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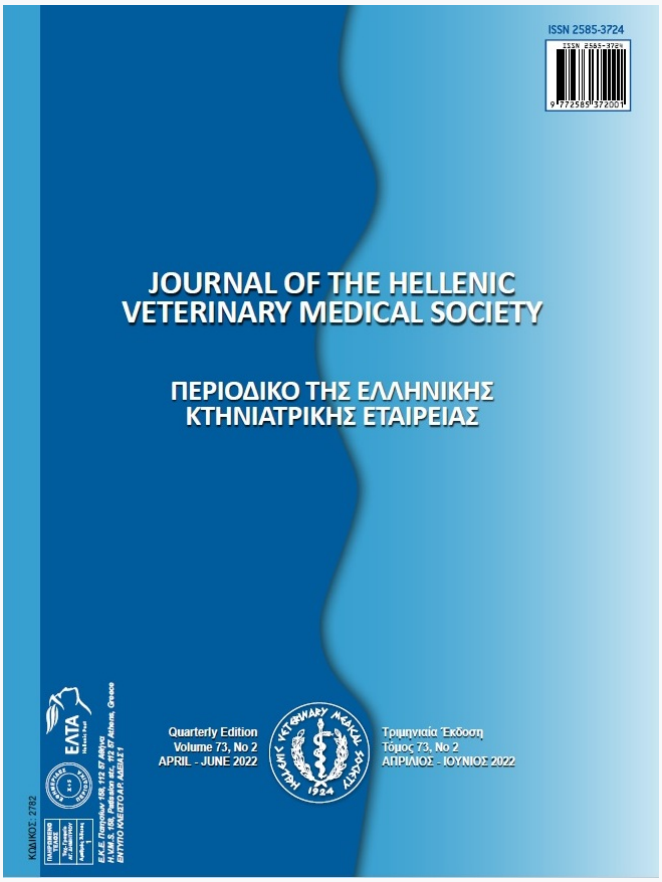

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Research article
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The Relationship of Clinicopathological Parameters and Formula for Predicting Malondialdehyde Level in Feline Immunodeficiency Virus (FIV) -infected Cats

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ABSTRACT: The objective of this study was to investigate clinicopathological parameters, malondialdehyde (MDA) and total antioxidant power for analyzing correlation and predicting MDA in plasma of feline immunodeficiency virus (FIV)-infected cats. Body condition score (BCS), mucous membrane color score (MMCS), vital signs, hematological parameters, MDA and ferric reducing antioxidant power (FRAP) of 20 FIV-infected cats were examined. The correlation and multiple regression models were then analyzed. The results revealed the following. The correlation between hemoglobin (Hb) vs. packed cell volume (PCV), total red blood cell (TRBC) vs. PCV, TRBC vs. Hb, mean corpuscular hemoglobin (MCH) vs. mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC) vs. MCH, body temperature (BT) vs. eosinophil, total white blood cell (TWBC) vs. eosinophil, MMCS vs. platelet, eosinophil vs. MDA, PCV vs. FRAP, Hb vs. FRAP, TRBC vs. FRAP and TRBC vs. FRAP were significantly positive correlated between pairs of these parameters ($P<0.05$), while BT vs. MCV, TRBC vs. MCV, heart rate (HR) vs. platelet, HR vs. platelet were significantly negatively correlated between pairs of parameters ($P<0.05$). A formula for predicting MDA was MDA (μmol/ml) = 0.12725 - 0.00036705*HR (beats/minute) + 0.00000138*EOS (cell) (MSE=0.00009511; $r^2=0.67$). The MDA level obtained from laboratory investigation and from calculation was found to be not significantly different ($P>0.05$). These results indicated that there were correlations between some clinicopathological parameters, MDA and total antioxidant power and a formula for predicting MDA levels in plasma of FIV-infected cat was obtained.

Keywords: FIV; clinicopathological findings; malondialdehyde; total antioxidant power; cat

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INTRODUCTION

Feline immunodeficiency virus (FIV) is a naturally occurring lentivirus of domestic cats and non-domestic feline species. FIV is believed to be transmitted from cat to cat via bite wounds during antagonistic or mating interactions (Miller et al., 2017). Domestic cats experimentally infected with FIV can develop marked immune dysfunction with severe and progressive respiratory and intestinal disease. Hematologic abnormalities are frequently reported in FIV-infected cats, both in the asymptomatic and symptomatic stages of infection. Nonregenerative anemia, leucocytosis, leucopenia, and thrombocytopenia have all been described. The mechanism of FIV-induced cytopenia is likely to be multifactorial and result from direct or indirect suppression of hematopoiesis or secondary factors such as opportunistic infections and neoplasia. Direct infection of the bone marrow stromal cells with FIV and subsequent changes in cytokine expression can result in suppression of hematopoiesis (White et al., 2011; Sakundech et al., 2021).

