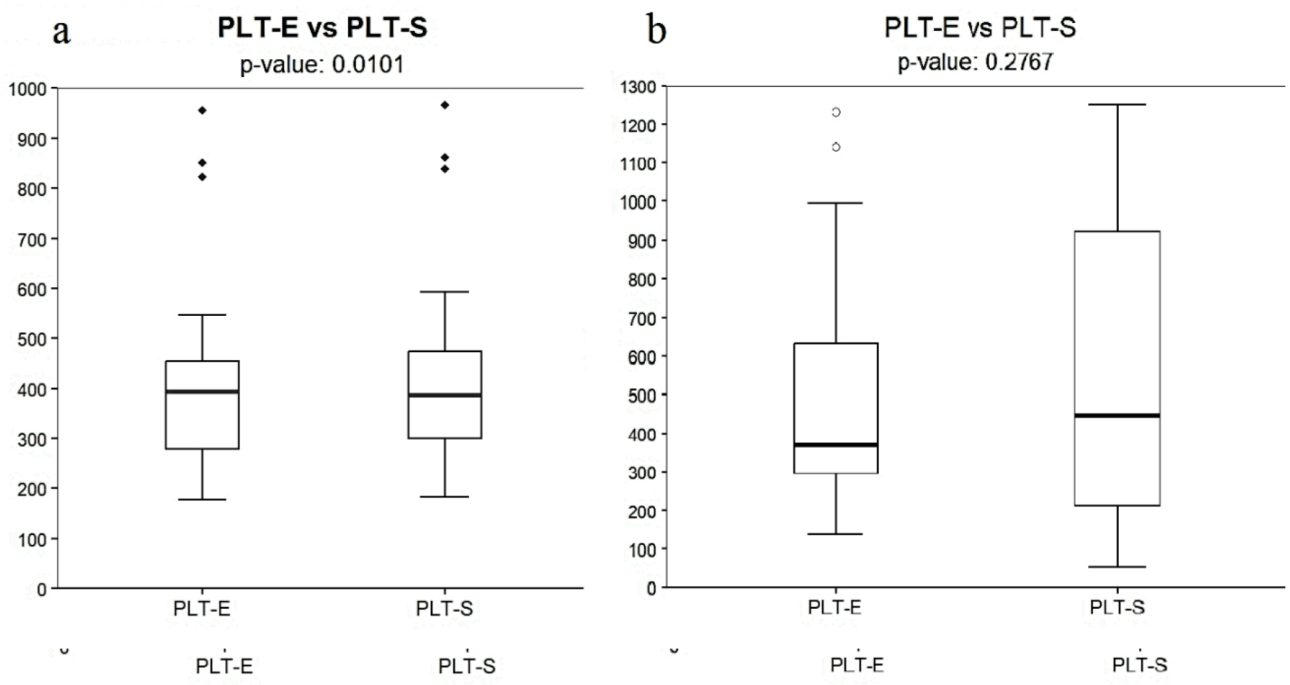


Figure 1

Box Plot comparing the total platelet counts (PLT, $\times 10^3/\mu L$ on the Y-axis) measured in canine (a) and feline (b) blood samples collected in K2-EDTA (PLT-E) and MgSO4
(PLT-S) tubes. The boxes represent the interquartile range (IQR), the central horizontal line indicates the median, and the whiskers denote the maximum and minimum values excluding outliers (individual points/diamonds).

