

Comparative Of Harderian Gland Of Dove And Native Chickens By Histology, Histochemical Analysis

Ibtessm Magad¹, Fatimah Swadi Zghair²

^{1,2}Department of Anatomy and Histology Veterinary Medicine, Al-Qadisiyah University
fatimah.zghair@qu.edu.iq

Abstract

Studying the histology and histochemical makeup of the Harderian gland in Dove and native chickens was the goal of this project. Samples from adult birds were collected, processed for histological analysis using hematoxylin and eosin (H&E) staining, and evaluated histochemically using periodic acid-Schiff (PAS) and Alcian blue stains to determine the mucopolysaccharide and glycoprotein content. The results demonstrate that the two species differ in glandular function and ecological niche adaptation, as demonstrated by distinct histological and histochemical differences. A multilobular tubular-alveolar exocrine glandular structure was seen in the Harderian glands of both domestic male chickens and male doves, according to histological and histochemical analysis. Whereas the dove's capsule is thinner and contains lymphocytes, the rooster's gland is encased in a thick capsule of connective tissue that is rich in blood arteries and nerve fibers. The interstitial connective tissue and the capsule both reacted differently to PAS and AB stains, suggesting that the two species' levels of glycoproteins and mucous components varied. The gland in the rooster showed a distinct lobular organization with large lobes and a cylindrical secretory epithelium containing lipid droplets, while the gland in the dove was smaller, more compact, with closer, more clearly defined lobules, and a cuboidal epithelium containing mucous vacuoles. Abundant lymphoid tissue was also observed within the parenchyma in both species, more so in the rooster, indicating immune activity. The excretory ducts were similarly organized in both species, ranging from intralobular ducts to the main excretory duct that drains into the third eyelid. These ducts reacted positively with AB-PAS stains, indicating mixed mucus secretions (acidic and neutral mucus).

Keyword: histology, native chicken, histochemical, dove

INTRODUCTION

The orbital cavity contains the complicated multilobular ocular gland known as the Harderian gland. It has a single duct that opens at the base of the nictitating membrane in the inner angle of the eye. It is found in the majority of terrestrial vertebrates, including reptiles, amphibians, birds and a few mammals (1, 2, 3, 4). The fowl HG may generate antibodies to both locally and systemically administered antigens and possesses sizable age-dependent populations of plasma cells (5). IgA, IgG, IgM, and IgY are the four types of immunoglobulins that are produced or transmitted by HG plasma cells (6, 7).

MATERIAL AND METHODS

Twenty apparently healthy local chicken and dove birds were used to produce the HGs used in the study. From September to March of 2024–2025, these birds were bought from regional vendors in the common markets of Aldwiyah province. The specimens used for histology investigations were taken from the body and duct of the birds under study. These samples were stored in sterile plastic containers after being carefully cleaned with 0.9% regular saline solution. A series of graded alcohols (70,80,90,95, and 100 percent) were used to dehydrate the specimens for two hours at each concentration after half of them had been fixed with 10% neutral buffered formalin (8, 9, 10) for two days. Following an hour of cleaning in xylene with two different changes, the specimens were penetrated with molten paraffin wax. Sections of the blocked specimens (Series MRS3500, Histo-Line laboratories Ltd, Italy) with a thickness of 5 micrometers were cut using a rotary microtome. These sections were then put on clean slides and tissue stainer than stained with Harris's hematoxylin and eosin, combined AB-PAS, Masson trichrome stains to show the tissues' overall histological components (11, 12, 13).

RESULT

Histological and histochemical finding in male local chicken (rooster) and male dove Our study revealed that the rooster and dove Harderian gland was represented an exocrine multilobular tubulo-alveolar gland which was consisted of :-

1 Stroma: (a) Capsule: In rooster, this gland was surrounded by somewhat thick connective tissue capsule which was consisted of connective tissue mainly collagenous fibers, blood vessels, nerve fibers and covered by a single layer of flattened endothelial cells (Fig. 1). The capsule showed negative for alcian blue stain and slight positive for pas stain (stains by combined alcian blue (PH_{2.5})) and PAS stains (Fig.2) while in dove same structure but thin fibrous connective tissue contain blood and nerve vessels, lymphocyte (Fig.3) The capsule showed negative for alcian blue stain and slight positive for pas stain (stains by combined alcian blue (PH_{2.5})) and PAS stains (Fig.4)