Reactive oxygen species (ROS) are generated through normal oxidative metabolism, and ROS in low concentrations are necessary for some physiological processes. Deleterious oxidative effects result from an oxidant/antioxidant imbalance, an excess of oxidants or a depletion of antioxidants (Kayar et al., 2015). Oxidative stress elevates intracellular ROS levels and damage lipids, proteins, and deoxyribonucleic acid (DNA) (Aengwanich et al., 2019; Arslan-Acaroz and Bayşu-Sozbilir, 2020). This condition has been implicated in the pathogenesis of many diseases and inflammatory conditions (Kayar et al., 2015; Acaroz et al., 2019). Lipid peroxidation (LPO) or the reaction of oxygen with unsaturated lipids produces a wide variety of oxidation products. The primary products of LPO are lipid hydroperoxides (LOOH). Among the many different aldehydes which can be formed as secondary products during LPO is malondialdehyde (MDA) (Ayala et al., 2014). The level of MDA serves as a reliable biomarker of LPO and usually serves as a marker of LPO (Acaros et al., 2018).

Generally, body condition score (BCS) is used for many purposes such as evaluating nutritional status (Sapowicz et al., 2016), or for diagnosis certain abnormalities or diseases (Teng et al., 2016). The normal mucous membrane color should be pale pink to pink. Abnormal mucous membrane colors such as pale, muddy, and white mucous membrane are commonly caused by poor peripheral perfusion, including vasoconstriction from shock or anemia (Norkus, 2019). Vital signs are the most critical objective parameters of the physical examination. A significant increase or decrease in the respiration or heart rate (HR) of the cat may be a sign of a major illness (Wang et al., 2020). Body temperature (BT) is frequently measured to assess the health status of cats. High BTs can result from infection, inflammation or overheating. Low BTs can result from septic shock, hypoperfusion, cardiac failure, cold exposure or anesthesia (Levy et al., 2015). Hematological parameters are considered to reflect the general health status of animals, including physiological condition and the function of important organs, and are commonly applied to evaluate the trend of physiological status (Hwang et al., 2015). Besides, the free radical oxidants such as ROS, reactive nitrogen species, and reactive sulfur species are produced in cells through various metabolic processes. The body is equipped with an antioxidant defense system that guards against oxidative damage caused by these reactive oxidants and plays a major role in protecting cells from oxidative stress and damage (Kayar et al., 2015; Aengwanich et al., 2019). The most common methods for investigation of antioxidants is ferric reducing antioxidant power (FRAP) (Lim and Lim, 2013). Most tests of ‘‘total antioxidant power’’ used to date have measured the ability of plasma to withstand the oxidative effects of reactive species (Benzie and Strain, 1996).

In the present study, we hypothesize that clinicopathological parameters, MDA and total antioxidant power are correlated and can be used for predicting MDA levels in FIV-infected cats. Therefore, the objective of this study was to test the hypothesis by investigating physical examination, hematological parameters, MDA and total antioxidant power in order to analyze correlation and develop a formula for predicting plasma MDA. The formula derived from this study will be important for treatment and palliative care of FIV-infected cats.

MATERIALS AND METHODS

Ethics approval

This study was approved by the Institution’s Ethics Committee on Animal Experimentation of Mahasarakham University (license number: IACUC-MSU-004/2020). All procedures were performed with the owner’s consent.

Animals and FIV testing

The test group for this study consisted of sixty-two

domestic short hair cats, aged 1 to 3 years. The cats were obtained from households and shelters in Mahasarakham Province, Thailand. All cats were fed with commercial foods. Cats were assessed for FIV infection at Veterinary Teaching Hospital, Faculty of Veterinary Science, Mahasarakham University between May-June 2020, using an Anigen Rapid FIV Ab / FeLV Ag Test Kit (Korea) (Sand et al., 2010). Cats tested for FeLV-positive were eliminated following the FIV infection test. Twenty cats (male = 12, female = 8, weight = 3.49 $\pm$ 0.92) were positive. All FIV-infected cats were non-pregnant or lactating, non-infected with internal or external parasites, and no drug administration or vaccination. All FIV-infected cats underwent a BCS and mucous membrane color score (MMCS) evaluation, vital signs investigation, and then blood was collected for laboratory analysis.

