





## Effects of Gender on Hematologic Parameters in Kangal Shepherd Dogs

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### ABSTRACT

Kangal Shepherd Dogs are highly valuable working dogs unique to Turkey, known for their robustness in withstanding harsh climatic conditions and possessing advanced herd protection and management abilities due to their genetic characteristics. Haematological values serve as crucial indicators of the physiological and physiopathological parameters in animals. A complete blood cell count (CBC) emerges as a powerful diagnostic tool when appropriately assessed. Given the establishment of pure breeds, it is essential to acknowledge that breed-specific hematologic values may exhibit variations influenced by factors such as gender, lifestyle, age, and geographical location. Therefore, understanding the physiological distinctions between genders becomes paramount. This study aims to assess the impact of gender on the complete blood count in Kangal Shepherd Dogs. Upon comparing hematologic parameters between female and male Kangal Shepherd Dogs, a statistically significant difference was observed in the mean platelet volume (MPV) value, which was higher in females than males ( $p < 0.05$ ). While the mean red blood cell (RBC), haemoglobin (HGB), and haematocrit (HCT) values were higher in males, no statistically significant results were found ( $p > 0.05$ ).

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## INTRODUCTION

It is known that the domestication of gray wolves produced the ancestors of the first dogs capable of herd protection (Galibert, 2011). Kangal shepherd dog breed is intensively preferred for both herd protection and guard duties in Turkey as in many countries around the world (Kockaya, 2019). Kangal shepherd dogs have a genotype and specific phenotypic characteristics unique to Turkey (Erdoğan *et al*, 2013). It is reported to be the only dog breed that is durable enough to withstand harsh climatic conditions in nature, has highly developed herd protection and management abilities, and can even neutralize wolves attacking the herd (Özbeyaz, 1994). Kangal shepherd dogs have a characteristic appearance with lioness-like body lines, a black mask around the mouth and eyes, and a tail curled up and forward (Yılmaz, 2007).

Hematological values are an important source of information about physiological and physiopathological parameters of animals (Barger, 2003). Complete blood cell count (CBC) is a powerful diagnostic tool (Dixon, 1997). When CBC is fully and appropriately evaluated, it allows a diagnosis to be made or a differential diagnosis list to be created (DeNicola, 2011). Reference values for animal species are important in this assessment (Etim *et al*, 2014). It has been stated in different studies that characteristics such as lifestyle, gender, age and climate may cause differences in hematological parameters (Connolly *et al*, 2020; Olayemi & Ighagbon, 2011). It is known that Kangal shepherd dogs are used as working dogs for herd protection and are raised as breeding dogs in various dog farms (Özbeyaz, 1994).

It is thought that increased intraspecies homogeneity, the presence of specific behavioral patterns and similarity of lifestyles may lead to increased interspecies genetic differences and variation in hematological reference values within species (Alilovic *et al*, 2022). It is thought that dogs used in herd management and guarding, such as Kangal shepherd dogs, may have hematologic differences compared to small breed dogs and domestic animals due to different lifestyles (Gavazza *et al*, 2012; Lee *et al*, 2020). Breed appears to be an important factor for the appropriateness of population-specific reference ranges and should be considered when performing health examinations including hematologic and biochemical parameters. It is thought that gender factor may also be an important variable in intra-breed evaluations.

This study was carried out to evaluate the effects of gender on hematologic parameters in kangals bred in farms and to establish reference values for complete blood count evaluation of kangal shepherd dogs.

## MATERIALS AND METHODS

### Animals

The scope of the study consisted of a total of 38 dogs, 20 healthy and adult dogs older than 6 months and younger than 6 years of age, 20 mother dogs and 17 breeding dogs, which were bred in the Anatolian Shepherd Dogs & Kangal Farm in Kangal farm in Eldivan District of Çankırı province. After the anamnesis of the dogs were taken, physical examinations were completed and those who were determined to be healthy were included in the study.

