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A stereological study of the renal and adrenal glandular structure of red-legged partridge (*Alectoris chukar*)

Renal, adrenal gland of *Alectoris chukar*

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Abstract

Background: Partridge is a major bird species that has recently gained an increasing importance as an alternative food source and a game animal in hunting tourism. Herein, we aimed to examine the histological structure and stereological properties of the renal and adrenal glands of red-legged partridge.

Materials and methods: It was used 7 healthy adult red-legged partridges (*Alectoris chukar*) of both genders in this study. The *Alectoris chukars* were perfused with the intracardiac method and were kept in 10% formaldehyde for 72 h for optimal fixation. The tissues were embedded in paraffin after routine light microscopic processes. Then, 5- μ m thick sections were taken, which were stained with Hematoxylin & Eosin, photographed, and examined in light microscope. Modified Cavalieri principle was used for volume calculation as stereological analysis. Total tissue volume ratios were calculated with the help of a point grid provided by the Shtereom 1.5 packaged program. Mann Whitney-U test was used for inter-group comparisons. The significance level was set at 5% SPSS (ver: 13) statistical software was used for all statistical analyses.

Results: Renal histological structure was found to resemble to those of other bird species and contained a mixed type (mammalian, reptilian) glomerular structure. It was also found that the adrenal histology was not in the form of separate layers but composed of cell groups with different properties. Stereological renal volume assessment revealed statistically similar right and left renal size ($p>0.05$). In adrenal gland volume assessment, the right and left adrenal volumes were also statistically similar ($p>0.05$).

Conclusions: The obtained data in the present study is thought contribute to the understand of the stereological, morphological and histological structure of the red-legged partridge (*Alectoris chukar*) kidney and adrenal gland.

Key words: adrenal gland, kidney, partridge, stereology

INTRODUCTION

The partridge is the common name for belonging to the *Alectoris* and *Perdix* species of the Phasianidae family [1]. The partridge is a major bird species that has recently gained an increasing importance as an alternative food source and a game animal in hunting tourism.

The kidneys are located on both sides of the abdominal aorta and inferior vena cava in the dorsal part of the abdominal cavity, and the adrenal glands are located on the cranial pole of both kidneys [2]. The kidneys of birds species has an intermediate structure between mammals and reptilians. The bird species do not possess a renal pelvis, urinary bladder, or urethra [3]. Histologically, their kidneys have a multilobulated pattern and isolated cortex and medulla which are separated by a fine connective tissue. They have two types of nephron structures. The first one is designated as the mammalian or medullary type and is composed of a renal corpuscle, a proximal convoluted tubule, a distal convoluted tubule, a collecting tubule, and thin and thick segments of Henle's loop. The second type is the reptilian type also called as the cortical type, which has a smaller renal corpuscle but no Henle's loop; additionally, there is a short section between proximal convoluted tubule and distal convoluted tubule, which is named the intermediate tubule [4]. Adrenal glands are anatomic structures that play an important role for accommodation to all types of stress and, by hormones they produce, they contribute to many metabolic processes. In bird species the shape of the adrenal glands is highly variable, with inter-species and even inter-individual

variations being possible. Experimental removal of adrenal glands results in death of birds [2]. It was showed in a review of the literature that there have been anatomic studies on red-legged partridge, but not specifically on its the renal and the adrenal gland structure. It was aimed to perform a detailed histological and stereological examination study of red-legged partridge's renal and adrenal glandular structures in this study.

MATERIALS AND METHODS

Seven healthy adult red-legged partridges (*Alectoris chukar*) of (*Alectoris chukar*) of the both genders in this study. The animals were obtained from Van Yuzuncu Yil University Wildlife Protection Center. It was approved by Van Yuzuncu Yil University Local Ethics Committee (27/06/2019/ 06) complied with the study procedures. The partridges were anesthetized with 10 mg/kg Xylazine HCL (Rompun %2, 50 ml, Bayer) and with 40 mg/kg i.m. Ketamine HCL (Ketalar 50 mg/ml, 10 ml vial, Pfizer). The animals were perfused with intracardiac method and dissected tissues were kept in 10% formaldehyde for 72 h for optimal fixation [5]. The tissues were embedded in paraffin after completion of routine histological light microscopy procedures. Then, 5- μ m thick sections were obtained. The first section was randomly selected and then the 40th section was taken. The sections were stained with Hematoxylin & Eosin, photographed, and examined in light microscope.

