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Macromorphological, histomorphological and histochemical studies of the spleen in guinea fowl (*Numida meleagris*)

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Abstract
The spleen of an adult Guinea fowl *(Numida meleagris*) bird was located at right lateral in abdominal cavity which observed to the junction between proventriculus and gizzard. Histomorphologically, the outer capsule had an unequal distribution of collagen, reticular and elastic fibers along with the few smooth muscle cells**.** Parenchyma were accommodated the poorly developed trabeculae with arteries and veins. Splenic parenchyma was composed of indistinctly demarcated white pulp and red pulp. The white pulp was predominantly constituted with small and medium-size lymphocytes, reticular cells and reticular fibers which was subdivided into periarterial lymphatic tissue, perivenous lymphatic tissue, periellipsoidal lymphatic tissue and ellipsoids. Germinal center was not observed in white pulp. The less capacious red pulp was evident with cords and narrow sinuses within network of reticular fibers. The sinuses were narrow and irregular spaces were lined by flattened endothelial cells. The cords were composed of lymphocytes, macrophages and granulocytes. Histochemically, the splenic capsule showed intense PAS positive activity while white pulp and red pulp showed moderate activity. Moderate acid phosphatase activity was observed in the capsule and white pulp, however the strong acid phosphatase activity was detected in red pulp. The capsule and white pulp has shown weak alkaline phosphatase activity but the red pulp has shown moderate activity of alkaline phosphatase.

Keywords: Ellipsoids, guinea fowl *(Numida meleagris*), histomorphology, periellipsoidal lymphatic tissue, red pulp, spleen, white pulp

Introduction
The largest peripheral lymphoid organ, spleen functions for hematogenesis, blood filtration, storage and immunity [1]. Most of the anatomical studies of this vital secondary lymphoid organ are studied in chickens and also put them on record. While widening the thrust area in examining macro morphological, histomorhological and histochemical anatomy of spleen in Guinea fowl *(Numida meleagris*), the present study has been undertaken.

Materials and Methods
Present study was conducted on spleen of 12 Guinea fowl (Numida meleagris) of either sex. The spleen samples were obtained at various meat shops at Udaipur by opting abdominal laparotomy and cranial displacement of sternum. The samples were washed immediately with normal saline and morphometrical measurement such as length, width, weight and volume of each of the organ were recorded. The collected samples were brought on ice in laboratory for further process. The samples were fixed in 10% Neutral Buffered Formalin and chilled pure Acetone, depending upon the different staining procedures to be adopted for histomorphological and histochemical studies. Thereafter, the tissues were processed after standard procedure of dehydration and clearing and paraffin sections were obtained on 5 µ thickness. The general histomorphology was studied by using Haematoxylin and Eosin stain. The Elastic, Reticular and Collagen fibres were detected after Silver Orcein and Aniline blue method [2]. The Weigert's method was also employed for detecting elastic and collagen fibres [2]. The histochemistry was studied on paraffin sections with Periodic acid Schiff`s (PAS) method for mucopolysaccharides, Gomori's alkaline phosphatase and acid phosphatase method [2].

Results and Discussion
The reddish brown coloured spleen in adult Guinea fowl birds was located at the right lateral

of abdominal cavity and close to the junction between the proventriculus and gizzard (Fig. 1). The present findings were concurrent with the findings in Kadaknath [3] and in adult indigenous fowl of Assam [4]. Pertaining to the shape of spleen, the present studies shown slightly elongated and ovoid shape with the hilus located at medial elongated surface (Fig. 2). These observances were in complete agreement with the observations recorded in adult indigenous fowl of Assam [4].

The comparison of weight of the spleen to the body weight in guinea fowl birds was observed. The mean of spleen weight was found to be 0.68 ±0.064 gm which was 0.041 ± 0.00049% to that of mean body weight of birds (Table-1). These observations were somewhat comparable with the findings submitted for the adult indigenous fowl of Assam [4], who were investigated the mean weight of spleen as 3.16±0.07 gm, which was 0.18±0.00% to that of mean body weight, might be it was attributed to the species difference of birds. Researcher also mentioned in 10 weeks age of fowl [5] weight 2.65 gm which was 2.00% of the body weight. However, the comparison of male and female native chicken of Bangladesh [6] were investigated, wherein an average weight of spleen in male was observed as 3.75±0.96 gm and in the female it was 2.67±0.82 gm. The findings in present study has shown the exceedingly smaller spleen, which might be indicating the strong defence mechanism of GuinFea fowl birds in comparison to the other birds which were studied and mentioned by other researchers.

