Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergikafkas.org
E-ISSN: 1309-2251

Kafkas Univ Vet Fak Derg
30 (1): 47-52, 2024
DOI: 10.9775/kvfd.2023.30363

Research Article

Evaluation of Colostral Passive Immune Transfer Success in Turkish Kangal Shepherd Dogs

Erdal KARA [1] Naci ÖCAL [1] Özkan DURU [2] Sibel YASA DURU [1] Yasin ŞENEL [1 (*)]

[1] Kırıkkale University, Faculty of Veterinary Medicine, Department of Internal Medicine, TR-71450 Kırıkkale - TÜRKİYE
[2] Kırıkkale University, Faculty of Veterinary Medicine, Department of Biochemistry, TR-71450 Kırıkkale - TÜRKİYE

(*) **Corresponding author:** Yasin ŞENEL
Phone: +90 318 357 4242/6055
E-mail: yasinsenel@kku.edu.tr

How to cite this article?
Kara E, Öcal N, Duru Ö, Yasa Duru S, Şenel Y: Evaluation of colostral passive immune transfer success in Turkish Kangal shepherd dogs. *Kafkas Univ Vet Fak Derg,* 30 (1): 47-52, 2024.
DOI: 10.9775/kvfd.2023.30363

Article ID: KVFD-2023-30363
Received: 02.08.2023
Accepted: 02.11.2023
Published Online: 22.11.2023

Abstract

The aim of this study was to investigate the passive immune transfer process and its effects in Turkish Kangal shepherd dogs. The material of the study consisted of 15 dams and their 138 offspring in the region of Central Anatolia, Türkiye. Blood samples were collected from the surviving puppies at 48±4 h postpartum and IgG levels were measured using the ELISA method. Before the blood samples were collected, 34 of the puppies (26.64%) died, 56 of the 104 puppies (40.58%) whose blood samples were collected and IgG analyses were done died prior to weaning, and 48 (34.78%) survived until weaning. All of the analyzed blood samples had IgG levels above 230 mg/dL and there was no passive transfer failure. The average blood IgG level of the puppies was 664.86 mg/dL. The effects of litter size and birth season on passive transfer success were statistically significant (P<0.05), while maternal age and the sex of the puppy were not significant (P>0.05). There was no correlation between the average blood IgG levels of the puppies and group mortality rates (r = -0.44). In conclusion, while the rate of passive transfer success for Turkish Kangal shepherd dogs was found to be high compared to other breeds, the high rate of mortality among the puppies suggested that dams and their litters should be cared for more carefully in the neonatal period.

Keywords: Canine neonatal period, Colostrum, ELISA, Ig G, Passive immune transfer, Puppy mortality, Turkish Kangal shepherd dogs

Introduction

Due to their endotheliochorial placental structure, dogs cannot transplacentally transfer macromolecular components such as IgG to their puppies during the intrauterine period [1]. Puppies are born with almost agammaglobulinemia or hypogammaglobulinemia, similarly to calves, foals, piglets, and kittens [2]. The immune components received through colostrum are of great importance in the survival of puppies born with almost completely unformed immune systems [3,4]. This immune transfer from mother to newborn via colostrum is called passive immune transfer [5].

Although many immune components are transferred in passive transfer, the success of this transfer is measured by IgG level [1,6]. While the blood IgG level in adult dogs is 800-2500 mg/dL, it is only 30 mg/dL when puppies are born [1]. Newborn puppies receive 85-95% of the IgG they need from colostrum, reaching an average IgG concentration of 600-1600 mg/dL at 2 days of age [7-9]. Even puppies that experience a successful passive immune transfer process only reach blood IgG levels 50-77% of that of adult dogs until their own Ig begins to be synthesized [1,10].

The critical period of puppies' first 3 weeks of life, before their own Ig levels reach a protective level, is called the neonatal period and the risk of death during this period is very high [11]. Survival in the neonatal period is directly related to the avoidance of passive transfer failure (PTF) [1,12]. The threshold for passive transfer insufficiency in dogs is an IgG concentration of 230 mg/dL and values below that are considered to be indicative of failure. While mortality in the neonatal period remains below 5% in puppies with successful passive transfer processes, it approaches 50% in puppies that experience PTF [10].

