



## Investigation of Morphological and Histological Structure of Red-Legged Partridge (*Alectoris Chukar*) Spleen

Adem KARA<sup>1a</sup>, Derviş OZDEMİR<sup>2b</sup>, Hulya BALKAYA<sup>2c</sup>,  
Hulya KARA<sup>2d</sup>, Zekeriya OZUDOGRU<sup>3e</sup>

1. Bingol University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Bingol, TURKEY.

2. Ataturk University, Faculty of Veterinary Medicine, Department of Anatomy, Erzurum, TURKEY.

3. Aksaray University, Faculty of Veterinary Medicine, Department of Anatomy, Aksaray, TURKEY.

ORCID: 0000-0002-5766-6116<sup>a</sup>, 0000-0002-6038-0485<sup>b</sup>, 0000-0001-6164-158X<sup>c</sup>, 0000-0002-7678-6471<sup>d</sup>, 0000-0002-0789 3628<sup>e</sup>

Geliş Tarihi/Received	Kabul Tarihi/Accepted	Yayın Tarihi/Published
21.09.2020	07.12.2020	26.04.2021

**Bu makaleye atıfta bulunmak için/To cite this article:**

**Kara A, Ozdemir D, Balkaya H, Kara H, Ozudogru Z:** Investigation of Morphologic and Histological Structure of Red-Legged Partridge (*Alectoris Chukar*) Spleen. *Atatürk University J. Vet. Sci.*, 16(1): 57-62, 2021. DOI: 10.17094/ataunivbd.797864

**Abstract:** The spleen of birds is located in the right side of the junction between the ventriculus and proventriculus, which is a lymphoid organ and responsible for many functions such as storing, production, and destruction of some blood cells. The aim of this study was to investigate the morphologic and histologic structures of the spleen in red-legged partridge (*Alectoris chukar*). In the present study, eight red-legged partridges were used. The spleens were removed from red-legged partridges and were measured in point of width, length, and weight for morphologic examination. The spleens were fixed into 10% formaldehyde solution for 72 hours. The fixed tissues were dehydrated and cleaned for histologic examinations. Then, these tissues were embedded in paraffin blocks and sectioned in a thickness of 5µm. Obtained sections were stained with Crossman Modified Triple staining and examined for their histologic structure. In the morphologic analysis, the average length of spleens was found as 10.32 ± 0.78 mm, the average width of spleens was found as 6.60 ± 0.50 mm, the average height of spleens was found as 5.55 ± 0.26 mm, and the average weight of spleens was found as 0.26 ± 0.06 g; in histologic analysis, spleen of red-legged partridge was surrounded with a thick capsule, and a few trabeculae were determined. Red and white pulps were scattered in the parenchyma; white pulps consisted of reticular cells, fibers, and lymphocytes. Red pulps consisted of macrophages, lymphocytes, venous sinuses, and reticular cells. In conclusion, morphologic features of the spleen in red-legged partridge were different from other bird species, but histologic structures were similar to chicken and quail spleens.