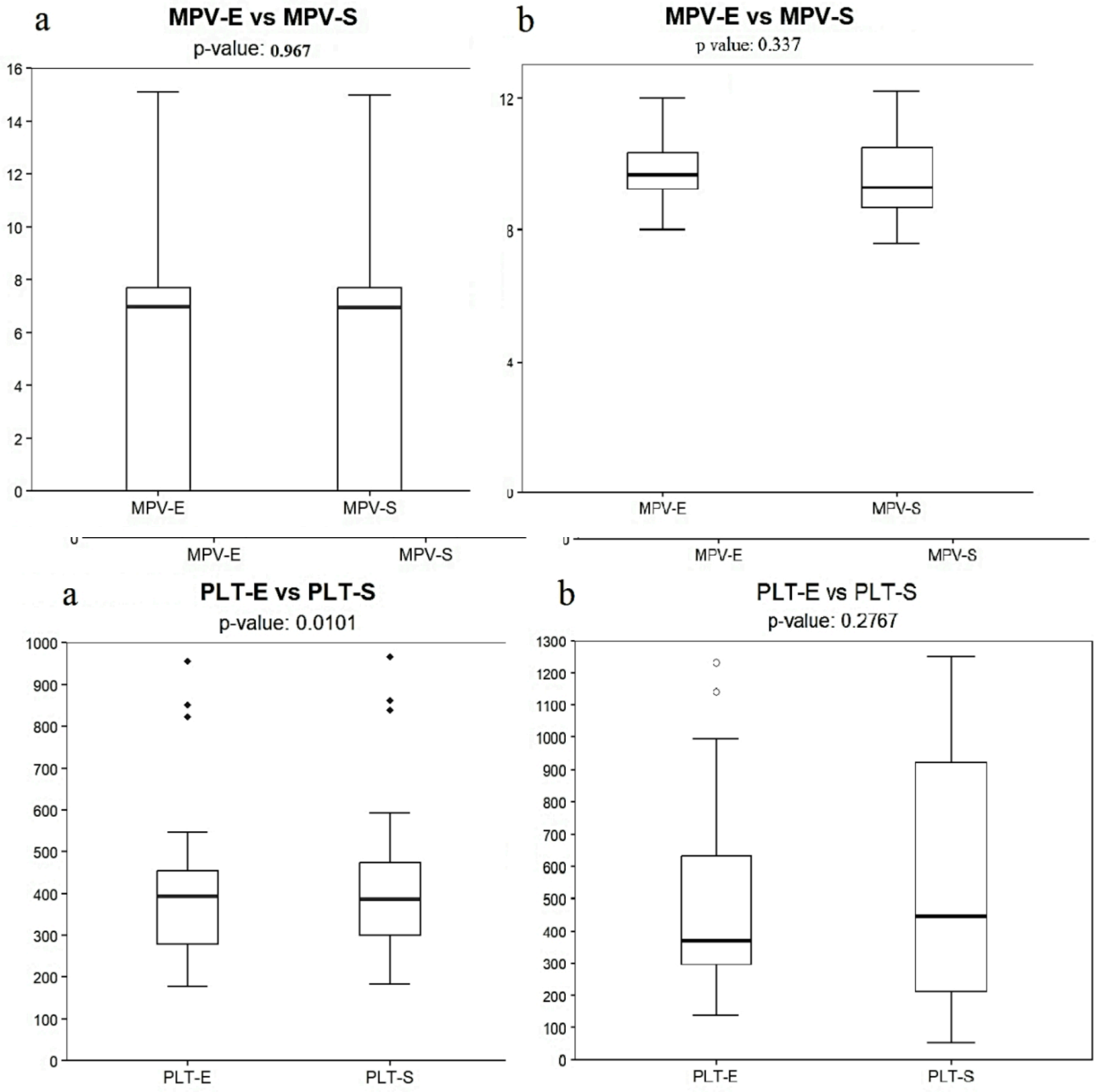


Figure 2

Box Plot comparing the mean platelet volume (MPV, fL on the Y-axis) measured in canine (a) and feline (b) blood samples collected in K2-EDTA (MPV-E) and MgSO4 (MPV-S) tubes. The boxes represent the interquartile range (IQR), the central horizontal line indicates the median, and the whiskers denote the maximum and minimum values excluding outliers (individual points/diamonds).

