International Journal of Veterinary Sciences and Animal Husbandry

ISSN: 2456-2912
VET 2019; 4(3): 05-08

www.veterinarypaper.com
Received: 04-03-2019
Accepted: 06-04-2019

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Comparisons of some blood hematological levels and biochemical parameters in pregnant and non-pregnant Kangal shepherd dogs

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DOI: https://doi.org/10.22271/veterinary.2019.v4.i3a.184

Abstract
The aim of this study is to compare some blood hematological levels and biochemical parameters obtained from pregnant and non-pregnant Kangal shepherd dogs. Blood samples were collected from 24 pregnant and 24 non-pregnant Kangal shepherd dogs that were aged between 3 to 5 years. Pregnant dogs were in their 35 to 52 days of their pregnancy. Non-pregnant dogs were beyond 30 or more days in their dioestrus. Automatic hemocytometer device was used to determine leucocyte count, erythrocyte count, hemoglobin, hematocrit, platelets, mean erythrocyte hemoglobin, mean erythrocyte hemoglobin concentration and mean erythrocyte volume levels in collected blood samples. Serum levels of total cholesterol, triglyceride, iron, high density lipoprotein and low density lipoprotein parameters were determined using biochemical analyzer device. Results of the study were presented significant increases for iron, hemoglobin, mean erythrocyte hemoglobin concentration and mean erythrocyte hemoglobin parameters in non-pregnant dogs and for cholesterol, low density lipoprotein and triglyceride parameters in pregnant dogs. Statistical significance was not determined for other parameters.

Keywords: Kangal shepherd dog, pregnancy, non-pregnancy, blood parameter

1. Introduction
Domestic dogs, which are descendants of the extinct grey wolf (Canis lupus) genetically predicted to be first used by humans in Eastern Asia 15 to 100 thousands years ago (Jensen, 2007) [9]. It was reported that breeds with herd protection capabilities were among the first dogs used in domesticated dog breeds (Sims and Dawydiak, 1990) [21]. The Kangal shepherd dog breed is greatly preferred for both herd protection and watchdog duties in Turkey as well as in many other countries in the world (Koçkaya and Şireli, 2015) [13]. This breed is called the "Kangal shepherd dog" due to the intense and the most typical presence of this breed in the Kangal town of the Sivas province in Turkey (Akçay, 2005; Özbeyaz, 1994; Yılmaz, 2008) [1, 17, 25]. Due to the great adaptability of these dogs, they are raised in many different countries, but, the literature about the pregnancy blood parameters for this breed was found insufficient (Reed, 2003) [19].

A typical pregnancy duration in dogs is 63 days. Delivery is considered as normal if it happens within 7 days earlier or later. Survival rates for pups were reported to be decreased in the case of significant changes in pregnancy duration (Taylor, 1993) [23]. Pregnancy is not visible in dogs until the 4th week. After the 4th week of the pregnancy, the abdomen starts to become enlarged and saggy, and also the udder starts to become enlarged. Towards the end of pregnancy, breast milk starts to flow from the udder (Horoz *et al*., 1996) [8]. Physical activity decreases in female dogs as the pregnancy progresses (Altınköprü, 1993) [3]. When the delivery time arrives, a pregnant dog starts to search for a safe spot, and the delivery generally occurs either at night or early in the morning (Browlie, 1988) [4]. Certain changes in both blood composition and biochemical parameters become apparent in dogs during the healthy pregnancy duration. Abnormal increases or decreases in these parameters during pregnancy are recognized as risk factors for some physiological disorders. Among these physiological disorders are anemia, hypertension, premature delivery, and lower birth weights. Monitoring the hematological and biochemical parameters during pregnancy is considered to be important for the healthy maintenance of pregnancy and for fetal development.

As with the other animal species, hematological parameters show great differences in dogs. These differences stem from various factors, including age, genetics, breed, pregnancy, and environmental factors. Even though there are many different studies regarding normal hematological values in Kangal shepherd dogs (Çınar *et al*., 2010; Kalaycıoğlu *et al*., 1995; Koçkaya *et al*., 2017) [6, 11, 14], the literature about hematological values during pregnancy is insufficient (Kaymaz and Baştan, 1997; Rushton, 1984) [12, 20].

The aim of the study was to compare some hematological and biochemical parameters obtained from pregnant and non-pregnant Kangal shepherd dogs.

2. Materials and Methods

Animal experimentations approval for the study was granted by the Local Ethics Committee for Animal Experimentations of Cumhuriyet University with issue number of 65202830-050.04.04-96 in October 28, 2016.