Methods used to evaluate passive transfer success in dogs include the determination of blood IgG levels at 2 days of age by ELISA, determination of blood gamma-glutamyl transferase levels, determination of antibody titers against specific diseases such as canine parvovirus 2, and early growth monitoring [1,13-15]. Although the success of passive transfer can be affected by variables such as maternal age, maternal behavior, litter size, birth weight, and live weight, the quality, quantity, and timing of colostrum consumption are the criteria that determine the success of passive immune transfer [1,10]. It is known that puppy mortality rates among large and giant dog breeds are higher than those of small breeds [16].

Turkish Kangal (Karabaş) shepherd dogs, together with Turkish Akbaş and Kars (Kafkas) shepherd dogs, are world-famous shepherd dogs of Türkiye and are bred in many countries including Belgium, France, Germany, and Slovenia [17,18]. Turkish Kangal shepherd dogs are large-sized dogs like many other breeds of Turkish shepherd dogs [19]. They are among the large breed dogs with an average adult live weight of 50 kg [17,20,21]. Kangal dogs give birth to an average of 6-9 puppies in a litter and the average birth weight of the puppies is 500-550 g [22-24].

This study aims to investigate the passive transfer success of Turkish Kangal shepherd dogs, a breed that is becoming increasingly popular in the world, and the variables affecting the passive transfer process. It is expected that this study will serve as a reference for the investigated markers of large-breed dogs.

Material and Methods

Ethical Statement

The study was carried out according to European Council Directive 2010/63/EU on the protection of animals used for scientific purposes and the relevant Turkish legislation (Law No. 5199, Regulation No. 28141) dated 13.12.2011, Kırıkkale University Animal Experiments Local Ethics Committee approval was obtained before the study (Approval No. 2020(4)/25).

Animals

The animal material of this study consisted of Turkish Kangal shepherd dogs raised as herd protection and guard dogs in the Kırıkkale region. A total of 138 puppies born to 15 dams were followed and 104 puppies that survived for 48 h were included in the passive transfer evaluation.

None of these traditionally fed dogs received professional feeding or dry food. A vegetable protein-based slurry called "yal," made from barley or wheat flour and sometimes with bran added, was used as chow. All of the puppies included in this study were born to dams who had received periodic vaccinations and antiparasitics.

The study was conducted in the 12-month calendar year from December 2020 to November 2021. Kırıkkale province, where the study was conducted, is located at 39.8392°N, 33.5089°E and has a continental climate. The puppies were evaluated within the 4 seasonal groups of winter, spring, summer, and autumn according to their birth dates. In the Kırıkkale region where the research was conducted, winter is December-February, spring is March-May, summer is June-August, and autumn is September-November.

Blood Sampling and IgG Analyses

Direct contact with the puppies was avoided by using disposable protective clothing and examination gloves. The puppies were taken from their mothers by their owners and given to the researchers. After blood sampling, puppies were rubbed on the dam's perineal area by the owner and then attached to the dam's teats to prevent the possibility of the dam rejecting her offspring and ensure that all puppies would be accepted by their mothers after sampling. Blood samples were obtained from the puppies 36-48 h after birth from the cephalic vein by breaking the plastic luer base of a 21-gauge needle. During blood sampling, the puncture area was not shaved. Asepsis and vasodilation were achieved with alcohol and then samples were collected into anticoagulant-free pediatric gel tubes of 1 mL with yellow caps that accelerated clotting with silica particles in the tube walls. Blood samples were centrifuged at 3000 rpm for 10 min, and serum was separated and kept in a freezer at -20°C until analysis was performed. Serum IgG was analyzed using a commercial ELISA kit (Dog IgG ELISA Kit, Lot: E44-128, 210428; Bethyl Laboratories, Montgomery, TX, USA).