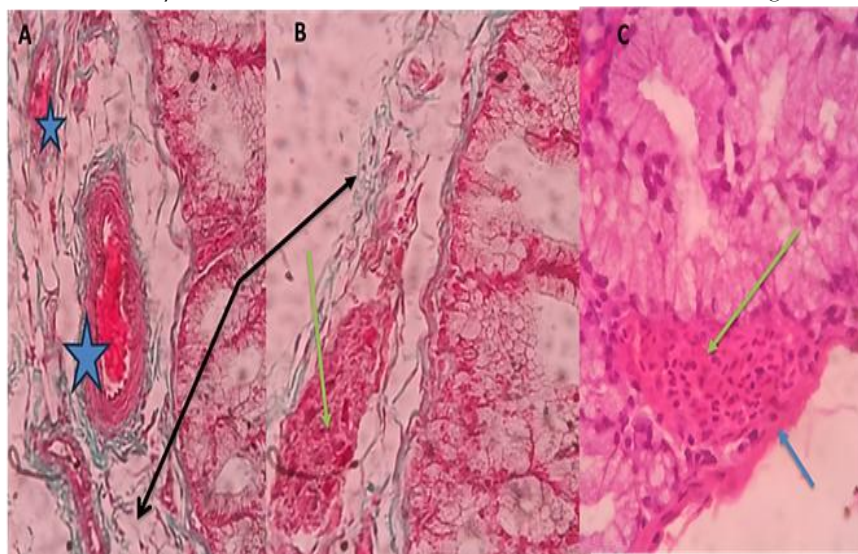


Fig. 1: A, B&C: microscopic image of harderian glands in rooster showed : Serosa with collagen fiber (black arrows), blood vessel (blue star), parasympathetic ganglian (green arrows), lymphocyte (blue arrow). Masson, trichrom and H&E: X400

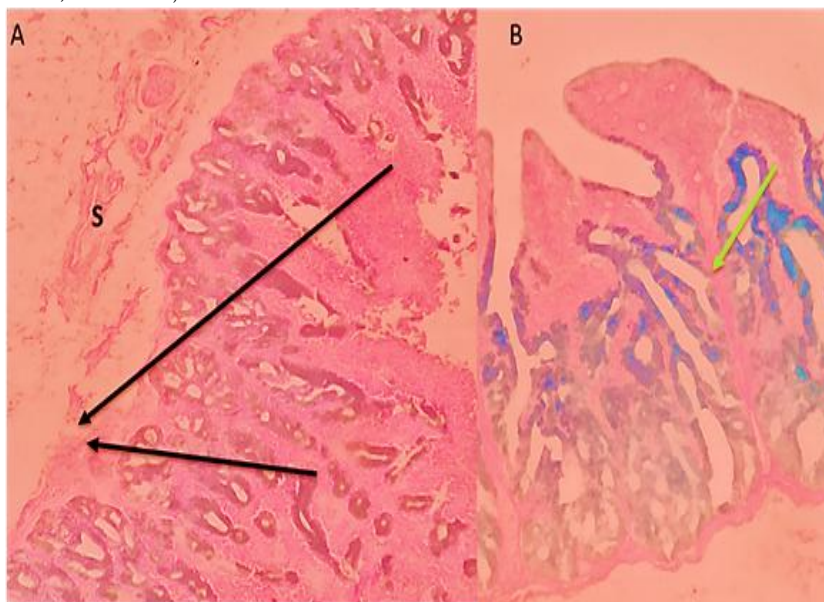


Fig. 2: A and B ; microscopic image of harderian glands In rooster showed: connective tissue of serosa negative stain with alcian blue and slight stain with pas stain (black arrows), also interlobular septa negative stain with alcian blue and slight stain with pas stain. Combined alcian and pas stains: A:X40 & B X 200