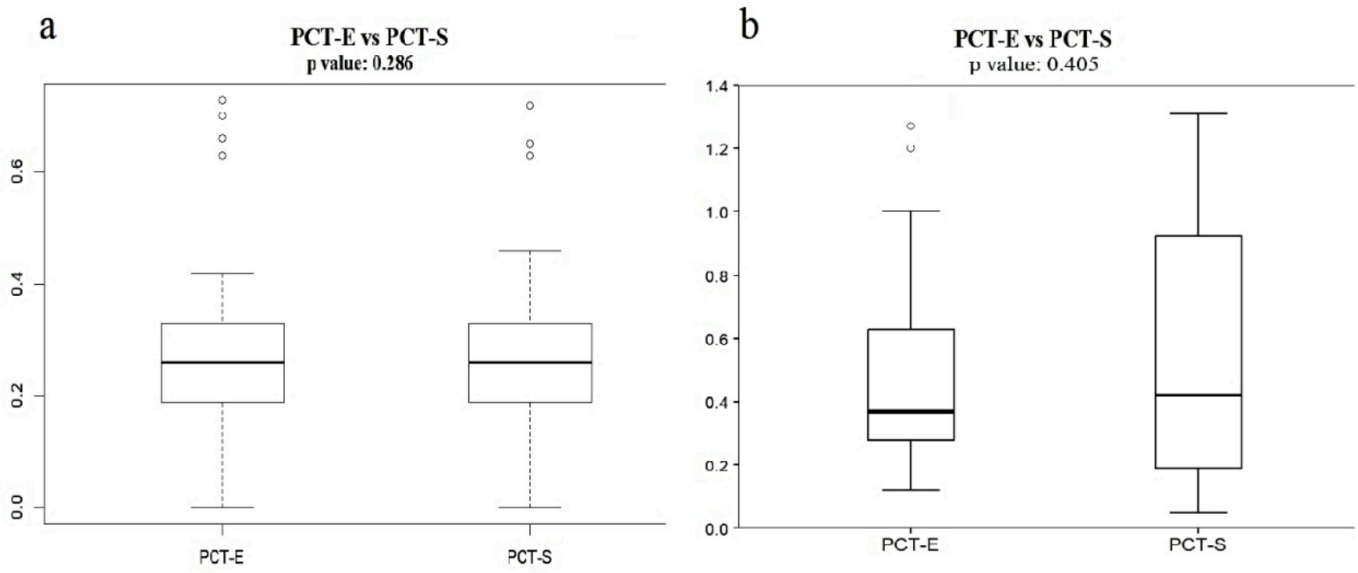


Figure 3

Box Plot comparing the plateletcrit (PCT, % on the Y-axis) measured in canine (a) and feline (b) blood samples collected in K2-EDTA (PCT-E) and MgSO4 (PCT-S) tubes. The boxes represent the interquartile range (IQR), the central horizontal line indicates the median, and the whiskers denote the maximum and minimum values excluding outliers (individual points/diamonds).

