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Histomorphological Study of the Spleen in the Iraqi Pin-Tailed Sandgrouse (*Pterocles Alchata*)

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ABSTRACT: This study was conducted with the Iraqi Pin-tailed Sandgrouse (*Pterocles alchata*). For this purpose, five adult birds weighing between 195–250 g were dissected and the spleen was removed. The organs were placed in 0.9% normal saline and fixed in 10% formalin and then preserved in 70% alcohol. Histological sections were prepared using the paraffin wax method, which involves several steps: Dehydration, clarification and embedding. The present results show that the spleen is surrounded by a capsule of connective tissue containing collagen fibres and smooth muscle. A series of connective tissue trabeculae extend from this capsule and penetrate the parenchyma of the spleen. The parenchyma consists of two main regions: the white pulp and the red pulp, which are randomly distributed due to the absence of a marginal zone between them. The white pulp is composed of central arteries, sheathed arteries and lymphatic tissue represented by the peri-arterial lymphatic sheath (PALS), the peri-ellipsoid lymphatic sheath (PELS) and lymphatic nodules of two types - spherical and ovoid. The red pulp is formed by venous sinusoids and splenic cords. Plasma cells, lymphocytes and macrophages were also observed.

KEYWORDS*:* Ellipsoid; PALS; PELS; Red pulp; White pulp.

INTRODUCTION

The Iraqi Pin-tailed Sandgrouse (*Pterocles alchata*) is the most common Sandgrouse species in Iraq. However, its population density decreases in winter as it tends to migrate to desert habitats to avoid rainfall, as this bird is very well adapted to dry environments. Taxonomically, it belongs to the order Pteroclidiformes, family Pteroclididae, species *Pterocles alchata* (Allouse,1962). Morphologically, Sandgrouse are slightly larger than pigeons. They have a compact body, relatively long and pronounced beaks and plumage that mimics the colours of their desert environment and thus camouflages them. The sexual dimorphism is obvious, as the males differ in appearance from the females. Their diet consists mainly of small seeds and grains, occasionally also green plants. They do not nest in trees, but lay their eggs directly on the ground. Normally the female does not lay more than three eggs at a time, hence the local name “Um Thalath” (mother of three). Breeding activity mainly takes place in spring and autumn, during which the incubation period and chick rearing takes place (Grzimek and McDade,2005)

The lymphatic system of birds plays a fundamental role in immune defence and protection against pathogenic microorganisms (Bach,1978;Getty,1975). Compared to mammals, the avian lymphatic system is characterised by a compact structure and less polymorphism and has certain anatomical peculiarities. In particular, the lymphoid nodule are absent in birds and are replaced by the bursa Fabricius, which only exists in birds (Lowenthal *et al*., 2013 ; Tizard, 1979). Functionally, the lymphatic system consists of central (primary) organs, namely the thymus, which is responsible for the synthesis and maturation of T lymphocytes, and the bursa of Fabricius, in which B lymphocytes develop and mature. Peripheral (secondary) lymphoid tissues include the spleen and the mucosa-associated lymphoid tissue (MALT), which is distributed along the respiratory, digestive and urinary tracts (Aguanta *et al*., 2018; Sultana *et al*., 2011).

Among these, the spleen is considered the largest and most important lymphoid organ in birds, especially due to the absence of lymphoid nodule (Zhang *et al*., 2015; Tiron and Vasilescu, 2008; Mebius and Kraal,2005). Although smaller in size compared to its mammalian counterpart, the avian spleen has similar immunological functions, with differences in the extent of immune responses (Jhon, 1994). Anatomically, it is located dorsal to the gonads, ventral to the liver and adjacent to the dorsal surface of the right lobe of the liver, close to the stomach (Scanes and Dridi,2021) Functionally, the spleen serves as the primary site of lymphocyte production and differentiation (Scanes ,2020) filtration of blood (Jhon, 1994). and phagocytosis of senescent or damaged erythrocytes, storing iron in the form of ferritin (Powers,2000). In addition, the spleen functions as a haematopoietic organ during embryonic development (Nicolas-Bolnet and is an important site for innate and adaptive immune responses as it is responsible for antibody production (Brendolan *et al*., 2007; Gumati *et al*., 2003)

Histologically, the spleen is enclosed by a connective tissue capsule from which trabeculae extend into the parenchyma (Kannan *et al*., 2015) The splenic parenchyma consists of two different regions: the white pulp and the red pulp. The white pulp consists of lymphoid tissue, including lymphoid progenitor cells, lymphocytes, reticular cells and Schweigger-Seidel sheaths. The red pulp, on the other hand, consists of venous sinusoids and splenic cords and contains plasma cells, mast cells and granulocytes (Sun *et al*., 2019 ; Hoffmann-Fezer *et al*., 1977).