**Keywords:** Morphology, Red-legged partridge, Spleen.

## Kınlı Keklik (*Alectoris Chukar*) Dalağının Morfolojik ve Histolojik Yapısının İncelenmesi

**Öz:** Dalak; ventriculus ve proventriculus'un birleştiği yerin sağında yer alan, bazı kan hücrelerinin depolanması, üretilmesi ve yıkımı gibi birçok görevi bulunan lenfoid bir organdır. Çalışmada kınlı keklik (*Alectoris chukar*)'lerde dalağın morfolojik ve histolojik özelliklerinin araştırılması amaçlanmıştır. Yapılan bu çalışmada 8 adet kınlı keklik (*Alectoris chukar*) kullanıldı. Kekliklerden diseksiyon ile dalak dokularının morfolojik yönden incelenmek üzere ağırlığı, eni, uzunluğu, yüksekliği ölçülerek %10'luk formaldehit çözeltisinde 72 saat süreyle tespit edildi. Tespit olan bu dokular histolojik incelemeler için dehidratasyon ve şeffaflaştırma işlemlerine tabii tutuldu. Daha sonra parafin bloklara gömülerek 5µm kalınlığında kesitler alındı. Alınan kesitler Crossman'ın modifiye üçlü boyası ile boyandı ve histolojik yapısı incelendi. Morfolojik ölçümlerde ise dalağın boyunun 10.32±0.78 mm, eninin 6.60±0.50 mm, yüksekliğinin 5.55±0.26 mm, ağırlığının 0.26±0.06 gr değerleri arasında olduğu belirlendi. Kınlı keklik dalağının kalın bir kapsül ile sarılmış olduğu gözlenip, içerisinde küçük trabeküllerin bulunduğu belirlendi. Kırmızı pulpa ve beyaz pulpanın paransim içerisinde dağınık halde olduğu, beyaz pulpa'nın retiküler hücreler, retiküler lifler ve lenfositlerden oluştuğu gözlemlendi. Histolojik incelemede, kırmızı pulpa'nın çok sayıda kan damarı, lenfosit, makrofaj, venöz sinüsler ile retiküler hücrelerin anastomozlarından oluştuğu belirlendi. Elde edilen veriler doğrultusunda kınlı keklik dalağının morfolojik yönden diğer kuş türleriyle farklılıklar gösterdiği bununla beraber histolojik olarak ise tavuk ve bıldırcın dalağıyla benzer olduğu gözlemlendi.

**Anahtar Kelimeler:** Dalak, Kınlı keklik, Morfoloji.

<sup>a</sup> Adem Kara

Bingol University, Faculty of Veterinary Medicine, Department of Histology and Embryology, Bingol, TURKEY.

e-mail: ademkara36@gmail.com

## INTRODUCTION

The Red-legged Partridges (*Alectoris chukar*) have a wide natural habitat in Turkey. However, the number of partridges in the natural area has reduced in the last years because of excessive hunting, habitat destruction, and unnecessary use of drugs as pesticides (1,2). Although the economic and ecological importance of these birds has been known, we identify less about bird's morphology.

The immune system consists of the central and peripheral lymphoid organs. These immune system organs can produce and develop lymphocytes. Thymus and cloacal bursa are the central lymphoid organs of birds, which produce, differentiate, and mature T and B lymphocytes, respectively. These lymphoid organs can provide normal immunological defense and resistance against diseases (3,4). The spleen is a principal organ of systemic immunity, and its importance in disease resistance is presumably accentuated by the scarcity of avian lymph nodes (5). Avian spleen tissue is one of the lymphoid organs, composed of the white and red pulp. The white pulp contains of small lymph nodes, and the red pulp consists of diffusely lymphoid cells and blood-filled sinusoids (6).

The spleen is located close to the right side of the junction between the proventriculus and the gizzard. It has pinkish-brown color in the fresh specimen (7). The spleen is the largest peripheral lymphoid organ, and that is the location of the immune response production in most organisms. Also, it has functions in hematogenesis, blood filtration, blood storage, and immune system response. Immunocompetent cells proliferate and differentiate in the spleen following the antigenic stimulation, and there are inter-specific differences in the morphology of the spleen. Among the immune organs of the animals, differences exist in terms of size, morphology, and structure (8,9).

Regarding the immunological point of view, the anatomical and histological structure of the spleen of

the red-legged partridge is essential (10). Although lymphoid organs of chicken (11), ostrich (12), quail (11), and the high-yielding birds (12) have been reported in many previous studies.

There is little information on the relative size of spleen in wild partridges compared with other domestic birds. The morphological, positional, and structural features of the spleen have not been described previously, therefore we aimed to define the anatomical and histological characteristics of the spleen of Red-legged partridge, based on morphometric and histological analysis.