Recorded Parameters

Information about the owners of the dams included in the study, puppies' birth dates, dams' ages, breeding purposes, the sex of the puppies, litter size, deaths in the first 48 h, deaths in the first 2 months, and numbers of surviving puppies were recorded. Since there are no previous studies in the literature on the individual marking of newborn Kangal puppies and the reactions of their mothers to such markings could not be predicted, individual marking and tracking of the puppies was not performed considering the possibility of the dams harming their offspring. The offspring of each dam were followed collectively and the IgG values of these groups were obtained by averaging the IgG levels of each individual offspring. Similarly, the mortality rates of the offspring of each dam were recorded, and the mean IgG values and mortality rates for each group were compared at the end of the study.

Informed consent has been obtained for all client-owned animals included in this study.

Statistical Analysis

One-way ANOVA testing was used to determine the differences between the groups in values determined from serum samples obtained in the study. The significance level was accepted as $P<0.05$ and the Tukey test, which is a post hoc test, was used to determine the groups from which the significant differences between groups arose. The obtained statistical results were presented as mean ± standard error values in tables. The means IgG levels and mortality rates of the groups were evaluated by Pearson correlation test [25].

Results

Within the scope of this study, 138 puppies were born to 15 dams. The mean number of puppies born in litters per dam was calculated as 9.2. Thirty-four (24.64%) of these puppies died within the first 48 h after birth and could not be included in the PTF evaluation because blood samples were not taken. The remaining 104 puppies were included in the PTF evaluation. Of the 104 pups evaluated for PTF, 56 (40.58% of the total puppies) died before weaning (2 months of age) and 48 (34.78% of the total puppies) survived (*Table 1*).

The IgG levels in blood serum samples obtained from the puppies were found to range between 419.27 and 1965.34 mg/dL with a mean value of 664.86 mg/dL (*Table 2*). When these blood IgG levels were evaluated, it was determined that all measurements were above the PTF threshold value of 230 mg/dL.

There was no statistical difference (P>0.05) in the Ig levels of the puppies according to maternal age (*Table 2*).

There was no statistically significant difference (P>0.05) between the Ig levels of the puppies according to sex (*Table 3*).

There was a statistically significant difference (P<0.05) in the Ig levels of the puppies according to the season of birth (*Table 4*). This statistically significant difference was observed to arise between puppies born in summer and those born in winter.

There was a statistically significant difference (P<0.05) in the Ig levels of the puppies according to litter size (*Table 5*). The blood IgG levels of the offspring of dams with litters containing 3-5 offspring were significantly higher than those of offspring in larger litters.

Table 1. *Number of puppies born per litter and puppy losses*

Number of dams in the study	Total number of puppies born	Mean number of puppies in a litter	Number of puppies that died in the first 48 h	Number of puppies that died at 2-60 days	Number of puppies surviving to 60 days of age
15	138	9.2	34 (24.64%)	56 (40.58%)	48 (34.78%)

Table 2. *Comparison of Ig levels of offspring according to maternal age*

Age of dams	Number of offspring (n)	Ig values (mg/dL) of puppies (Mean±S_E)	p
Group I (2 years)	30	654.70±63.32	0.914
Group II (3 years)	38	697.68±56.26	
Group III (4 years)	17	674.28±84.12	
Group IV (5 and above)	19	632.79±79.57	
Total	104	664.86±35.86	

Table 3. *Comparison of Ig levels according to the sex of the puppies*

Groups	Number of offspring (n)	Ig values (mg/dL) of puppies, mean ± standard error	p
Group I (male)	58	667.465±40.75	0.944
Group II (female)	46	672.289±56.56	

Table 4. *Comparison of Ig levels according to birth season of the puppies*

Season	Number of offspring (n)	Ig values (mg/dL) of puppies, mean ± standard error	p
Group I (spring)	21	613.488±46.23	0.046
Group II (summer)	32	554.026±35.26	
Group III (autumn)	36	694.795±56.19	
Group IV (winter)	15	870.236±155.46	