Physical examination

BCS and MMCS assessment, and vital signs were performed within a laboratory. The BCS of cats was determined using a nine-point scale that reported by Bjornvad et al. (2011). The MMCS was investigated using a method adapted from the description of Englar (2019) as follows: White mucous membrane = 0, pale pink mucous membrane = 1, and pink mucous membrane = 2. BT measured using an Auricular infrared thermometer (Humm and Kellett-Gregory, 2016). The respiratory rate was measured by the method as follows: One breath is counted each time the chest rises and falls. Count the number of breaths in a minute (Duguma, 2016). The HR was measured by placing a stethoscope on the left chest and counting the number of beats in 60 seconds to get the number of beats per minute (Taylor, 2020).

Blood sample collection, hematological and biochemical analysis

4 mL of blood samples of experimental groups were taken from a cephalic vein using a butterfly needle into vacuum ethylenediaminetetraacetic acid (EDTA) (for hematological analysis) and vacuum heparin tubes. The vacuum heparin tube was centrifuged using a centrifuge at 2500 rpm for 5 min. The heparinized plasma is stored in the freezer at −20 °C before biochemical analysis. Blood samples with EDTA as anticoagulants were analyzed by the IDEXX ProCyte Dx hematology analyzer (USA) for RBC count, white blood cell (WBC) count, platelet count, hematocrit, and hemoglobin (Hb). The blood films were prepared, fixed with methanol, and stained with a Giemsa-Wright solution, and then used for a white blood cell differential count.

MDA in plasma was investigated by using the following procedure. A 0.01 mL sample was assayed by the addition of 3 mL (0.05 mol/L) of HCl and 1 mL (0.67%) of thiobarbituric acid. The mixtures were heated for 30 mins at 100 °C, cooled with running tap water, and then 4mL of n-butyl alcohol was added. The mixture was shaken in a vortex mixer and centrifuged using a refrigerated centrifuge at 3,000 rpm for 10 min. The absorbance at 532 nm was measured by a microplate reader. 1,1,3,3-tetramethoxypropane was used as the standard (Aengwanich et al., 2019).

Plasma total antioxidant power was evaluated by the procedure as follows: 300 mmol/L of acetate buffer (pH 3.6), 10 mmol/L of 2,4,6-tri-pyridyl-s-triazine in 40 mmol/L of HCl; and 20 mmol/L of $FeCl_3.6H_2O$ were prepared. 20 mL of acetate buffer, 2.5 mL of 2,4,6-tri-pyridyl-s-triazine, and 2.5 mL of $FeCl_3.6H_2O$ yielded the working FRAP reagent, and then 0.5 mL of plasma, 0.5 mL of deionized distilled water, and working FRAP reagent were mixed. After exactly 6 minutes at room temperature, the absorbance at 593 nm was measured by a microplate reader. $FeSO_4.7H_2O$ was used as the standard.

Statistical analysis

Data were prepared with Excel software. The normal distribution of data was tested. The correlation analyses for estimating the level of association between quantitative variables were performed using Pearson's correlation test. A p-value <0.05 was accepted as significant. The multiple regression model was analyzed by using the stepwise method for selection of significant independent variables ($P < 0.05$).

RESULTS

The Pearson correlation coefficients of relationships between physical examination parameters, hematological values, and biochemical biomarkers in FIV-infected cats are presented in Table 1. Hb vs. packed cell volume (PCV), total red blood cell (TRBC) vs. PCV, TRBC vs. Hb, mean corpuscular hemoglobin concentration (MCHC) vs. BT vs. eosinophil, total white blood cell (TWBC) vs. eosinophil, MMCS vs. platelet, eosinophil vs. MDA, PCV vs. FRAP, Hb vs. FRAP and TRBC vs. FRAP, TRBCvs. FRAP all had a significant positive correlations between the stated pairs of these parameters ($P<0.05$),