Table 1: Gross parameter on the spleen of 12 Guinea Fowl (*Numida meleagris*) birds with following parameter

Sample No.	Total Body Weight of Bird	Total length	Width of spleen	Volume of spleen	Weight of spleen	Weight percent of spleen to total body weight
Sample 1	1.478K.g.	13.18mm	09.70mm	0.4ml	0.638gm	0.043
Sample 2	1.628k.g.	13.72mm	10.54mm	0.9ml	0.655gm	0.040
Sample 3	1.737k.g.	13.65mm	10.00mm	0.8ml	0.684gm	0.039
Sample 4	1.560k.g.	13.62mm	09.60mm	0.7ml	0.648gm	0.041
Sample 5	1.648k.g	13.76mm	09.70mm	0.9ml	0.659gm	0.039
Sample 6	1.720k.g.	13.80mm	10.69mm	0.8ml	0.673gm	0.038
Sample 7	1.428k.g.	13.11mm	08.80mm	0.4ml	0.623gm	0.043
Sample 8	1.659k.g.	13.56mm	09.95mm	0.9ml	0.662gm	0.040
Sample 9	1.470k.g.	13.16mm	09.10mm	0.5ml	0.628gm	0.042
Sample 10	1.731k.g.	13.63mm	10.34mm	0.8ml	0.680gm	0.039
Sample 11	1.681k.g.	13.78mm	10.40mm	0.7ml	0.663gm	0.039
Sample 12	1.430k.g.	13.08mm	08.70mm	0.4ml	0.625gm	0.043
Average	1.912 k.g.	13.50 mm	09.79 mm	0.68 ml	0.64 gm	0.041
SD	0.1188	0.2841	0.6614	0.2037	0.02249	0.0017
SE	0.0342	0.0820	0.1908	0.0588	0.0064	0.00049

Histomorphologically the guinea fowl spleen was composed of capsule, trabeculae, white pulp and the red pulp. The thick white fibrous capsule was covering the spleen. The capsule was composed of collagen, elastic and reticular fibers with some smooth muscles cells. The firmness to the organ was provided by the fibers, of which the thickness was more at hilus (Fig. 3). The present descriptions on microscopical findings were in similarity with the mentionings in Japanese quails, broiler chicken and indigenous fowl of Assam and in Japanese quails exceptionally observance of thin capsule was there [4, 7, 8, 9].

Present investigations in Guinea fowl spleen showed poorly developed trabeculae within the splenic parenchyama. The parenchyma was made up of a reticular network with much small white pulp area surrounded by red pulp area. The white and red pulp had no demarcative line. The red pulp was consisted of cords and sinuses in the network of reticular cells and reticular fibers (Fig. 6). The sinuses were narrow and irregular spaces which were lined by flattened endothelial cells. The cords were composed of lymphocytes, macrophages and granulocytes. These observations were in accordance with the findings of in Japanese quail, Assam fowl, Ostrich Chicks and chicken [1, 4, 10, 11].

The tissues of white pulp were spread diffusely and related to parenchymal vasculature. The white pulp was predominantly constituted of lymphocytes and subdivided into periarterial lymphatic tissue, perivenous lymphatic tissue, periellipsoidal lymphatic tissue and ellipsoids (Fig. 4). These findings were in complete agreement with the different findings put on record in ostrich chicks [1], Assam fowl [4], quail [9], duck [12] and Japanese quail [10, 13].

The periarterial lymphatic and perivenous lymphatic tissue was composed of aggregated lymphocytes and few macrophages around the ellipsoids. The convergence of two to four ellipsoids was visible. The dense network of reticular fibers was located around the parenchymal vasculature (Fig. 5). Some researcher had reported the lymphocytic aggregations around the central arteries and collecting veins at splenic parenchyma in duck [12]. The present study was exhibited the absence of marginal zone in guinea fowl which was in harmony with the reports mentioned by Baishya *et al* [4] in Assam fowl and in chicken by Olah *et al* [14].