Table 5. *Comparison of serum Ig levels according to litter size*

Groups	Number of offspring (n)	Ig values (mg/dL) of puppies, mean ± standard error	p
Group I (3-5 puppies)	8	1024.127±273.41	0.022
Group II (6-8 puppies)	28	642.336±54.009	
Group III (9-11 puppies)	12	600.910±107.97	
Group IV (12-14 puppies)	56	647.302±31.57	

Table 6. *Correlation of serum IgG levels and mortality rates of the maternal groups*

Parameters	N	Mean group IgG values (mg/dL)	Group mortality percentages	p
Mean group IgG values (mg/dL)	15	1	-0.44	0.877
Group mortality percentages	15	-0.44	1	

The mean IgG levels and mortality rates of the offspring of each dam were calculated. The r value was found to be -0.44 and the P value was found to be 0.877, indicating that there was no correlation between the mean IgG levels and mortality rates *(Table 6)*.

Discussion

Although IgG is not the only immune component present in the colostrum of dogs, it is commonly considered as a reference molecule in the evaluation of passive transfer success [1,9,26]. Gooding and Robinson [27] reported the prevalence of PTF in dogs as 5%. Mila et al.[10], in a study of 149 puppies, found that 26 puppies had IgG concentrations below the threshold value and they subsequently reported the rate of PTF in dogs as 17.4%. Chastant and Mila [1] reported that the IgG concentration was below 230 mg/dL in only 4 puppies among the unpublished data of their evaluations of 90 Labrador puppies; they found the PTF rate to be 4.4%. In the present study, blood samples from 104 puppies were evaluated. It was observed that blood IgG levels were above 230 mg/dL at 48 h of age in all cases and no PTF was observed for any of the evaluated puppies. The results obtained here are similar to those of Gooding and Robinson [27] and Chastant and Mila [1] but considerably lower than the values reported by Mila et al.[10]. Although the rate of PTF was reported to be between 4.4% and 17.4% in other studies of dogs. PTF was not detected in any puppies in the present study. Although the rate of 0% obtained in this study is relatively close to the rates of ≤5% reported by some researchers, it is still a lower value compared to all previous studies in the literature to date. Further research is needed to determine whether this absence of PTF is a breed-specific characteristic of Turkish Kangal shepherd dogs or a finding unique to the present study.

It was previously reported that the average litter size of Turkish Kangal shepherd dogs ranges from 5.9 to 8.9 [23]. In our study, the mean litter size was found to be 9.2, slightly greater than the range stated in the literature.

Mila et al.[10] reported that the effects of variables such as breed size, sex, season of birth, colostrum IgG concentration, and litter size on passive transfer success in puppies were insignificant on a dog farm where different dog breeds were bred. In the present study, the effects of maternal age and the sex of the puppies on blood IgG levels at 2 days of age were similarly found to be insignificant for passive transfer success, while the effects of birth season and litter size were significant, in contrast to the findings of Mila et al.[10]. In this study, with a mean litter size of 9.2, the reason for the negative effect of increasing litter size on passive transfer success may be that the colostrum of dams with larger litters may not be sufficient for all of the offspring, while the colostrum of dams with 5 or fewer offspring is more likely to be sufficient and higher passive transfer success will likely be achieved. In addition, dams with 5 or fewer puppies have more opportunities to care for their offspring on an individual basis, whereas dams with larger numbers of puppies may be exhausted due to the prolonged birth process and more extensive feeding, leaving them unable to care for each puppy individually and negatively affecting the success of passive transfer. Considering the effect of the season of birth on passive transfer success, it was observed that the rate of passive transfer success was higher in winter compared to summer in the present study. This may have been due to the fact that the dams were more affected by heat stress in summer and processes requiring close physical contact such as feeding were kept to shorter durations as the dams tried to prevent their body temperatures from rising even more, indirectly affecting the success of passive transfer. While the mean IgG levels in spring and fall were found to be quite close to the general mean value obtained in this study, they were highest in winter, when the dams were in constant contact with the newborn puppies to maintain their body temperatures. The dam's effort to remain in constant contact with the puppies in winter to protect them from hypothermia would give the puppies more opportunities to suckle during the course of that contact. However, this hypothesis can only be confirmed by future studies that compare ambient temperatures and suckling times of puppies in the first 48 h of life.