### Blood samples

In order to eliminate the changes that may be caused by stress in the dogs included in the study,

the dogs were taken to the areas where they normally lived with people they knew and trusted. The dogs were allowed to get used to and trust them to researchers before blood sampling and spent 20 minutes in the cage. During this period, anamnesis was obtained from the animal caregiver and a physical examination was performed. After the physical examination was completed, blood was collected aseptically from *V. cephalica* into tubes containing K2 EDTA as anticoagulant and blood was counted in a fully automatic blood counting device (Abacus Junior Vet5). With this blood count, 22 different values measured with the device at one time were obtained. These are respectively; White blood cells (WBC), Lymphocytes (LYM), Monocytes (MON), Neutrophils (Neu), Eosinophils (EOS), Lymphocyte percentage (LYM%), Monocyte percentage (MO%), Neutrophils percentage (NE%), Eosinophils percentage (EO%), Basophils percentage (BA%), Red Blood Cell (RBC), Hemoglobin (HGB), Haematocrit (HCT), Mean Corpuscular Volume (MCV), Mean corpuscular hemoglobin (MCH), Mean corpuscular hemoglobin concentration (MCHC), Red cell distribution width (RDW), Platelets (PLT), Platelet-crit (PCT), Mean platelet volume (MPV), Platelet distribution width (PDW).

### **Statistics**

Descriptive statistics of the data were calculated and Arithmetic Mean, Standard Error, Standard Deviation, Median, Minimum and Maximum values were calculated and presented in a table. Parametric assumptions were tested by Shapiro-Wilk Test and Levene's Test. Since MON, NEU, MO%, RDWc, PLT, PCT variables did not meet the parametric assumptions, Mann-Whitney U test was performed for differences between gender groups. Since the other variables met the parametric assumptions, Student's T test was performed for the differences between gender groups. The analysis was performed at 0.05 significance level. IBM SPSS 23.0 statistical package program was used for all statistical analyses.

### **RESULTS**

This study focused on the effect of gender on complete blood count values in healthy Kangal shepherd dogs. The hematologic values obtained from the dogs included in the study are given in Table 1. When hematologic parameters were compared between female and male Kangal shepherd dogs, it was observed that mean platelet volume (MPV) value was statistically significantly higher in females than males ( $p < 0.05$ ). The mean RBC, HBG and HCT values were higher in males, but no statistically significant result was determined ( $p > 0.05$ ). The data we obtained were compared with the cbc reference values of dogs. In the comparison, it was determined that RBC, HBG, HCT, PDW parameters of male dogs and WBC, EOS, HGB, PCT and PDW parameters of female dogs were outside the reference range (Table 1).

### **DISCUSSION**

CBC is one of the most basic hematologic diagnostic methods used by clinicians (Dixon, 1997). When evaluated appropriately, it provides important information about the health status of the animal, definitive diagnosis of diseases and preparation of a differential diagnosis list (DeNicola, 2011). It is important to know the specific reference values for species in the evaluation of complete blood count (Etim *et al*, 2014). Today, the breeding of pure breeds within species and the awareness of breeders about the importance of preserving the genotypes of breeds have led to an increase in genetically determined differences between breeds. It is thought to have breed-specific hematological values due to the creation of homogeneous populations in terms of phenotype, genotype and behavioral characteristics

Table 1. The effect of gender on total blood count results in Kangal shepherd dogs.