Table1. Correlation between pair of parameters in FIV-infected cats

Parameters	*P*-value	Pearson's correlation coefficient between parameters (r)
1. BT vs. PCV	0.0430	-0.4566
2. BT vs. Eosinophil	0.0375	0.4680
3. HR vs. Platelet	0.0375	-0.5827
4. HR vs. MDA	0.0103	-0.5827
5. Hb vs. PCV	<0.0001	0.9530
6. TRBC vs. PCV	<0.0001	0.9658
7. TRBC vs. Hb	<0.0001	0.8851
8. TRBC vs. PCV	0.0256	-0.4975
9. MCH vs. PCV	0.0002	0.7361
10. MCHC vs. MCH	<0.0001	0.9024
11. TWBC vs. Eosinophil	0.0159	0.5316
12. MMCS vs. Platelet	0.0159	0.6171
13. Eosinophil vs. MDA	0.0334	0.5610
14. PCV vs. FRAP	0.0201	0.6115
15. Hb vs. FRAP	0.0185	0.6182
16. TRBC vs. FRAP	0.0147	0.6348

Table 2. Comparison of MDA from laboratory measurement and calculation from HRand eosinophil of FIV-infected cats

Parameter	Laboratory measurement (n=20)	Calculation using formula (MDA (µmol/ml) = 0.12725 -0.00036705*HR (beats/minute) + 0.00000138*EOS (cell) (MSE=0.00009511; r^2=0.67), HR, and eosinophil data of each FIV-infected cats (n=20)	*P*-value
MDA* (µmol/ml)	0.067±0.012	0.067±0.009	0.9576

MDA*=mean±SD

while BT vs. PCV, TRBC vs. PCV, HR vs. platelet, HR vs. MDA, and TRBC vs PCV had significant negative relation between pairs of parameters (P<0.05), respectively (Table 2).

In addition, after the multiple regression model was analyzed. A formula for predicting MDA levels in FIV-infected cat was constructed; MDA (µmol/ml) = 0.12725 - 0.00036705*HR (beats/minute)+ 0.00000138*EOS (cell) (MSE=0.00009511; r^2=0.67). A reverse test was then used to check the reliability of the formula by using HR and eosinophil data from laboratory measurement of FIV-infected cats for calculation of the MDA level of each FIV-infected cat. The normal distribution of MDA data was tested and a comparison of MDA level obtained from laboratory investigation and from calculation was found to be not significantly different (P>0.05) using a *t*-test (Table 3).

DISCUSSION

Vital signs are indicators of circulatory, respiratory, neural and endocrine function (Teixeira et al., 2015). Respiratory rate, HR and temperature are the simplest, cheapest and probably most important vital signs (Brekke et al., 2019). These indicators are a mechanism to universally communicate a patient's condition and severity of disease (Teixeira et al., 2015). In the present study, BT of FIV-infected cats was negatively correlated with PCV and positively correlated with eosinophil. Besides, HR was negatively correlated with platelet and MDA. In the case of BT of FIV-infected cats, that was negatively correlated with PCV and was both similar and different with the study of Boyd et al. (2016), who found that PCV of steers were positively correlated for the summer trial but negatively correlated for the winter trial. This phenomenon indicated that BT had influence on the PCV. Alam et al. (2011) reported that eosinophil of goats maintained at high environmental temperatures increased. This finding was consonant with the correlation between BT and eosinophil of FIV-infected cats in the present study. We found that the BT of FIV-infected cats was positively correlated with eosinophil. The negative correlation between HR and platelet of FIV-infected cats in the present study was in accordance with the report of Ozdemir et al. (2004), who found that the autonomic nervous system had in-

fluence on the changing of platelet profile. Moreover, the negative correlation between HR and MDA of FIV-infected cats in the present study was similar to the study of Mostafa et al. (2019). They found that the mean HR was negatively correlated with MDA level.

Blood parameters, i.e., red blood cell parameters, white cell parameters and platelets are helpful in assessing the health status of animals. Turkson and Ganyo (2015) and Velguth et al. (2010) reported that PCV correlated with Hb. McManus et al. (2009) found that PCV correlated with Hb, TRBC; TRBC correlated with Hb and PCV; PCV correlated with MCHC, respectively. Furthermore, Tawari et al. (2013) reported that Hb correlated with PCV. In the present study, we found that Hb vs. PCV, TRBC vs. PCV, TRBC vs. Hb, TRBC vs. PCV and MCH vs. PCV were correlated and were in agreement with reports of Turkson and Ganyo (2015) and Velguth et al. (2010), McManus et al. (2009) and Tawari et al. (2013). Whereas, correlations between MCHC vs. MCH, TWBC vs. eosinophil, MMCS vs. platelet, eosinophil vs. MDA, PCV vs. FRAP, Hb vs. FRAP, TRBC vs. FRAP of FIV-infected cats have not been reported.