MATERIALS AND METHODS

Five male Iraqi pintail chickens (Pterocles alchata) aged approximately 6–8 weeks and weighing between 195–250 g were used for histological examination of the spleen. The spleens were removed after anaesthesia and dissection of the birds through the ventral approach. Ethical standards for the use of laboratory animals were strictly adhered to and an official authorisation form confirming compliance with animal welfare regulations was included. Samples were placed in 0.9% normal saline and then fixed in 10% formalin for 48 hours. They were then washed under running water for 10 minutes and preserved in 70% ethanol (Coles *et al*., 2007). The histological sections of the spleen were prepared using the paraffin embedding technique, which involves a series of sequential steps. First, dehydration was performed by soaking the samples in ethanol of increasing concentration (70–100%) for one hour. Subsequently, the samples were cleaned with xylene for 15 minutes and then embedded in paraffin wax. The tissue blocks were cut into 7 µm thick sections using a rotary microtome. The sections were stained with haematoxylin and eosin (H\&E) (Bancroft *et al*., 2012). Microscopic examination was performed using a light microscope, and histological images were captured using a Wi-Fi digital camera connected to Image View software. This software provides advanced imaging and editing capabilities, including a negative mode that inverts pixel values to simulate effects similar to those of high-resolution microscopy.

RESULTS

Histological examination of the spleen of the Pin-tailed Sandgrouse revealed that the organ is surrounded by a single-layered capsule consisting of collagenous fibres interspersed with smooth muscle fibres. The outer surface is covered by an epithelial layer, the mesothelium. A series of trabeculae, consisting of collagen fibres and connective tissue, extend from the inner surface of the capsule and penetrate and run through the spleen parenchyma.

The parenchyma of the spleen is divided into two main regions: the white pulp and the red pulp, which are irregularly distributed due to the lack of a distinct marginal zone between them. The current findings show that the white pulp consists of medium and small sized arteries together with lymphatic tissue. The trabeculae give rise to 2–3 central arteries characterised by relatively wide lumina and a thicker tunica media of smooth muscle fibres. These arteries are surrounded by connective tissue, while the central artery itself is enveloped by lymphatic tissue known as the periarterial lymphatic sheath (PALS). Small arterioles branch off from the central artery. These vessels have narrow lumina, lack an outer tunic and are instead surrounded by lymphoid cells, while their endothelial lining comes into direct contact with reticular cells. At this stage, such vessels are referred to as sheathed arterioles or ellipsoid capillaries.

The presence of structures known as Schweigger-Seidel envelopes (or ellipsoids) was observed in the white pulp. These appear as rosette-like formations consisting of one or two layers of rounded cells surrounding the terminal branches of the arterial system, known as penicillary arterioles. This structure is surrounded by lymphatic tissue that forms the so- called periellipsoid lymphatic sheath (PELS). The lymphatic tissue of the white pulp appeared in two forms: either as a sheath surrounding the blood vessels (PALS and PELS) or as lymphatic nodules. Two types of nodules were distinguished: (1) spherical lymphoid nodules surrounded by a connective tissue capsule with a central artery at its edge, and (2) ovoid lymphoid nodules, which are less organized and surrounded by a thin capsule without blood vessels at their edge.

Histological sections also showed that the red pulp consists of venous sinusoids and splenic cords. The venous sinusoids were located near trabeculae and larger arteries, but were absent in the intermediate areas between the white and red pulp. These sinusoids appeared as irregularly shaped vascular channels of varying diameters lined by endothelial cells, and their lumina contained erythrocytes and occasionally mature blood cells. Apart from these sinusoids, no other vascular structures were observed in the red pulp. The splenic cords consisted of cellular aggregates occupying the splenic parenchyma, composed primarily of lymphocytes, reticular cells, and macrophages.