## MATERIALS and METHODS

### Animal Slaughter and Tissue Collecting

A total of eight red-legged partridges (four months old) were obtained from commercial partridge farm in Amasya Province, Turkey. This study is in compliance with the principles of scientific research and publication ethics according to ethical norms approved by the Local Animal Care Committee of Bingöl University Veterinary Faculty (85680299/020). Under the ether anesthesia, all birds were killed by dislocation of the neck and by cutting the jugular vein. Then, the cavum abdominis was opened from the median line of the body, and then the spleen tissues in the abdominal cavity were photographed with a digital camera (Sony, Tokyo). Subsequently, the spleens were removed from the body and were measured the length, width, height, and weight sizes by electronic caliper. Following the anatomically morphometric assessment, the spleen tissues were fixed in 10% formaldehyde solution for 72 hours (13).

### Histologic Analysis

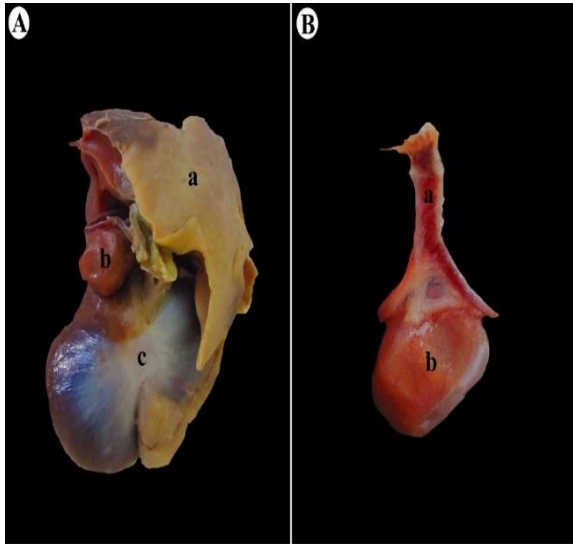
After the fixation period, all spleen tissues were washed under tap water and dehydrated with ascending alcohol series (50%, 70%, 95%, and absolute) and cleared with double xylene solution

series. Then, each spleen tissue was embedded in paraffin, and a 5- $\mu$ m section of each sample was placed on a glass slide and stained with Mallory's Triple stain for examination with a light microscope (Nikon Eclipse i50, Japan).

## RESULTS

### Anatomical and Morphometric Results

The spleens of Red-legged partridge were in dark red color and ellipse-shaped. They were located close to the proventriculus, ventriculus, and liver. It was located on the right side of the gastric isthmus (Figure 1-A, B).



**Figure 1.** Macroscopic views of Red-legged partridge's spleen, **A**; Spleen and its neighbor organs of Red-legged partridge, (a); liver, (b); spleen, (c); ventriculus, **B**; Spleen of the Red-legged partridge; (a); artery splenicae, (b); spleen.

**Şekil 1.** Kınalı keklik dalağının makroskopik görünümü, **A**; Kınalı keklik dalağı ve komşuluğundaki organlar, (a); Karaciğer, (b); Dalak, (c); Ventrikulus, **B**; Kınalı keklik dalağı; (a); Splenik arter, (b); Dalak.

In the morphometric analysis of the spleen, it was found that the average length was  $10.32 \pm 0.78$  mm, the average width was  $6.60 \pm 0.50$  mm, the average height was  $5.55 \pm 0.26$  mm, and the average weight was  $0.260 \pm 0.06$  g (Table 1).