After multivariate regression was analyzed, we derived a formula for predicting MDA level. For this equation, r^2 was quite high, which meant that the formula was reliable. After back-testing the formula for effectiveness and reliability, it was found that the formula gave results that were not different from laboratory measurement. Researchers or practitioners could use common parameters during clinical examination, i.e., HR and eosinophil of FIV-infected cats for plasma MDA level calculation. Lastly, the limitation of this formula is only used in FIV-infected cats, and readers should use this formula carefully.

CONCLUSION

Clinicopathological parameters, MDA and total antioxidant power of FIV-infected cats were determined and then analyzed using correlation and multivariate regression to demonstrate correlation between pairs of these parameters. After the multiple regression model among parameters was analyzed, a formula for calculation plasma MDA level was MDA (μmol/ml) = 0.12725 - 0.00036705*HR (beats/minute) + 0.00000138*EOS (cell) (MSE=0.00009511; r^2=0.67).

CONFLICT OF INTEREST

None declared by the author.

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REFERENCES

Acaroz U, Ince S, Arslan-Acaroz D, Gurler Z, Kucukkurt I, Demirel HH, Arslan HO, Varol N, Zhu K (2018) The ameliorative effects of boron against acrylamide-induced oxidative stress, inflammatory response, and metabolic changes in rats. *Food* ChemToxicol 118: 745-752.

Acaroz, U., Ince, S., Arslan-Acaroz, D., Gurler, Z., Demirel, H. H., Kucukkurt, I., Eryavuz A, Kara R, Varol N, Zhu, K. (2019) Bisphenol-A induced oxidative stress, inflammatory gene expression, and metabolic and histopathological changes in male Wistar albino rats: protective role of boron. Toxicol Res 8 (2): 262-269.

Aengwanich W, Sakundech K, Chompoosan C, Tuchpramuk P, Boonsorn T (2019) Physiological changes, pain stress, oxidative stress, and total antioxidant capacity before, during, and after castration in male dogs. J Vet Behav32: 76-79

Alam MM, Hashem MA, Rahman MM, Hossain MM, Haque MR, Sobhan Z,Islam MS (2011) Effect of heat stress on behavior, physiological and blood parameters of goat. Progress Agric22 (1-2): 37-45.

Arslan-Acaroz D, Bayşu-Sozbilir N (2020) Ameliorative effect of boric acid against formaldehyde-induced oxidative stress in A549 cell lines. Environ SciPollut Res 27 (4): 4067-4074.

Ayala A, Muñoz MF, Argüelles S (2014) Lipid peroxidation: Production, metabolism, and signaling mechanisms of malondialdehyde and 4-Hydroxy-2-Nonenal. Oxid Med Cell Longev.Volume 2014, Article ID 360438

Benzie IFF, Strain JJ (1996) Thererric reducing ability of plasma (FRAP) as a measure of ‘‘antioxidant power’’: The FRAP assay. Anal Biochem239: 70-76.

Bjornvad CR, Nielsen DH, Armstrong PJ, McEvoy F, Hoelmkjaer KM, Jensen KS, Pedersen GF, Kristensen AT (2011) Evaluation of a nine-point body condition scoring system in physically inactive pet cats. Am J VetMed Res72: 433-437.

Boyd BM, Mader TL, Bittner CJ, Burken DB, Hilscher FH, WijffelsG, Gaughan JB, Sullivan ML, Cawdell- Smith AJ, Erickson GE (2016). Metabolic and Body Temperature Responses to Environmental Conditions Across Seasons in Finishing Steers. Nebraska Beef Cattle Reports. Paper 861.

Brekke IJ, Puntervoll LH, Pedersen PB, Kellett J,Brabrand M (2019) The value of vital sign trends in predicting and monitoring clinical deterioration: A systematic review. PLoS One14 (1): e0210875.https://doi.org/10.1371/journal. pone.0210875

Duguma A (2016) Practical manual on veterinary clinical diagnostic approach.J Vet SciTechnol7: 337. https: //doi:10.4172/2157-7579.1000337

Englar RE (2019) Common Clinical Presentations in Dogs and Cats, John Wiley & Sons, Inc, New Jersey.

Humm K,Kellett-Gregory L (2016) Monitoring Small Animal Patients in the Intensive Care Unit. In Pract38 (Suppl 4): 12-17.