Present study doesn't shown the germinal center in the white pulp, which was contradictory with the findings in spleen of Japanese quail [10] of four weeks and above age who were mentioned the appearance of germinal center in the spleen.

Histochemically, splenic capsule of guinea fowl showed intense PAS positive activity while white pulp and red pulp showed moderate activity (Fig. 7). Positive PAS activity showing components involved in the optimal function for which the glycogens act as the source of energy. Moderate acid phosphatase activity was observed in the capsule. The red pulp showed strong acid phosphatase activity and white pulp showed moderate acid phosphatase activity (Fig. 8). Blood vessels showed the weak activity (Fig. 9). However, Mishra and Meshram 2019 [15] had mentioned the studies on organ component of pecten oculi where the activity of alkaline phosphatase was near to negative and they had

quoting on their inference that organ components which shown less activity must be performing extremely vigorous and in the active mode. The intensity of acid phosphatase might be taken inversely proportional to the functional supremacy of that organ component. The present results were not different than the mentioning in chickens spleen which was evident of acid-phosphatase activity and possess considerable numbers of primary lysosomes [16] and in mammals, follicular dendritic cells were negative acid phosphatise activity which possessed only a few primary lysosomes [17, 18].

The present findings have revealed less intense alkaline phosphatase activity in guinea fowl splenic capsule. Moreover, the very mild activity of alkaline phosphatase noticed in white pulp and red pulp and blood vessels show moderate activity (Fig. 10 and 11). The intensity of alkaline phosphatase might be taken inversely proportional to the functional supremacy of that organ component. Very weak alkaline phosphatise activity was reported in chicken [19] which was similar to the present findings.

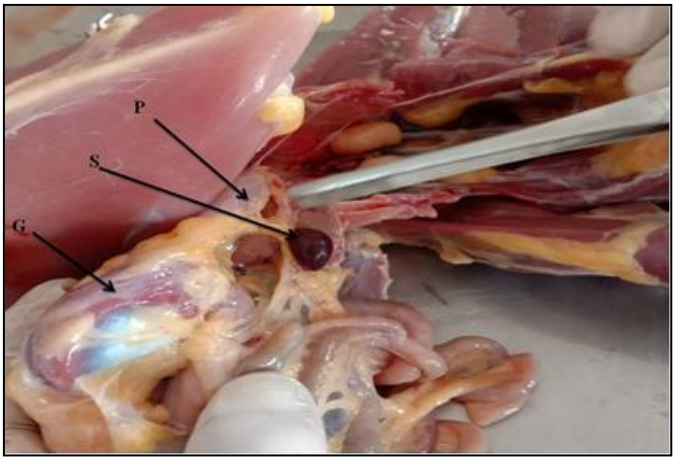

Fig.1: Photograph showing Spleen (S), Proventriculus (P) and Gizzard (G).

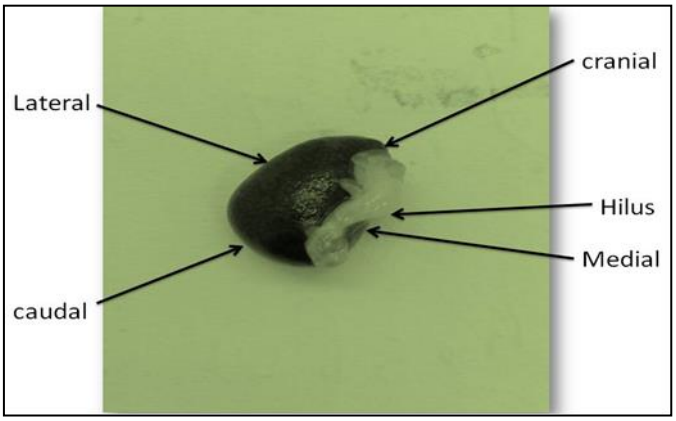

Fig 2: Photograph showing different parts of spleen and hilus on the medial elongated surface.