**Table 1.** The morphometric analysis of the spleen of the Red-legged partridge.

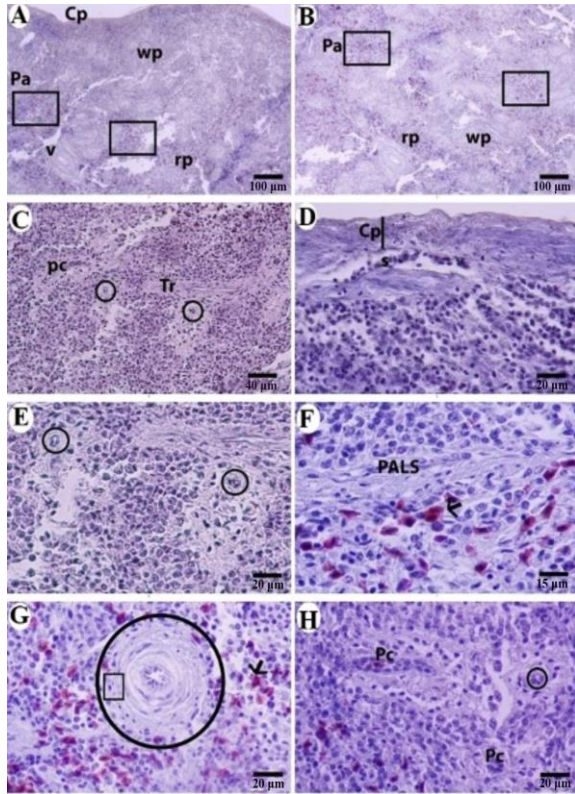
**Tablo 1.** Kınalı keklik dalağının morfometrik analizi.

	Length (mm)	Width (mm)	Height (mm)	Weight (g)
1st Red-legged partridge	11.27	7.53	6.00	0.378
2nd Red-legged partridge	11.51	6.76	5.25	0.321
3rd Red-legged partridge	9.09	6.56	5.75	0.259
4th Red-legged partridge	10.41	6.41	5.55	0.236
5th Red-legged partridge	10.21	6.32	5.35	0.215
6th Red-legged partridge	9.97	6.98	5.46	0.250
7th Red-legged partridge	9.78	5.86	5.72	0.196
8th Red-legged partridge	10.29	6.34	5.28	0.218
Average	10.32	6.60	5.55	0.260
Standard Deviation (SD)	0.78	0.50	0.26	0.060

### Histological Results

The spleen of the Red-legged partridge was covered by a thick splenic capsule. The capsule was extended with a small amount number of trabecula into the parenchyma. The white pulps were more distinct in parenchyma (Figure 2-A, B). The red pulps were less distinct and scattered among the white pulp. The white pulps were involved in reticular fibers and reticular cells, which were covered with sheathed arteries and lymphatic nodules (Figure 2-F). Various sized lymphocytes and plasma cells were diffusely distributed in the white pulps (Figure 2-D, E). The wall of the trabecular artery was moderately distinct and was bordered with numerous muscle layers (Figure 2-C, G). The central arteries were also moderately distinct and were bordered with a single muscle layer (Figure 2-E). The red pulp consisted of macrophages, lymphocytes, venous sinuses, the anastomosing cord of reticular cells, and erythrocytes (Figure F). The penicilliform capillary had a thin basement membrane and cubic epithelial cells were lined in the capillary mucosa. The mesenchymal cells were ellipsoidal reticular cells and

located around the penicilliform capillary (Figure 2- H).



**Figure 2.** Illustration of the Red-legged partridge spleen, Crossman Modified Triple staining, **A, B**; Histological structures of Red-legged partridge spleen at low magnification; (Cp); a thick capsule covers the spleen, and there are few trabeculae, (Pa); parenchyma of spleen, (rp); red pulp, (square); granulocytes area; (v); vessel; (wp); white pulp, **C**; Histological structures of Red-legged partridge spleen at high magnification: (pc); penicilliform capillary, (circles); central artery in white pulp, (Tr); trabeculae. **D, E**; Higher magnification of white pulp: (Cp); capsule of the spleen, (circles); central artery in the white pulp, (R); reticular fibers, (s); sinusoid. **F, G, H**; Higher magnification of spleen histology, (arrow); granulocytes, (circle); mesenchymal cell around, (R); reticular fibers, (RC); reticular cells, (PALS); periarteriolar lymphatic sheath, (pc); penicilliform capillary, (square); trabecular artery.