| Parameters | Units              | GENDER    |      |      |       |         |         |           |      |      |       |         | P       |       |
|------------|--------------------|-----------|------|------|-------|---------|---------|-----------|------|------|-------|---------|---------|-------|
|            |                    | Male      |      |      |       |         |         | Female    |      |      |       |         |         |       |
|            |                    | $\bar{x}$ | SE   | S    | Mean  | Minimum | Maximum | $\bar{x}$ | SE   | S    | Mean  | Minimum | Maximum |       |
| WBC        | $\times 10^9/L$    | 15,89     | 0,81 | 3,34 | 16,67 | 10,85   | 21,32   | 17,89     | 1,12 | 5,00 | 17,09 | 10,83   | 30,24   | 0,169 |
| LYM        | $\times 10^9/L$    | 3,37      | 0,52 | 2,16 | 2,30  | 0,65    | 7,09    | 3,21      | 0,35 | 1,55 | 3,24  | 1,02    | 5,59    | 0,799 |
| *MON       | $\times 10^9/L$    | 0,67      | 0,09 | 0,36 | 0,54  | 0,13    | 1,61    | 0,89      | 0,13 | 0,57 | 0,78  | 0,26    | 2,40    | 0,175 |
| *NEU       | $\times 10^9/L$    | 11,27     | 0,66 | 2,71 | 10,98 | 6,50    | 16,69   | 12,81     | 0,87 | 3,91 | 12,89 | 6,60    | 23,76   | 0,180 |
| EOS        | $\times 10^9/L$    | 0,48      | 0,06 | 0,23 | 0,52  | 0,09    | 1,01    | 0,66      | 0,10 | 0,43 | 0,53  | 0,19    | 1,72    | 0,124 |
| BAS        | $\times 10^9/L$    | 0,10      | 0,02 | 0,08 | 0,08  | 0,01    | 0,29    | 0,14      | 0,02 | 0,10 | 0,12  | 0,03    | 0,45    | 0,145 |
| LY%        | %                  | 20,6      | 2,9  | 12,0 | 17,2  | 5,3     | 42,6    | 18,4      | 1,8  | 8,3  | 18,6  | 5,0     | 35,1    | 0,531 |
| *MO%       | %                  | 4,3       | 0,5  | 2,2  | 4,0   | 0,8     | 9,8     | 5,0       | 0,5  | 2,3  | 5,1   | 1,5     | 9,2     | 0,329 |
| NE%        | %                  | 71,5      | 2,8  | 11,7 | 71,1  | 48,6    | 87,7    | 71,7      | 2,3  | 10,3 | 71,2  | 53,6    | 84,8    | 0,965 |
| EO%        | %                  | 3,1       | 0,3  | 1,3  | 3,2   | 0,6     | 5,2     | 4,1       | 0,6  | 2,8  | 3,3   | 1,1     | 11,8    | 0,160 |
| BA%        | %                  | 0,6       | 0,1  | 0,5  | 0,5   | 0,1     | 1,5     | 0,8       | 0,1  | 0,4  | 0,8   | 0,2     | 1,5     | 0,198 |
| RBC        | $\times 10^{12}/L$ | 8,84      | 0,25 | 1,04 | 8,90  | 6,77    | 10,49   | 8,39      | 0,31 | 1,37 | 8,35  | 6,21    | 10,42   | 0,271 |
| HGB        | g/dl               | 20,1      | 0,7  | 2,7  | 20,2  | 15,1    | 23,9    | 19,3      | 0,8  | 3,6  | 18,8  | 14,3    | 24,5    | 0,454 |
| HCT        | %                  | 55,30     | 2,31 | 9,50 | 58,41 | 28,43   | 65,68   | 54,54     | 2,05 | 9,15 | 55,19 | 41,67   | 67,95   | 0,806 |
| MCV        | $fL$               | 64,5      | 0,7  | 2,6  | 64,5  | 59,0    | 70,0    | 65,0      | 0,6  | 2,7  | 65,0  | 60,0    | 71,0    | 0,618 |
| MCH        | pg                 | 22,70     | 0,15 | 0,60 | 22,75 | 21,70   | 24,00   | 23,06     | 0,23 | 1,02 | 22,80 | 21,00   | 25,70   | 0,221 |
| MCHC       | g/dl               | 35,0      | 0,5  | 2,0  | 34,8  | 30,6    | 38,6    | 35,3      | 0,3  | 1,2  | 35,0  | 33,6    | 38,2    | 0,552 |
| *RDW       | %                  | 17,1      | 0,2  | 1,0  | 17,0  | 15,9    | 19,4    | 16,8      | 0,1  | 0,6  | 16,5  | 16,0    | 18,1    | 0,433 |
| *PLT       | $\times 10^9/L$    | 220       | 35   | 140  | 185   | 31      | 534     | 246       | 23   | 102  | 247   | 37      | 445     | 0,152 |
| *PCT       | %                  | 0,25      | 0,04 | 0,17 | 0,20  | 0,03    | 0,65    | 1,46      | 1,19 | 5,31 | 0,27  | 0,03    | 24,00   | 0,161 |
| MPV        | $fL$               | 10,3      | 0,2  | 0,8  | 10,3  | 9,4     | 11,9    | 11,0      | 0,2  | 1,1  | 11,0  | 8,9     | 13,1    | 0,023 |
| PDW        | %                  | 39,8      | 0,8  | 3,1  | 40,5  | 30,2    | 43,8    | 41,4      | 0,3  | 1,3  | 41,6  | 39,2    | 44,0    | 0,054 |