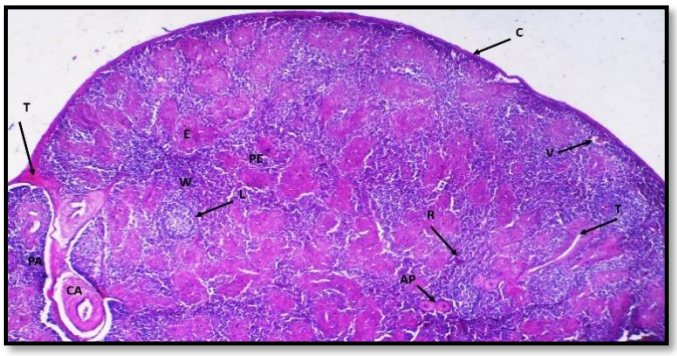

Figure1: Microscopic image of the spleen in Iraqi pin tailed sandgrouse showing: AP (Arteria Penicillata), C (Capsule), CA (Central Artery), E (Ellipsoid), L(Lymph nodule), PA (Peri-Arterial Lymphoid Sheath), PE (Peri ellipsoid Lymphoid sheath), R (Red Pulp), T(Trabeculae), V (Venous Sinusoid), W(White Pulp); H&E,4X.

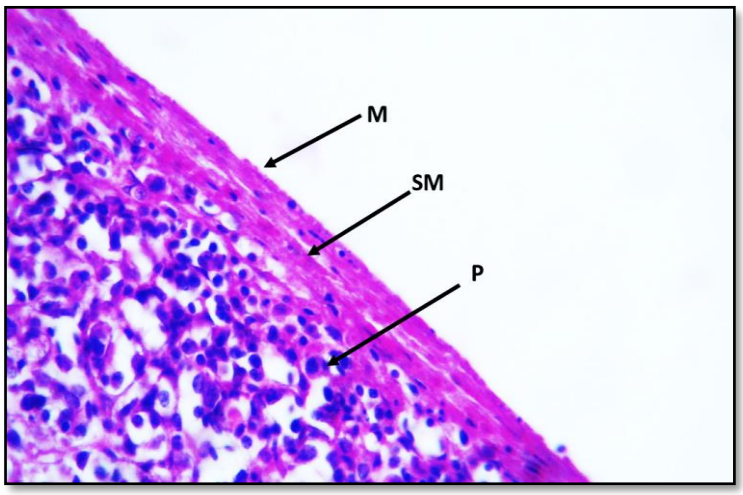

Figure.2: Microscopic image of the spleen in Iraqi pin tailed sandgrouse showing capsule: M (Mesothelium), SM (Smooth Muscles), P (Plasma cell); H&E,40X.

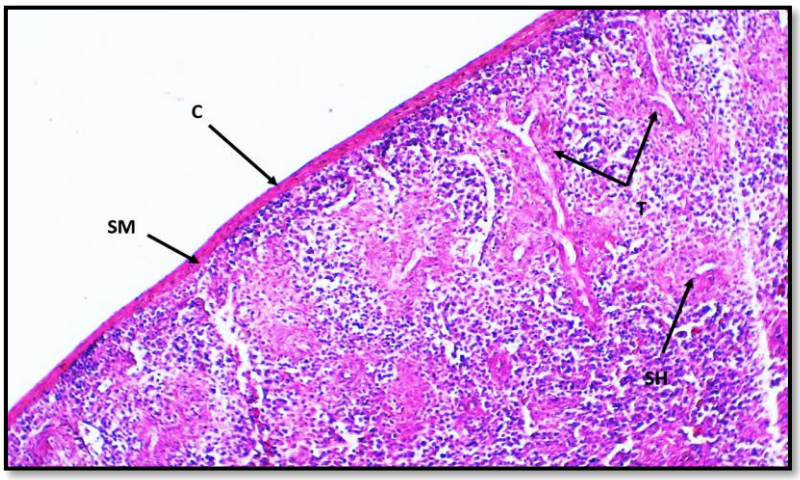

Figure 3: Microscopic image of the spleen in Iraqi pin tailed sandgrouse showing: C (Capsule), SH (Sheathed Capillary), SM (Smooth Muscles), T (Trabeculae); H&E,10x.