**Şekil 2.** Kınalı keklik dalağı histolojik görüntüsü, Crossman'ın modifiye üçlü boyası, **A, B**; Kınalı keklik dalağının küçük büyütmedeki histolojik yapısı; (Cp); Dalağı saran ince kapsül ve birkaç trabekül, (Pa); Dalak paransması, (rp); kırmızı pulpa, (kare); granulosit alanları, (v); kan damarı, (wp); beyaz pulpa, **C**; Kınalı keklik dalağının büyük büyütmedeki histolojik yapısı, (pc); penisiliform kılcalları, (Tr); trabekül. **D, E**; Beyaz pulpa büyütülmüş görüntüsü,

(Cp); dalak kapsülü, (çember); beyaz pulpa sentral arteri, (s); sinuzoid. **F, G, H**; Dalağın büyük büyütmedeki histolojik yapısı, (ok); granulosit, (çember); mezensimal hücre, (PALS); periarteriolar lenfatik kılıf, (pc); penisiliform kılcalı, (kare); trabekular arter.

## DISCUSSION and CONCLUSION

The spleen is the major secondary lymphatic organ, which filters the blood and provides the immune responses against diseases, has a major role in the immune system of the organism. Also, the spleen undertakes erythropoiesis in the fetal period of life (8,9).

The spleen of birds has differences in the size and number among domestic animals (14-16). In the anatomic analysis of the present study, the spleen of Red-legged partridges was dark red and elliptical. It was located close to the proventriculus, ventriculus, and liver on the right side of the gastric isthmus. Besides, Akter et al. (17) report that the spleen is a rounded and reddish-brown organ that lies close to the right side of the junction between the proventriculus and gizzard in chicken (8). Song et al. (9) report that the spleen is dark red and elliptical and wedged against the right kidney, the posterior of the caudal vein, and the proventriculus in the ostrich (5).

In the morphometric analysis, the average length was  $10.32 \pm 0.50$  mm, the average width was  $6.60 \pm 0.50$  mm, the average height was  $5.55 \pm 0.26$  mm, and the average weight was  $0.260 \pm 0.06$  g in the spleen of Red-legged partridges. The ostrich spleen was found about 4.8 cm in length and 1.5 cm in diameter, and  $2.378 \pm 0.0620$  g in weight (9). Also, Liman and Bayram (8) suggested that the weight of the quail spleen was about  $138.125 \pm 30.073$  g in post-hatching 60<sup>th</sup> day. The spleen weight was about  $1.974 \pm 0.0497$  g, and the length was about  $18.93 \pm 0.394$  mm in 28 days old chicken (11). The morphometric differences of spleen between the bird species can be related to body weight and individual variation in the feeding and live habitats.

The results of the study revealed that the red pulps of the spleen were less district and white pulps of the spleen were involved reticular fibers and reticular cells and covered with sheathed arteries

and lymphatic nodules in the red legged partridges. Similar to our study, the same similar histologic structure is reported in indigenous ducklings of Bangladesh (18), in ostrich (5), in post-hatching quails (4), and chicken (19).

The mesenchymal cell around the penicilliform capillary known as Schweigger-Seidel sheath is well-developed ellipsoids in avian species. In the present study, the ellipsoids observed in the red-legged partridge's spleen located around the penicilliform capillary. Similar to chicken and guinea fowl, the Schweigger-Seidel sheath covered with two similar cell layers were reported (20,21).

In conclusion, although the morphologic features of the spleen in the Red-legged partridge were different from other bird species, histologic structures were similar to chicken, ostrich, and quail spleens.

#### Conflict of interest

The authors declare that they have no conflict of interest.