Stereological analysis

Stereology, random and systematic sampling is utilized to gain unbiased and quantitative information. Among stereological analyses, modified Cavalieri principle was used for volume calculation. Then, total tissue volume ratios were calculated using a point grid provided by the Shtereom 1.5 packaged program (Fig. 1). Volume calculation was carried out using the following formula:

$$V(\text{obj}) = t \times a/p \times \Sigma p$$

$V(\text{obj})$ is the volume of the objective, t is the section thickness, a/p represents the area of each point on the point counting grid and Σp is the total number of points hitting the renal

and adrenal areas [6, 7]. CE and CV were used to determine the optimum sample size in each group in the standard stereological approach [8]. Our study's the coefficient of error (CE) and Coefficient of variation (CV) values of this study were in accordance with the literature data.

Statistical analysis

Descriptive statistics included median, mean, standard deviation, minimum and maximum. Inter-group differences were analyzed with Mann Whitney-U test. The significance level was set at 5%. All statistical analyses were performed with SPSS (ver.13) statistical software package program.

RESULTS

The stereological analysis revealed that the left and right kidneys had similar sizes. A stereological volume calculation showed a right renal volume of 2.28 mm³ and a left kidney volume of 3.02 mm³. Comparison of both renal volumes revealed a slight but statistically non-significant difference between both kidneys ($p > 0.05$) (Table 1). The microscopic examination of the histological sections demonstrated that the kidney was composed of lobules, each being composed of the cortical and medullary parts and encapsulated by connective tissue containing blood vessels. The renal structure was composed of 60% cortex and 40% medulla on the right side and 80% cortex and 20% medulla on the left kidney. The nephrons had structure two type of nephrons that the reptile and mammalian nephrons (Fig. 2). The number of reptilian nephrons was larger than the mammalian nephrons. The two nephron types also had different distributions so that they were more numerous in the cortex but there were only tubal structures in the medulla. The mammalian type nephrons were located in deep cortex, close to the medullary cone, whereas the reptilian nephrons were located in the peripheral and middle parts of the cortex, aligned in horseshoe shape around the central vein (Fig. 3).

Examination of the adrenal glandular structure showed that the left and right adrenal glands had some differences in shape but not in size, and a stereological volume assessment revealed that the volume of both adrenal glands was 0.02 mm³ ($p > 0.05$) (Table 1). A general

histological the examination showed that the adrenal gland had no three distinct different layers but only cellular differentiation (dark, light colored; having different nuclear structures); the glands were encapsulated by a connective tissue containing blood vessels capsule separating them from the kidneys. The adrenal gland had a separate vascular supply. (Fig. 4).

DISCUSSION

It was showed that the renal structure of red-legged partridge was similar to those of other bird species in this study. Red-legged partridge had two kidneys encapsulated by connective tissue containing blood vessels sheaths and composed of multiple lobules containing the cortical and medullar parts. The stereological volume assessment has shown that the right and left renal size were similar statistically. Previous studies on the right and left renal size of bird species have revealed variable results. As a result of a study reported that there were no difference between the right and left renal size [9] whereas another study reported difference between the left and right renal size [10]. Similarly, studies assessing the cortical and medullary volumes of bird species have reported variable results. A study has reported that 90% of the total volume of bird kidney was composed of the cortex and 2% of the medulla [11]; another has reported that corresponding figures of 77% and 10%, respectively [12]. It was founded that the cortical ratio similar to the literature data, but the medullary ratio was slightly higher in this study. Casotti & Braun [13] have reported that renal volume varied by the living environment of the bird species. Literature data suggest that a bird's renal shape, size, and structure change by a its diet, phylogeny, and environmental factors [10]. All animals were in the same region and climatic conditions (maximum temperature 30-33 ° C). The basic renal structure is composed of nephrons. The bird kidney is different from the mammalian one because it is composed of a 2 types of nephrons resembling mammalian and reptilian nephrons [14,15]. The present study similarly was observed two types of nephrons. Prior studies have shown that more numerous reptile nephrons are located in the periphery of the lobule, a small renal corpuscle, and no loop of Henle. It has been reported that the mammalian nephrons are larger and had a more complex renal corpuscle and are located deep in the cortex neighboring the medulla; additionally, they contain a loop of Henle, and had a smaller number [16]. Herein, it was showed that a similar number and distribution of nephrons. The nephrons are responsible for filtration, reabsorption,

and secretion, thereby forming urine. The birds nephrons are composed of a glomerulus, a proximal tubule, a distal tubule, and collecting ducts [3]. This study also has shown revealed similar results.