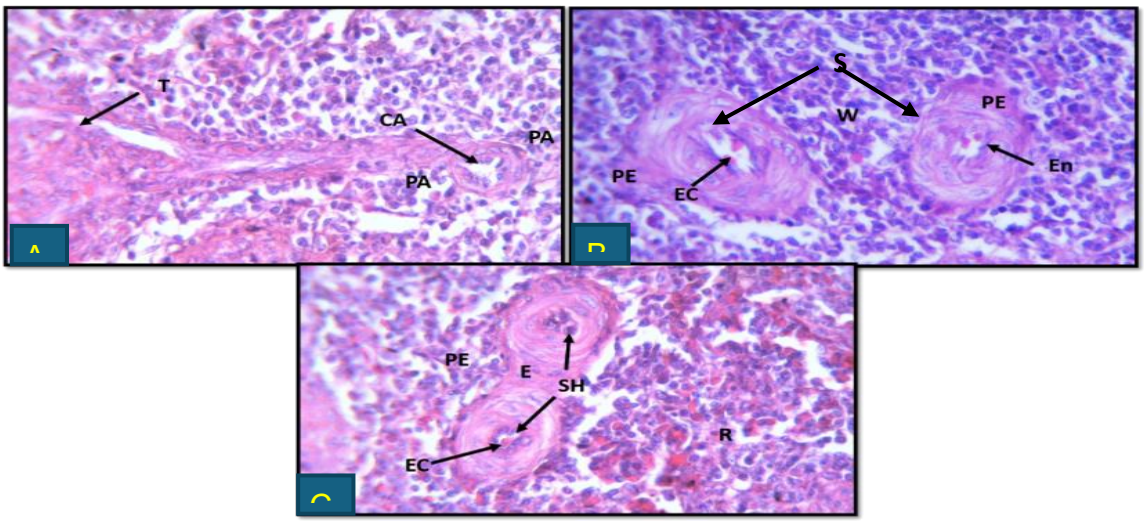

Figure 4: Microscopic image of the spleen in Iraqi pin tailed sandgrouse showing the White Pulp structure: (A) showing: CA (Central Artery), PA (Peri-Arterial Lymphoid Sheath), T (Trabeculae); (B) showing: EC (Erythrocyte), En(Endothelium),PE(Peri- Ellipsoid Lymphoid Sheath), SH (Sheathed Capillaries), W (White Pulp); (C)showing: E (Ellipsoids), EC(Erythrocyte), PE (Peri-Ellipsoid Lymphoid Sheath), SH (Sheathed Capillaries), R (Red Pulp); A&B&C: H&E,40X

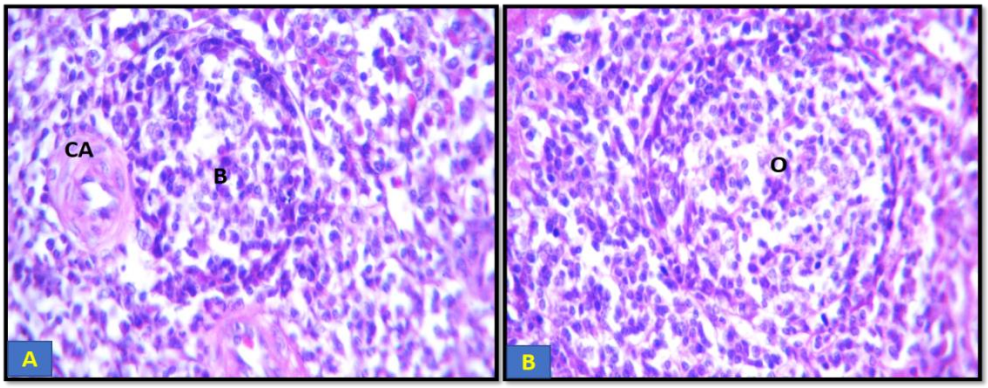

Figure 5: Microscopic image of the spleen in Iraqi pin tailed sandgrouse showing the Lymphatic tissue of the White pulp: (A) showing: B (bulbous lymph nodule), CA (Central Artery); (B) showing: O (Oval Lymph nodule) ; A&B: H&E,40X.