Mila et al.[10] evaluated the factors affecting neonatal mortality and found that the effects of the sex of the puppies, breed, colostrum IgG concentration, and litter size were insignificant while blood IgG levels at 2 days of age were significant (P=0.018). Tønnessen et al.[16] found the effect of maternal age on puppy mortality to be statistically significant. In the present study, individual follow-up was not possible because the puppies were not marked. In our study, the mortality rate of the offspring of 15 dams was obtained by calculating the ratio of the puppies surviving until weaning (2 months of age) to the total number of offspring included in the study. No correlation was found between passive transfer success and survival until weaning. However, Mila et al.[10] found a correlation between passive transfer success and mortality when they followed puppies individually and evaluated mortality within 21 days, in contrast to our study. Furthermore, our study was conducted under field conditions in an environment in which biosecurity measures were very limited, unlike the controlled study of Mila et al.[10], in which biosecurity measures were applied on a professional farm. This may explain the discrepancies between the results of these studies. Under field conditions, puppies are exposed to many factors that may increase the mortality rate, particularly including infective agents [16]. In the present study, since the 15 dams were divided into 4 age groups and there was a small number of dams in each group, the effect of maternal age on puppy mortality was not statistically evaluated. Tønnessen et al.[16] reported that maternal age had a significant effect on puppy mortality in their comprehensive study of 58.439 puppies born to 10.810 dams. The Turkish Kangal shepherd dogs were quite tolerant of the manipulations performed on their offspring during the present study and it is anticipated that the individual marking of puppies will not be a problem in future studies. In this way, it will be possible to follow the health parameters of individual puppies and compare them in terms of all variables, allowing more detailed and reliable results to be obtained.

Mila et al.[10] reported that 34 (17.43%) of 195 puppies born on a farm with dogs of different breeds died in the first 48 h after birth. The differences between the puppy mortality rates in this study and the rates of previous studies may be due to many different factors including farm and breeding conditions, litter size, and susceptibility to diseases according to the size of the breed [16].

The survival rate of Kangal puppies in our study was 34.78% at the end of 2 months, while Tepeli and Çetin [28] reported it as 87.05% and Kırmızı [24] reported it as 41% for the spring and summer months and 39.1% for autumn and winter. Oğrak [29] reported that this rate varied between 62.5% and 94.37% for puppies born in different months. These differences in the findings of the survival of Kangal puppies until weaning may be affected by variables including epidemics at different times and in different regions, care and feeding conditions, and the implementation of biosecurity measures. Although some of the studies discussed here were conducted on breeding farm, our study was conducted under field conditions with dogs fed by non-professional breeders where biosecurity measures were not applied, and so the higher mortality rates were an expected finding.

In conclusion the present study has offered the first evaluation of passive transfer success in Turkish Kangal shepherd dogs. It was found that the blood IgG levels of all surviving puppies were above the PTF threshold value, but further studies are needed to determine whether this is a result specific to the region or whether the passive transfer process is more successful for Kangal dogs than other breeds. It was also observed that passive transfer success was affected by season of birth and litter size, while maternal age and the sex of the puppies had no statistically significant effects. As another remarkable finding, although the passive transfer success rate was well above the average values, the puppy mortality rate was higher than the ranges reported in the literature data, revealing that dog owners who breed Turkish Kangal shepherd dogs, and particularly those around Kırıkkale, do not manage the processes of the neonatal period well and their biosecurity and management practices are inadequate. For this reason, it is necessary to raise awareness among the breeders in this region about neonatal protocols, the neonatal period, biosecurity, and preventive medicine practices that should be applied for puppies.

Availability of Data and Materials

The authors declare that the data and materials are available on request from the corresponding author.