$\bar{x}$ : Arithmetic mean, SE: Standard Error, S: Standard Deviation, \*: Variables for which Mann-Whitney U test was performed.

within pure breeds and the production of strong breeders in this direction (Alilovic *et al*, 2022). It should also be kept in mind that breeds can be affected by various variables such as age, gender, lifestyle and geographic location (Gavazza *et al*, 2012; Lee *et al*, 2020). It is important to consider the physiological differences between genders. In this study, we evaluated the effects of gender on complete blood count in kangal shepherd dogs.

Mean platelet volume (MPV), which represents the average platelet size, is a routinely studied parameter (Bommer *et al*, 2008). Several studies have been conducted on the diagnostic value of MPV in dogs. It was reported that MPV increased significantly in experimentally induced endotoxemia in dogs (Yilmaz *et al*, 2008). It was also reported that MPV increased in dogs with babesiosis (Žvorc *et al*, 2010). Schneider and Mischke (2016) and Nikolic *et al*. (2022) reported that gender affected the MPV parameter statistically significantly ( $p < 0.05$ ) in healthy dogs in accordance with our study. However, while higher MPV values were determined in male dogs compared to female dogs in the reported studies, a statistically significant higher value was determined in females in our study. However, in contrast to these studies, Bommer *et al* (2008) reported that gender had no significant effect on MPV in a study with 80 control and 159 thrombocytopenic dogs. In our study, although MPV was statistically significant, no significant results were observed in PLT, PCT and PDW parameters (Table 1). It is thought that interpreting only MPV for platelet size and distribution may be misleading by the authors.

Kockaya *et al*. (2021) investigated the effects of gender and age on hematologic parameters in Kangal shepherd dogs and reported that there was no significant relationship between sex, age and age-sex relationship in hematologic parameters, which is partially consistent with our study. In accordance to our study, it was reported that MPV in adult Kangal shepherd dogs was higher in females, but gender was not statistically significant (Kockaya *et al*, 2021). It is thought that the increase in the number of samples in our study was effective in the emergence of a statistically significant expression.

According to the similar study conducted by Kockaya *et al*. (2021) in Kangal shepherd dogs, when the CBC parameters were compared on the basis of gender with our study, it was determined that RBC, HGB, MCHC, RDW, MPV and PDW parameters were higher in both male and female Kangal dogs and PCT parameter was higher only in female Kangal dogs. It is thought that this situation may be related to complete blood count device.

## **CONCLUSION**

The Kangal shepherd dog is a working dog native to Turkey with a very high herding ability. Hematologic data may vary in various situations. This study investigated the effects of gender on CBC. The results showed that gender had no statistically significant effect, except for the MPV parameter. More studies are needed to better understand the physiology and physiopathology of Kangal shepherd dogs. Further comprehensive and different studies may help veterinarians to avoid misinterpretation of laboratory results in the diagnostic process, prognosis and therapeutic monitoring.

## **Ethical Approval**

The Kırıkkale University Animal Experiments Local Ethics Committee (2023/04-15) approved and deemed the study's animal experimentation protocols compliant.

## **Conflict of Interest**

The authors declared that there is no conflict of interest. Material preparation and data collection were carried out by Halime Kara and Yasin Şenel. Statistical analysis was carried out by Ali Alparslan Sayım.

The first draft of the manuscript was written by Mustafa Güven, and all authors commented on previous versions of the manuscript. All authors read and approved the final version of the manuscript.

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