2.1. Animals

Kangal shepherd dogs used in the study were obtained from breeders in the Sivas province of Turkey, and they comprised 24 pregnant and 24 non-pregnant dogs aged between 3 and 5 years. The pregnant dogs were chosen in their 35 to 52 days into pregnancy. The non-pregnant dogs were chosen from dogs that were into 30 days or more in their dioestrus periods. The dogs were selected on the basis of anamnesis provided by their breeders and ultrasonography results.

2.2. Blood sampling

During the course of the study, the animals were kept in their familiar environment and allowed to perform their routines in order to prevent stress and potential alterations in their hematological parameters. Establishments were chosen according to having to offer a similar dietary provisions to dogs in order to prevent dietary changes in both hematological and biochemical parameters.

Blood samples from each dog were collected from antebrachial cephalic vena into two tubes which were lithium heparin containing tubes and yellow capped anticoagulant-free gel coated biochemistry tubes. Stress occurrence in dogs was prevented by prior familiarization of dogs to blood sampling and by familiarization of dogs to the person who will collect samples. Blood samples were delivered into the laboratory in shortest time possible. Blood samples were centrifuged for 10 min at 4000 rpm for obtaining serums. Obtained serums were stored at -20ºC until the analysis. Biochemistry analyzer device (Mindray BS200, Mindray, P.R.C.) was used to determine serum total cholesterol, triglyceride, iron (Fe), high density lipoprotein (HDL), and low density lipoprotein (LDL) levels. Automatic hemocytometer device (Hematologic Analyzer System 9000, Serono Diagnostics) was used to determine hematological parameters.

2.3. Statistics

Dogs were divised into 2 study groups as pregnant dogs and non-pregnants dogs, and intergroup statistical analyses were conducted by using Mann-Whitney U test. Statistics were performed by using SPSS v.15 for Windows (SPSS Inc, IBM, USA) [22] software suite.

3. Results

Obtained hematological values from pregnant and non-pregnant Kangal shepherd dogs were presented in Table 1. Hemoglobin (HGB), mean erythrocyte hemoglobin concentration (MCHC) and mean erythrocyte hemoglobin (MCH) values for hematological comparisons of study groups were determined as statistically significant (P<0,001; P<0.01). These values were observed as higher in non-pregnant dogs compared to pregnant dogs.

Table 1: Determined hematological values in study groups.

Groups			
Parameter	**Pregnant (n=24) median**	**Non-Pregnant (n=24) median**	**P-Value**
HGB (g/dL)	10.65	12.95	***
WBC (10^9/L)	12.7	12.5	Nss.
RBC (10^{12}/L)	5.96	6.23	Nss.
HCT (fL)	34.95	36.8	Nss.
MCV	64.45	65.65	Nss.
MCH (pg)	22.15	22.85	**
MCHC (g/dL)	33.45	34.6	***
PLT (10^9/L)	316	312	Nss.

L:liter, dL: deciliter, fL: femtoliter, g: gram, pg: picogram

Nss: statistically insignificant (P>0.05), **:P<0.01 ***: P<0.001

Obtained serum biochemistry values from pregnant and non-pregnant Kangal shepherd dogs were presented in Table 2. Intergroup serum biochemistry comparisons revealed that all values excluding HDL (Fe, triglyceride, LDL, and cholesterol) were determined as statistically significant (P<0.001; P<0.01; P<0.05). Triglyceride, LDL, and cholesterol values were determined as higher in pregnant dogs whereas only Fe values were determined as higher in non-pregnant dogs.

Table 2: Determined serum biochemistry values in study groups

Groups			
Parameter	**Pregnant (n=24) median**	**Non-Pregnant (n=24) median**	**P-Value**
Cholesterol (mg/dL)	160.16	159.16	*
HDL	99.33	99.33	**Nss.**
LDL	15.89	15.33	**
Fe (µg/dL)	21.21	22.05	***
Triglyceride (mg/dL)	55.98	53.07	***

dL: deciliter, mg: milligram, µg: microgram

Nss: statistically insignificant (P>0.05), *:P<0.05, **:P<0.01 ***: P<0.001

4. Discussion

Even though there are many different studies regarding the normal hematological values in Kangal shepherd dogs (Çınar *et al*., 2010; Kalaycıoğlu *et al*., 1995; Koçkaya *et al*., 2017) [6, 11, 14], the literature about hematological values during the pregnancy was insufficient (Kaymaz and Baştan, 1997; Rushton, 1984) [12, 20]. Therefore, results of this study would be fulfilling an important gap in literature by comparing hematological parameters of Kangal shepherd dogs both in pregnancy and dioestrus periods.

HGB values in the study were assessed as 12.95 g/dL and 10.65 g/dL in non-pregnant and pregnant Kangal shepherd dogs, respectively. Statistically significant decreases in HGB values of pregnant dogs (P<0.001) were thought to be associated with the increase of plasma volume exceeding the erythrocyte mass index in pregnant dogs. Obtained result was in accordance with the other studies (Concannon *et al*., 1977; Rushton, 1984) [5, 20].