Funding Support

This work was supported by the Scientific Research Projects Coordination Unit of Kırıkkale University BAP (Project Number 2020/087).

Ethical Statement

Kırıkkale University Animal Experiments Local Ethics Committee approval was obtained before the study (Approval No. 2020(4)/25).

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

Author Contributions

All authors contributed to the design of the study and the writing of the manuscript. EK, NÖ, SYD, and YŞ carried out blood sampling and health monitoring of the puppies. ÖD performed the laboratory analyses. NÖ performed the statistical analyses. All authors read and approved the final manuscript.

References

1. Chastant S, Mila H: Passive immune transfer in puppies. *Anim Reprod Sci*, 207, 162-170, 2019. DOI: 10.1016/j.anireprosci.2019.06.012

2. Bouchard G, Plata-Madrid H, Youngquist RS, Buening GM, Ganjam VK, Krause GF, Allen GK, Paine AL: Absorption of an alternate source of immunoglobulin in pups. *Am J Vet Res*, 53 (2): 230-233, 1992.

3. Grundy SA: Canine neonatal health. *Vet Clin North Am Small Anim Pract*, 53 (5): 1161-1193, 2023. DOI: 10.1016/j.cvsm.2023.05.008

4. Pereira M, Bolas A, Marques C, Alexandre-Pires G, Fonseca I, Santos-Gomes G: Development of dog immune system: From in uterus to elderly. *Vet Sci*, 6 (4):83, 2019. DOI: 10.3390/vetsci6040083

5. Lombard J, Urie N, Garry F, Godden S, Quigley J, Earleywine T, McGuirk S, Moore Branan DM, Chamorro M, Smith G, Shivley C, Catherman D, Haines D, Heinrichs AJ, James R, Maas J, Sterner K: Consensus recommendations on calf- and herd-level passive immunity in dairy calves in the United States. *J Dairy Sci*, 103 (8): 7611-7624, 2020. DOI: 10.3168/jds.2019-17955

6. Godden SM, Lombard JE, Woolums AR: Colostrum management for dairy calves. *Vet Clin Food Anim Pract,* 35 (3): 535-556, 2019. DOI: 10.1016/j.cvfa.2019.07.005

7. Chastant-Maillard S, Freyburger L, Marcheteau E, Thoumire S, Ravier JF, Reynaud K: Timing of the intestinal barrier closure in puppies. *Reprod Domest Anim*, 47, 190-193, 2012. DOI: 10.1111/rda.12008

8. Day MJ: Immune system development in the dog and cat. *J Comp Pathol*, 137, 10-15, 2007. DOI: 10.1016/j.jcpa.2007.04.005

9. Poffenbarger EM, Olson PN, Chandler ML, Seim HB, Varman M: Use of adult dog serum as a substitute for colostrum in the neonatal dog. *Am J Vet Res*, 52 (8): 1221-1224, 1991.

10. Mila H, Feugier A, Grellet A, Anne J, Gonnier M, Martin M, Rossig L, Chastant-Maillard S: Inadequate passive immune transfer in puppies: Definition, risk factors and prevention in a large multi-breed kennel. *Prev Vet Med*, 116 (1-2): 209-213, 2014. DOI: 10.1016/j.prevetmed.2014.05.001

11. Mugnier A, Brévaux J, Mila H, Lyazrhi F, Mariani C, Adib-Lesaux A, Chastant-Maillard S, Saegerman C, Grellet A: Low birth weight as a risk factor for early neonatal puppy mortality. *Prev Vet Med,* 171:104746. 2019, DOI: 10.1016/j.prevetmed.2019.104746

12. Rossi L, Lumbreras AEV, Vagni S, Dell'Anno M, Bontempo V: Nutritional and functional properties of colostrum in puppies and kittens. *Animals*, 11 (11):3260, 2021. DOI: 10.3390/ani11113260

13. Center SA, Randolph JF, ManWarren T, Slater M: Effect of colostrum ingestion on gamma-glutamyltransferase and alkaline phosphatase activities in neonatal pups. *Am J Vet Res*, 52 (3): 499-504, 1991.