Hwang J, Gottdenker N, Min MS, Lee H, Chun MS (2015) Evaluation of biochemical and haematological parameters and prevalence of select-

ed pathogens in feral cats from urban and rural habitats in South Korea. J Feline Med Surg2015: 1-9.https://doi: 10.1177/1098612X15587572

Kayar A, Dokuzeylul B, Kandemir FM, Kirbas A, Bayrakal A, Or ME (2015) Total oxidant and antioxidant capacities, nitric oxide and malondialdehyde levels in cats seropositive for the feline coronavirus. Vet Med-Czech60: 274-281.

Levy JK, Nutt K, Tucker SJ (2015) Reference interval for rectal temperature in healthy confined adult cats. J Feline Med Surg 17: 950-952.

Lim CSH, Lim SL (2013) Ferric reducing capacity versus ferric reducing antioxidant power for measuring total antioxidant capacity. Lab Med44: 51-55.

McManus C, Paludo GR, Louvandini H, Gugel R, Sasaki LCB,Paiva SR (2009) Heat tolerance in Brazilian sheep: Physiological and blood parameters. Trop Anim Health Prod 41: 95-101.

Miller C, Boegler K, Carver S, MacMillan M, Bielefeldt-Ohmann H,VandeWoude S (2017) Pathogenesis of oral FIV infection. PLoS One 12 (9): e0185138. https://doi.org/10.1371/journal. pone.0185138

Mostafa M, Ferdousi S, Sultana S, Akhter A (2019) Autonomic impairment and oxidative stress: Relationship in PCOS patients. J Bangladesh SocPhysiol14: 82-88.

Norkus CL (2019). Veterinary Technician's Manual for Small Animal Emergency and Critical Care. 2ed, Wiley Blackwell, PA.

Ozdemir O, Alyan MSO, Geyik B, Demir AD, Aras D, Cihan G, Cagirci G, Kacmaz F, Balbay Y, Sasmaz H,Korkmaz S (2004) Association between mean platelet volume and autonomic nervous system functions: Increased mean platelet volume reflects sympathetic overactivity. ExpClinCardiol 9: 243-247.

Sakundech K, Kullaya A, Aengwanich W (2021) Evaluation of the difference between feline immunodeficiency virus (FIV)-infected and healthy cats by using clinicopathological parameters, oxidative stress, and total antioxidant power. Comp ClinPathol 30:149-154.

Sand C, Englert T, Egberink H, Lutz H, Hartmann K (2010) Evaluation of a new in-clinic test system to detect feline immunodeficiency virus and feline leukemia virus infection. Vet ClinPathol39: 210-214.

Sapowicz SA, Linder DE, Freeman LM (2016) Body condition scores and evaluation of feeding habits of dogs and cats at a low cost veterinary clinic and a general practice. Sci World JArticle ID 1901679

Taylor SM (2020)Small Animal Clinical Techniques, Elsevier,*Missouri*.

Teixeira CC, Boaventura RP, Souza ACS, Paranaguá TT de B, Bezerra ALQ, Bachion MM,Brasil VV (2015) Vital signs measurement: An indicator of safe care delivered to elderly patients. TextoContextoEnferm24: 1071-1078.

Teng KT, McGreevy PD, Toribio JAML, Raubenheimer D, Kendall K,Dhand NK (2018) Strong associations of nine-point body condition scoring with survival and lifespan in cats. J Feline Med Surg20: 1110-1118.

Tiwari LM, Kotwal CJ, Kotwal A, Mishra MP, Dutta BV, Chopra BS (2013) Correlation of haemoglobin and red cell indices with serum ferritin in Indian women in second and third trimester of pregnancy. Med J Armed Forces India 69: 31-36.

Turkson PK,Ganyo EY (2015) Relationship between haemoglobin concentration and packed cell volume in cattle blood samples. Onderstepoort J Vet Res 82 (1), 863.

Velguth KE, Payton ME, Hoover JP (2010) Relationship of hemoglobin concentration to packed cell volume in avian blood samples. J Avian Med Surg24: 115-121.

Wang P, Ma Y, Liang F, Zhang Y, Yu X, Li Z, An Q, Lv H,Wang J (2020) Non-contact vital signs monitoring of dog and cat using a UWB radar. Animals, 10: 205. https://doi: 10.3390/ani10020205

White J, Stickney A, Norris JM (2011) Feline immunodeficiency virus: Disease association versus causation in domestic and nondomestic felids. Vet Clin Small Anim41: 1197-1208.