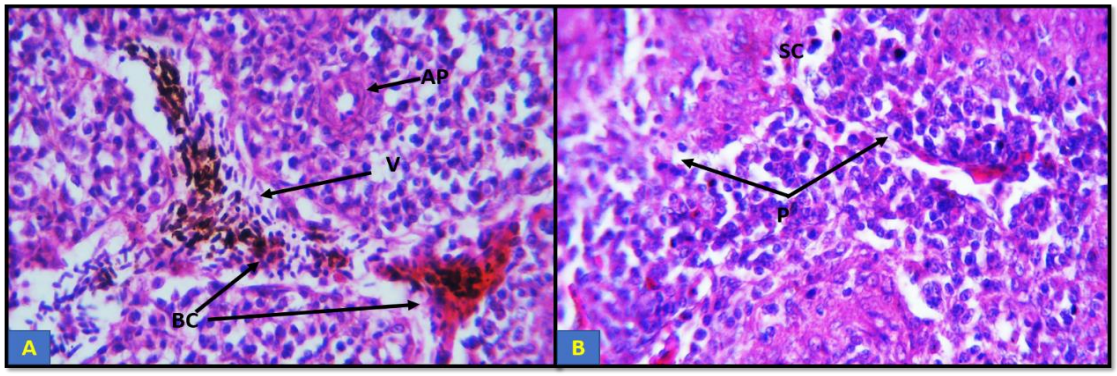

Figure 6: Microscopic image of the spleen in Iraqi pin tailed sandgrouse showing the Red Pulp structure: (A) showing: Ap (Arteria Penicillata), BC (Blood Cells), V (Venous sinusoid); (B) showing: P (Plasma Cells), SC (Splenic Cord); A&B:H&E,40X.

DISCUSSION

The present histological findings in the spleen of the Iraqi Pin-tailed Sandgrouse demonstrated that the organ is surrounded by a capsule consisting of a single layer, which is consistent with the observations of Mohammed *et al*. (2020) in African quail and

(Reshag and Hamza, 2017; Khan *et al*.,2014) in chickens. However, these results contradict those of (Kozlu *et al*., 2011), who reported that the spleen of falcon and ostrich is surrounded by a capsule consisting of two layers: an outer layer of smooth muscle fibers and an inner layer of collagen fibers. In the ostrich, however, the arrangement was reversed, with smooth muscle fibers forming the inner layer and collagen fibers forming the outer layer.

The current study also showed the presence of trabeculae, which consist of connective tissue and extend into the splenic parenchyma. These findings are in agreement with those of (Ahmed and Sarma ,2023; Mohammed *et al*. 2020; Sultana *et al*., 2011; Hamza and Balash. 2005) who reported trabeculae originating from the capsule and composed of collagen and reticular fibers. In contrast, Kozlu *et al*., (2011) found that no trabeculae were present in the falcon spleen.

Examination of the splenic parenchyma showed two main regions, the white pulp and the red pulp, between which there is no clear boundary due to the absence of a marginal zone. This agrees with the findings of (Mohammed *et al*. 2020; Reshag and Hamza, 2017; Sultana *et al*., 2011) in Galliformes and (Kozlu *et al*., 2011) in ostriches, eagles and falcons. Similarly, Zhang *et al*., 2016 reported comparable observations in turtles, which emphasised the absence of a marginal zone and thus indicated an evolutionary relationship between reptiles and birds. However, the present results contradict those of (Aldabagh and Nuzuz,1963) , who found that the spleen of the Scottish red grouse has a distinct marginal zone between the white and red pulp, consisting of small lymphocytes and a transition zone that extends into the red pulp.