14. Mila H, Grellet A, Desario C, Feugier A, Decaro N, Buonavoglia C, Chastant-Maillard S: Protection against canine parvovirus type 2 infection in puppies by colostrum-derived antibodies. *J Nutr Sci*, 3:e54, 2014. DOI: 10.1017/jns.2014.57

15. Mila H, Grellet A, Feugier A, Desario C, Decaro N, Buonavoglia C, Mariani C, Chastant-Maillard S: General and type 2 parvovirus-specific passive immune transfer in puppies-evaluation by early growth. *Reprod Domest Anim,* 53, 96-102, 2018. DOI: 10.1111/rda.13334

16. Tønnessen R, Borge KS, Nødtvedt A, Indrebø A: Canine perinatal mortality: A cohort study of 224 breeds. *Theriogenology*, 77 (9): 1788-1801, 2012. DOI: 10.1016/j.theriogenology.2011.12.023

17. Yilmaz O, Ertugrul M: Turkish Kangal (Karabash) shepherd dogs raised in Europe. *Can J Pure Appl Sci,* 9 (2): 3393-3397, 2015.

18. Koçkaya M: Effect of exercise on electrocardiography and stress behavior of Kangal shepherd dogs with ankyloglossia. *Kafkas Univ Vet Fak Derg,* 27 (4): 511-515, 2021. DOI: 10.9775/kvfd.2021.25797

19. Yılmaz O, Ertuğrul M: Native dogs breeds and types of Turkey. *Iğdır Univ J Inst Sci Tech,* 2 (1): 99-106, 2012.

20. Atasoy F, Unal N: Body weight and some body measurement of Kangal dogs. *Lalahan Hay Araşt Enst Derg,* 45 (1): 33-39, 2005.

21. The Kennel Club Limited, Kennel Club, UK: Anatolian Shepherd Dog. https://www.thekennelclub.org.uk/breed-standards/pastoral/anatolian-shepherd-dog; *Accessed:* 31.07.2023.

22. Sokolowski JH, Stover DG, Van Ravenswaay F: Seasonal incidence of estrus and interestrous interval for bitches of seven breeds. *J Am Vet Med Assoc*, 171 (3): 271-273, 1977.

23. Erol B, Atasoy F: Reproductive traits, viability and growth of Kangal shepherd dogs. *Ankara Univ Vet Fak Derg,* 57 (1): 55-59, 2010. DOI: 10.1501/Vetfak_0000002310

24. Kırmızı E: Comparison of Turkish Shepherd Dog and German Shepherd Dog in terms of Fertility, Rate of Puppies Raised, Growth, and Body Measurements. *PhD Thesis.* Istanbul University Institute of Health Sciences, 1991.

25. Kalaycı Ş: SPSS Uygulamalı Çok Değişkenli Istatistik Teknikleri. Asil Yayın Dağıtım, Ankara, 2010.

26. Chastant-Maillard S, Marcheteau E, Freyburger L, Fontbonne A, Bergamo P, Ravier JF, Reynaud K: Identification and quantification of immunoglobulins in canine colostrum–Quantification of colostral transfer. **In,** *Proceedings of 7th congress of European Veterinary Society for Small Animal Reproduction*, 14-15 July, Louvain-La-Neuve, Belgium, 2010.

27. Gooding GE, Robinson WF: Maternal antibody, vaccination and reproductive failure in dogs with parvovirus infection. *Aust Vet J*, 59 (6): 170-174, 1982. DOI: 10.1111/j.1751-0813.1982.tb15997.x

28. Tepeli C, Çetin O: Determination of growth, some body measurements and reproductive traits of Kangal Turkish Shepherd Dogs. II. Reproductive traits. *Eurasian J Vet Sci,* 16 (1), 17-25, 2000.

29. Oğrak YZ: Early puppy survival rate in Kangal breed of Turkish shepherd dogs in their homeland province. *J Anim Plant Sci,* 24 (4): 1050-1055, 2014.