MCH and MCHC values were determined in non-pregnant Kangal shepherd dogs as 22.85 pg and 34.6 g/dL, respectively. Same values were assessed in pregnant dogs as 22.15 pg and 33.45 g/dL, respectively. Statistically significant decreases in MCHC (P<0.001) and MCH (P<0.01) in pregnant dogs were though to be associated with the decreases in hemoglobin levels due to iron deficiency. These results were similar to results provided from other studies (Allard *et al*., 1989; Kaymaz and Baştan, 1997; Milman *et al*., 2000) [2, 12, 15].

Serum Fe and ferritin levels, and total iron binding capacity in serum become decreased during the pregnancy. Serum Fe levels in the study were determined as 22.05 µg/dL and 21.21 µg/dL in non-pregnant and pregnant Kangal shepherd dogs, respectively. Statistically significant decreases in serum Fe levels in pregnant dogs (P<0.001) were thought to be associated with the expenditure of reserved iron in the body for fetal development and placenta maintenance. This result was found as similar to other studies (Mungan, 2002; Palmer *et al*., 1986; Truswell, 1985) [16, 18, 24].

Plasma LDL levels are progressively increasing during the normal pregnancy, and this increase manifests itself in increases in cholesterol and triglyceride levels. Estrogen is thought to be playing major role in these changes of lipoprotein levels during the pregnancy. Increase in placental lipoprotein lipase activity during the pregnancy facilitates the transfer of maternal lipids into fetus through physiological adaptation. Serum cholesterol, triglyceride, and LDL levels were determined in non-pregnant Kangal shepherd dogs as 159.16 mg/dL, 53.07 mg/dL, and 15.33 mg/dL, respectively. These values were determined in pregnant dogs as 160.16 mg/dL, 55.98 mg/dL, and 15.89 mg/dL, respectively. Statistically significant increases in serum cholesterol (P<0.05), triglyceride (P<0.001), and in LDL (P<0.01) levels in pregnant dogs were thought to be associated with the mechanism of estrogen in pregnant dogs. These results were in accordance with the previous studies (Herrera, 2002; Jimenez *et al*., 1988) [7, 10].

5. Conclusions

It was assessed from the results that HGB, MCH, and MCHC levels were found to be significantly lower in pregnant Kangal shepherd dogs compared to non-pregnant dogs, and these results were associated with the decrease in Fe levels. Decrease in serum Fe levels in pregnant dogs is an important indicator of reserved Fe depletion. Therefore, it is thought that monitoring the Fe levels during the pregnancy would be beneficial for maintaining a healthy pregnancy duration.

It was determined from the results that serum cholesterol, triglyceride, and LDL levels in pregnant Kangal shepherd dogs were significantly higher compared to non-pregnant dogs. Therefore, it would be important to control dietary cholesterol context of pregnant dogs for maintaining a healthy pregnancy.

In conclusion, certain changes in blood hematological parameters in pregnant Kangal shepherd dogs were determined, and monitoring these parameters for the duration of the pregnancy in order to maintain a healthy pregnancy was thought be beneficial. Additionally, it can be recommended to take into consideration of these hematological parameters in pregnant dogs in cases of treatments and operations.

6. List of abbreviations

WBC: Leucocyte
RBC: Erythrocyte
HGB: Hemoglobin
HCT: Hematocrit
PLT: Platelets
MCH: Mean Erythrocyte Hemoglobin
MCHC: Mean Erythrocyte Hemoglobin Concentration
MCV: Mean Erythrocyte Volume
Fe: Iron
HDL: High Density Lipoprotein
LDL: Low Density Lipoprotein
L:liter
dL: deciliter
fL: femtoliter
g: gram
mg: milligram
µg: microgram
pg: picogram
Nss: statistically insignificant

7. Acknowledgements

This study will be presented as an oral presentation at the 2nd International Scientific and Vocational Studies Congress – Health and Sport Sciences (BILMES HL-SP 2018), 5–8 July 2018, Ürgüp, Nevşehir, Turkey.

8. Ethics approval and consent to participate

Approval for animal experimentations the study was granted by the Local Ethics Committee for Animal Experimentation of Cumhuriyet University permit no 65202830-050.04.04-96 issued on 28 October 2016. Written informed consent was obtained from the dogs' owners for publication of this manuscript.

9. Funding

This work was supported by the Scientific Research Project Fund of Cumhuriyet University under project number **V-061**.

10. Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

11. Author contributions

MK conceived and designed the study. MK collected the samples and performed the analysis. MK write manuscript.

12. Consent for publication
All data obtained from this work were approved by author for publication.

13. Competing Interest
There is no conflict of interest in this work.

14. References

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