The current study has shown that the white pulp is composed of small and medium-sized arteries, including central and sheathed arteries, together with lymphoid tissue that may be diffusely organized or in lymphoid nodules. These findings are similar to those reported by (Kozlu *et al*., 2011; Sultana *et al*., 2011; King and McClelland ,1984). In addition, the presence of Schwigger-Seidel sheaths (ellipsoids) was noted, which appear as rosette-like structures of round or oval cells surrounding the terminal branches of the penicillar arteries. Similar structures were described by Biro *et al*.(2011) in chickens and by(Hamza and Balash,2005) in quails, while (Olah and Glick,1982) observed these sheaths in the red pulp. These differences could be related to the absence of a marginal zone and the irregular distribution of the two pulp types. According to (Zhang *et al*.,2015, Bao *et al*., 2009)these sheaths are vascular structures found in reptiles, birds and some mammals.

Microscopic evaluation also confirmed that the white pulp contains lymphoid tissue in three forms: peri-arterial lymphoid sheaths (PALS), peri-ellipsoid lymphoid sheaths (PELS), and lymphoid nodules, which are both spherical and ovoid in shape. These observations are consistent with those of Mohammed *et al*. (2020) in quails, (Reshag and Hamza, 2017) in chickens and (Sugimura and Hashimoto, 1980) in ducks, all of which reported a central artery surrounded by small lymphocytes and reticular cells. The current findings are also in agreement with (Reshag and Hamza, 2017 ; Jeurissen, 1991) , who noted lymphoid tissue around the sheathed arterioles and with Khan *et al*., 1996, who described PELS as consisting of 2–3 layers of large round cells intermingled with collagen fibres and lymphocytes.

The occurrence of lymphoid nodules in the white pulp is also confirmed by (Sultana *et al.*, 2011) in ducks and (Mohammed *et al*., 2020) in quails, who reported that these nodules lack germinal centers. Similarly, Hamza and Balash (2005) described two types of nodules, spherical and ovoid, both lacking germinal centers in the spleen of native quails. In contrast, Nagy (1970) found that white and brown Leghorn chickens had two types of lymphoid nodule in which a central artery was located, a finding inconsistent with the present results in which the central artery was peripherally located. In addition, (Kozlu *et al*., 2011 reported that the spleens of eagles, falcons and ostriches develop lymphoid follicles that differentiate the white pulp from the red pulp. These follicles had no germinal centers in falcons, few in eagles and abundant in ostriches, similar to mammals. Based on these observations, it is assumed that these follicles correspond to the lymphoid nodules present in the spleen of the Iraqi Pin-tailed Sandgrouse.

In addition, (Reshag and Hamza, 2017; Liman and Bayram, 2011; Sultana *et al.* 2011) observed lymphoid follicles in the spleens of chickens and quails in the third week after hatching; initially these follicles lacked germinal centers, which appeared after one month. This differs from the current results, in which the nodules consistently lacked germinal centers.

In the present study, it was observed that the red pulp was irregularly distributed with the white pulp due to the absence of a marginal zone. It consisted of venous sinusoids and splenic cords, which is consistent with the findings reported by (King and Mclelland, 1975) in birds, (Mohammed *et al*., 2020) in guinea fowl, (Kadam *et al*., 2019) in Japanese quail, (Sultana *et al.,* 2011) in ducks, (Kannan *et al*., 2015) in chickens and (Ahmed and Sarma, 2023) in lizards. Determining which of the two pulps predominates was difficult due to the mixing of their histological components. In the present study, the red pulp was found to contain macrophages, reticular cells and plasma cells, which is consistent with the findings of (Hamza and Balash, 2005) in European quail, (Sultana *et al*., 2011) in ducks and (Rose, 1981) in farm animals.

CONCLUSION

The spleen of the Iraqi Sandgrouse (*Pterocles alchata*) is surrounded by a thin, single-layered capsule and contains trabeculae that extend into the parenchyma. The parenchyma consists of white and red pulp, which is irregularly distributed due to the absence of a marginal zone. The white pulp comprises peri-arterial and peri-ellipsoid lymphoid sheaths and spherical and ovoid lymphoid nodules, while the red pulp consists of venous sinusoids and splenic cords containing lymphoid, reticular, macrophage and plasma

cells. These results highlight both common and unique histological features of the spleen of P. alchata and provide a basis for further comparative and evolutionary studies in avian species.

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