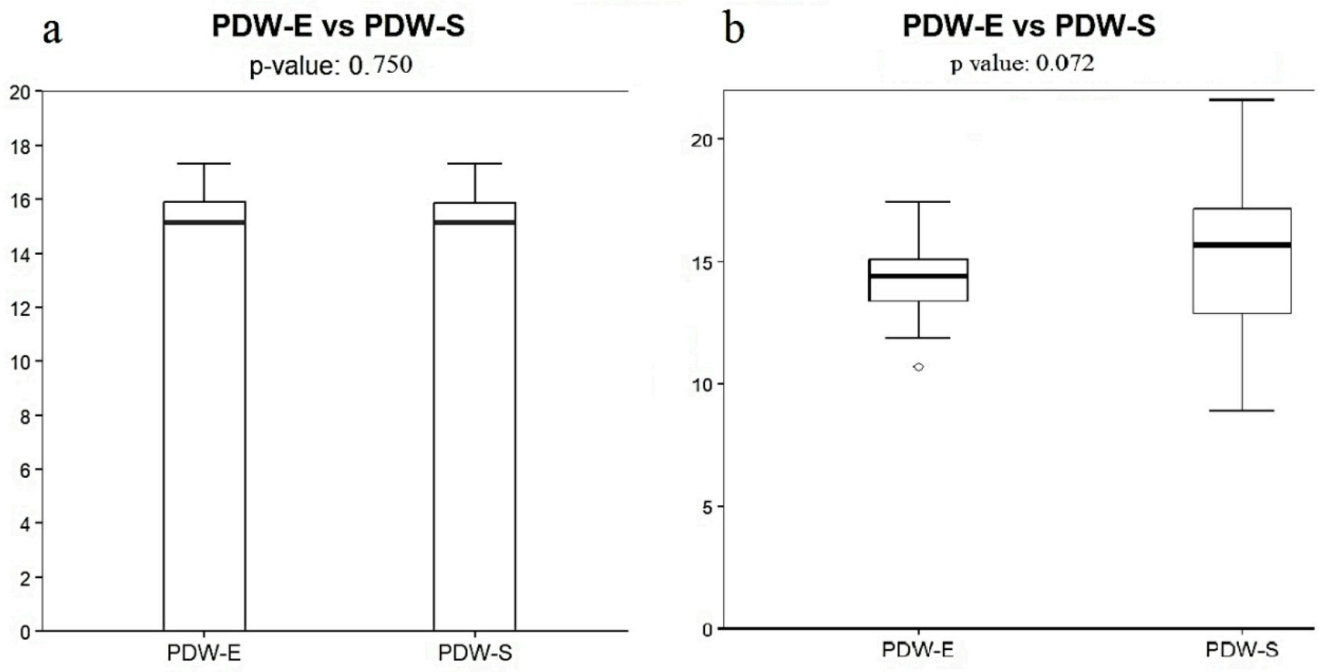


Figure 4

Box Plot comparing the platelet distribution width (PDW, % on the Y-axis) measured in canine (a) and feline (b) blood samples collected in K2-EDTA (PDW-E) and MgSO4 (PDW-S) tubes. The boxes represent the interquartile range (IQR), the central horizontal line indicates the median, and the whiskers denote the maximum and minimum values excluding outliers (individual points/diamonds).

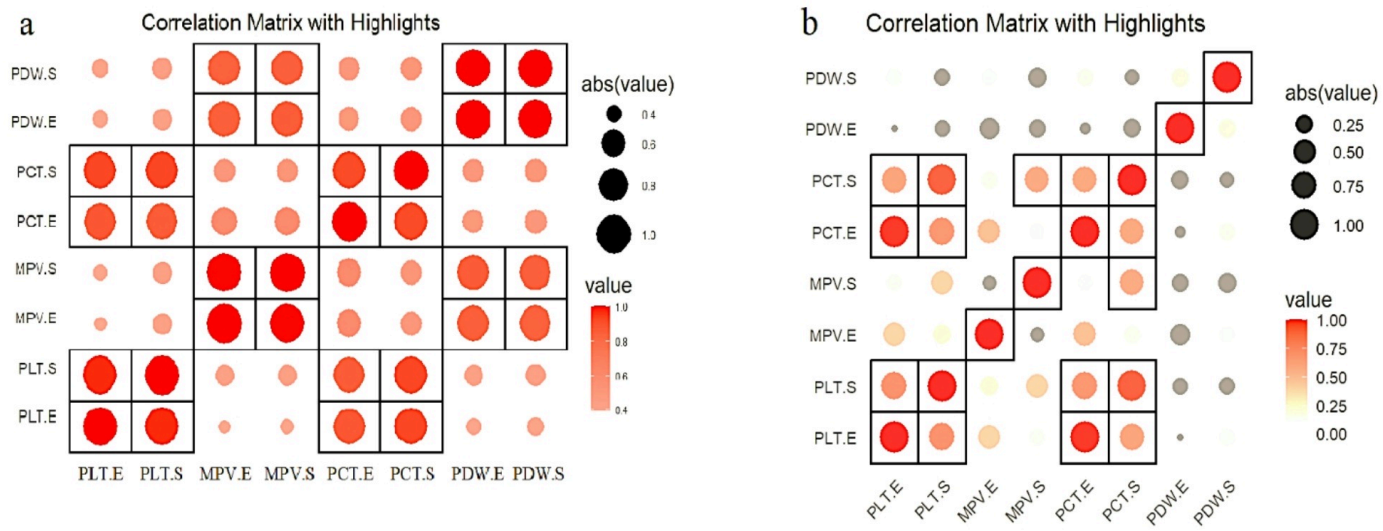


Figure 5

Correlation matrix illustrating the bivariate correlation coefficients (r) between platelet count (PLT), Plateletcrit (PCT), Mean Platelet Volume (MPV), and Platelet Distribution Width (PDW) measured in canine (a) and feline (b) blood using two anticoagulants: K2-EDTA (indicated by the suffix 'E') and MgSO4 (indicated by the suffix 'S'). For straightforward interpretation, the size of each circle directly reflects the absolute strength of the correlation (abs (value)). At the same time, the color intensity (from light pink to bright red) clearly shows the sign and magnitude of that relationship (value), meaning a larger, darker red circle represents a stronger positive connection.