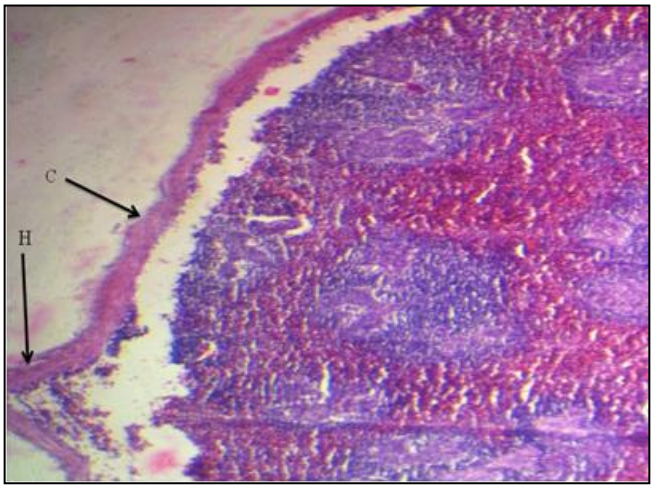

Fig 3: Photomicrograph of Spleen showing Capsule (C) and Hilus (H). H&E- 4x

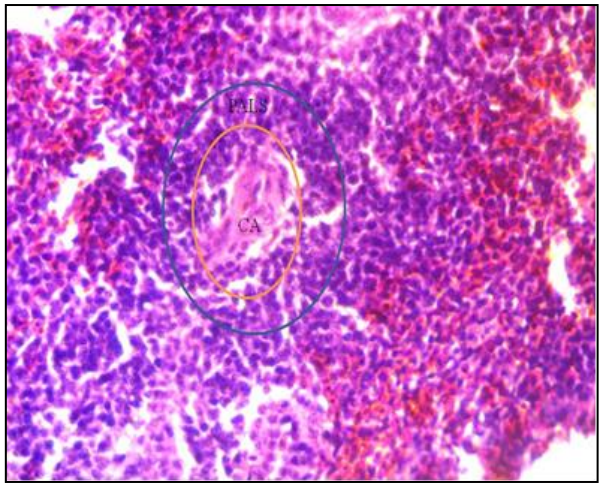

Fig 4: Photomicrograph of Spleen showing White pulp (WP), Central artery (CA) and Periarterial lymphatic sheath (PALS). H&E-40x