The present study demonstrated that the adrenal glands were located at the cranial pole of the kidneys and medial to them, as Nickel et al. [17] has reported. It was found the adrenal histology was not in the form of separate layers but composed of cell groups with different properties (having a dark or light color and varying nuclear structure). It was also shown that the right and left adrenal glands showed the difference in shape and there were also inter-individual differences. The adrenal gland was separated from the kidney tissue with a connective tissue sheath and had a separate vascular supply. The result of this study are in accordance with the previous studies on birds species [2]. Among the studies has performed on adrenal gland size, Humayun et al. [18] and Wells and Wight [19] has reported that the left adrenal gland was larger than the right one in chickens of both genders whereas Ozudogru et al. [2] as well as Elbajory [20] has reported that the right adrenal gland was larger than the left one. The weight, length, width, and thickness of the adrenal glands vary by a number of factors, such as species, breed, age, health status, and environment [18]. In this study it was detected that the right and left adrenal glands have no different values about volume calculation stereologically.

CONCLUSIONS

The obtained data in the present study is thought contribute to the understand of the stereological, morphological and histological structure of the red-legged partridge (*Alectoris chukar*) kidney and adrenal gland. We think that it will be a guide for the studies about red-legged partridge (*Alectoris chukar*).

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TABLE 1 Descriptive statistics and comparison results

| | Right | | | | | Left | | | | | p |
|--|------------|------|-----------------|------|------|--------|----------|-----------------|------|-------|-------|
| | Media n | Mean | St. Dev . | Min. | Max. | Median | Mea n | St. Dev . | Min. | Max. | |
| Kidney tot.vol. (mm³) | 2,28 | 3,34 | 2,18 | 1,61 | 6,88 | 3,02 | 2,61 | 0,85 | 1,52 | 3,80 | 0,499 |
| Cortex vol. (mm³) | 1,55 | 2,02 | 1,14 | 1,03 | 3,94 | 2,43 | 2,09 | 0,88 | 0,70 | 3,08 | 0,735 |
| Kidney tot.vol./Cortex vol.(mm³) | 1,67 | 1,60 | 0,27 | 1,22 | 1,91 | 1,24 | 1,36 | 0,38 | 1,06 | 2,17 | 0,310 |
| Medulla vol. (mm³) | 0,91 | 1,32 | 1,08 | 0,30 | 2,94 | 0,59 | ,53 | 0,24 | 0,18 | 0,82 | 0,091 |
| Kidney tot.vol /Medulla vol. (mm³) | 2,50 | 3,24 | 1,53 | 2,10 | 5,52 | 5,14 | 6,60 | 4,93 | 1,85 | 16,74 | 0,176 |
| Cortex vol. /Medulla vol. | 1,50 | 2,24 | 1,53 | 1,10 | 4,52 | 4,14 | 5,60 | 4,93 | 0,85 | 15,74 | 0,259 |
| Adrenal gland tot. vol. (mm³) | 0,02 | 0,02 | 0,01 | 0,01 | 0,03 | 0,02 | 0,02 | 0,01 | 0,02 | 0,03 | 0,499 |

Figure 1 Transverse section of kidney photographed (x4 objective) and a point counting grid superposed onto it, for the volume calculation.

Figure 2. a. Hematoxylin and eosin (H&E) staining. Kidney nephron structure. The reptilian type nephrons (black arrows). Scale bar = 50 μm ; **b.** H&E staining. The mammalian type nephron. Scale bar = 50 μm

Figure 3. a. Hematoxylin and eosin (H&E) staining. The general appearances, lobular structure and vascularization of kidney. Scale bars = 500 μm **b.** H&E staining. The histological structure of kidney. Medulla (white asterisk), cortex (black asterisk), horseshoe-shaped reptile glomerular structure (black arrow). Scale bars = 500 μm

Figure 4. a. Hematoxylin and eosin (H&E) staining. The general appearance of the adrenal gland (arrow). Scale bar = 500 μm ; **b.** H&E staining. The adrenal gland structure and vascularization. Scale bar=100 μm .