#### REFERENCES

1. Yamak US., 2015. Artificial breeding of wild birds in Turkey: Partridge breeding case. *Indian J Anim Res*, 49, 258-261.
2. Karabag K., Alkan S., Mendes M., 2010. Classification tree method for determining factors that affecting hatchability in chukar partridge (*Alectoris chukar*) eggs. *Kafkas Univ Vet Fak Derg*, 16, 723-727.
3. Nasu T., Shimizu K., Nakai M., 1992. Morphological study of the dove spleen. *Poultry Sci*, 71, 1527-1530.
4. Rautenfeld V., Berens D., Budras KD., 1982. The bursa cloacae (Fabricii) of struthioniforms in comparison with the bursa of other birds. *J Morphol*, 172, 123-138.
5. Kozlu T., Sari EK., Bozkurt YA., Kurtdede N., 2019. Immunohistochemical staining of cd3, cd79 $\alpha$ c and s-100 on bursa fabricius, thymus and spleen of turkeys (*Meleagris gallapavo*). *Indian J Anim Res*, 53, 1450-1454.
6. Powers LV., 2000. The Avian Spleen: Anatomy, Physiology and Diagnostics Compendium on Continuing Education for the Practising. *Veterinarian-North American Edition*. 22, 838-843.
7. Islam MN., Khan MZI., Jahan MR., 2017. Shinoda K., Developmental trajectory of the prenatal lymphoid organs in native chickens: a macro anatomical study. *Asian J Med Biol Res*, 3, 432-436.
8. Liman N., Bayram GK., 2011. Structure of the quail (*Coturnix coturnix japonica*) spleen during pre-and post-hatching periods. *Rev Med Vet*, 162, 25-33.
9. Song H., Peng KSM., Li SH., Wang Y., Wei L., Tang L., 2012. Morphological characterization of the immune organs in ostrich chicks. *Turk J Vet Anim Sci*, 36, 89-100.
10. Özüdoğru Z., Balkaya H., Kara H., Özdemir D., 2019. Morphologic and Histologic Observation of Red-Legged Partridge's (*Alectoris Chukar*) Liver. *Van Vet J*, 30, 159-161.
11. Rahman ML., Islam MR., Asaduzzaman M., Khan MZI., 2003. Lymphoid tissues in the digestive tract of Deshi Chicken (*Gallus domesticus*) in Bangladesh. *Pak J Biol Sci*, 6, 1145-1150.
12. Bejdic P., Avdic R., Amidzic L., Cutahija V., Tandir F., Hadziomerovic N., Mlaco N., 2018. Ultrastructure of plasma cells in harderian gland of laying hen. *Anat Histol Embryol*, 47, 46-50.
13. Matsuda Y., Fujii T., Suzuki T., Yamahatsu K., Kawahara K., Teduka K., Kawamoto Y., Yamamoto T., Ishiwata T., Naito Z., 2011. Comparison of fixation methods for preservation of morphology, RNAs, and proteins from paraffin-embedded human cancer cell-implanted mouse models. *J Histochem Cytochem*. 59, 68-75.
14. Millan J., Gortazar C., Villafuerte R., 2001. Marked differences in the splanchnometry of farm-bred and wild red-legged partridges (*Alectoris rufa* L.). *Poultry Sci*, 80, 972-975.
15. Kwak H., Austin RE., Dietert RR., 1999. Influence

- of dietary arginine concentration on lymphoid organ growth in chickens. *Poultry Sci*, 78, 1536-1541.
16. Puvadolpirod S., Thaxton JP., 2000. Model of physiological stress in chickens 3. Temporal patterns of response. *Poultry Sci*, 79, 377-382.
17. Akter S., Khan MZI., Jahan MR., Karim MR., Islam MR., 2006. Histomorphological study of the lymphoid tissues of broiler chickens. *Bangladesh J Vet Med*, 4, 87-92.
18. Sultana N., Khan MZI., Wares MA., Masum MA., 2012. Histomorphological study of the major lymphoid tissues in indigenous ducklings of Bangladesh. *Bangladesh J Vet Med*, 9, 53-58.
19. Khan MZI., Masum M., Khan MZI., Aziz AR., Nasrin M., Siddique MNH., 2014. Histomorphology of the lymphoid tissues of broiler chickens in Kelantan, Malaysia (Histomorfologi Tisu Limfa Ayam Pedaging di Kelantan, Malaysia). *Sains Malays*, 43, 1175-1179.
20. Mast J., Goddeeris BM., 1997. CD57, a marker for B-cell activation and splenic ellipsoid-associated reticular cells of the chicken. *Cell Tissue Res*, 291, 107-115.
21. Olah I., Kupper A., Ducz P., 1993. Schweigger-Seidel sheath or ellipsoid in the spleen of guinea hen. *Acta Biol Hung*, 45, 375-386.