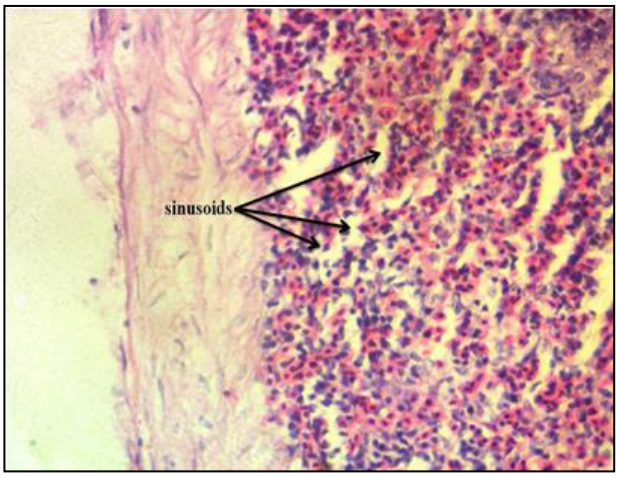

Fig 5: Photomicrograph of Spleen showing Sinusoids H&E-40x

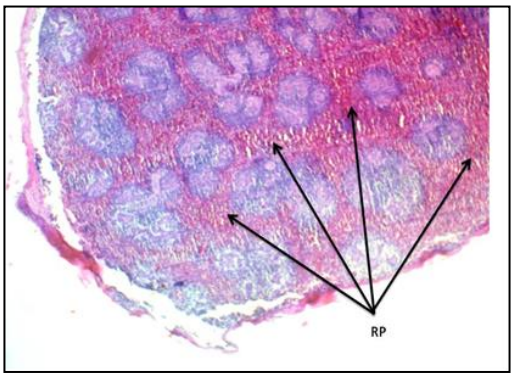

Fig 6: Photomicrograph of Spleen showing Red pulp (RP) H&E- 40x

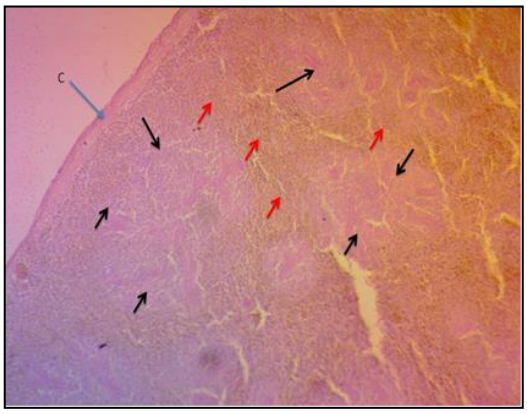

Fig 7: Photomicrograph of Guinea Fowl spleen showing, Capsule (c) PAS positive activity, Black arrow- PAS positive reaction in white pulp and Red arrow- Less activity in red pulp. Periodic acid Schiff's (PAS)- 40X

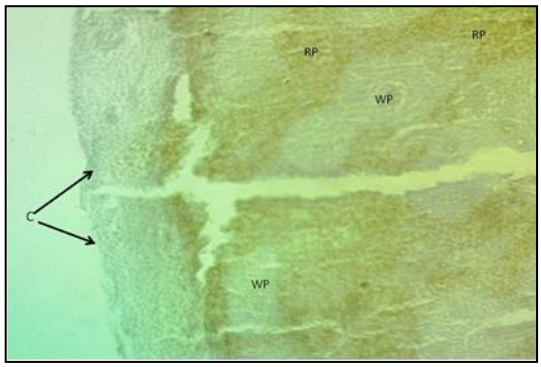

Fig 8: Photomicrograph of Guinea Fowl spleen showing moderate activity in capsule(C). moderate activity in white pulp (WP) and strong activity in red pulp(RP). Acid phosphatases- 100X

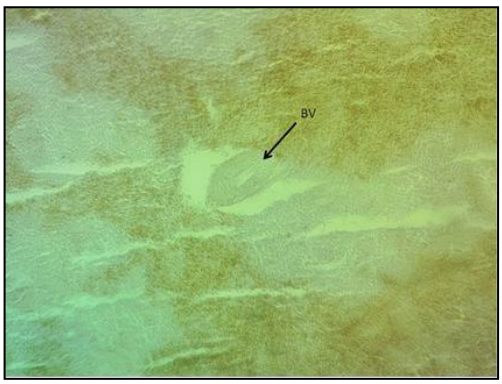

Fig 9: Photomicrograph of Guinea Fowl spleen showing weak activity in Blood vessels (BV). Acid phosphatases- 100X

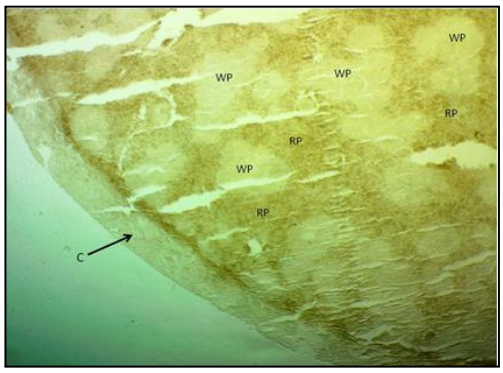

Fig 10: Photomicrograph of Guinea Fowl spleen showing weak activity in ruptured capsule(C), very weak strength of alkaline phosphatase activity in white pulp (WP) and moderate activity in red pulp (RP). Alkaline phosphatase- 100X

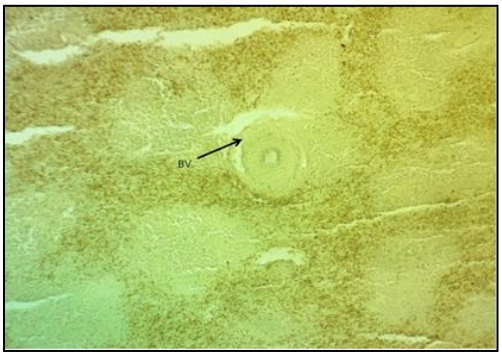

Fig 11: Photomicrograph of Guinea Fowl spleen showing weak activity in blood vessel. Alkaline phosphatase- 100X

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