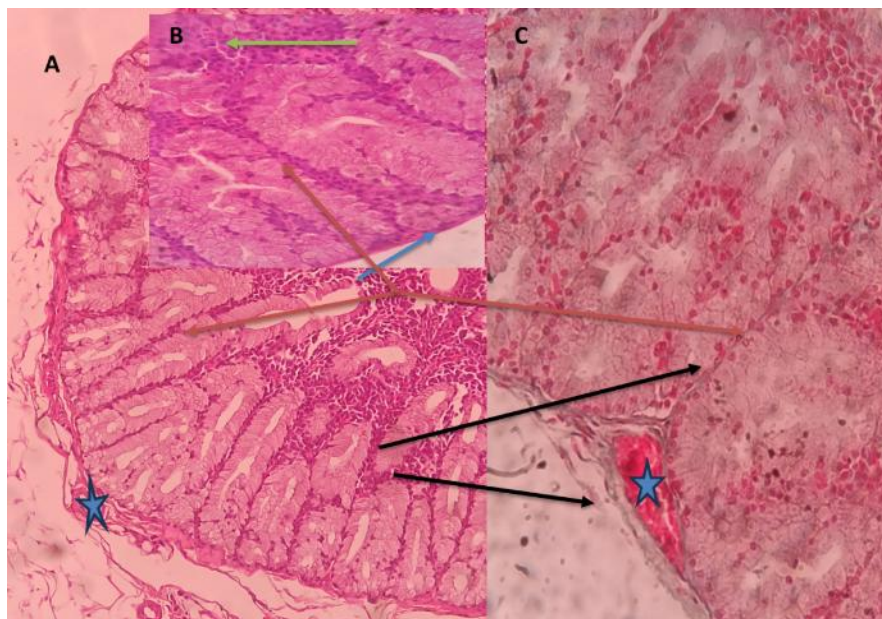


Fig.3: Microscopic image of hardrian glands in dove connective stain of with collagen fiber (black arrows), and interlobular septa (red arrows), blood vessel (blue star), nerve (green arrows), lymphocyte (blue arrow), interlobular septa (red arrows),

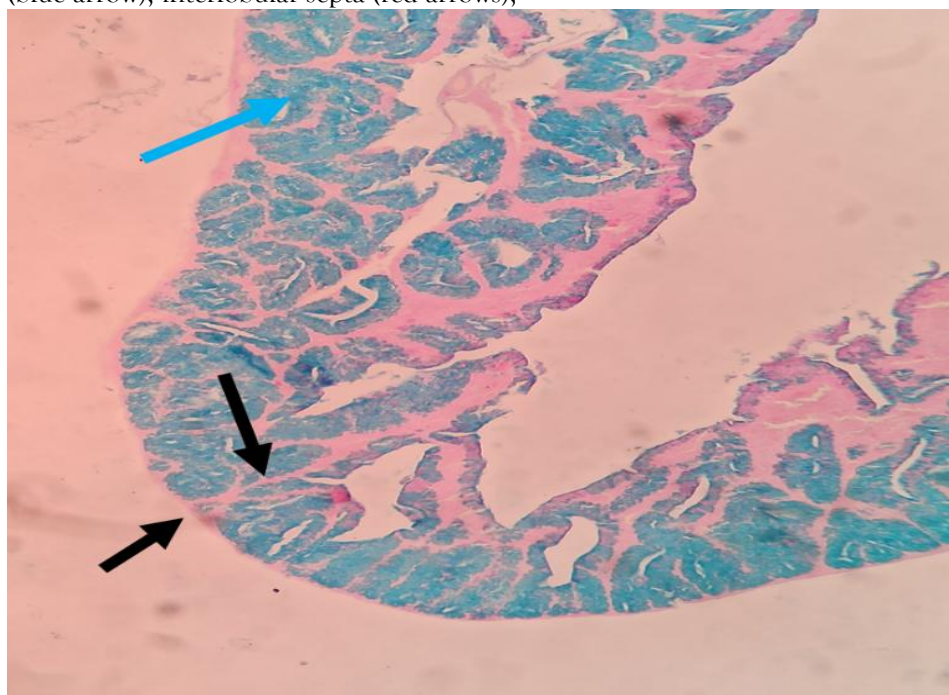


Fig. 4; microscopic image of harderian glands In dove showed: connective tissue of serosa negative stain with alcian blue and slight stain with pas stain (black arrows), also interlobular septa negative stain with alcian blue and slight stain with pas stain. Combined alcian and pas stains : X 400

(b) Septa & Interstitial connective tissue:

In two species, the capsule sent folds of connective tissue septa that were somewhat thick at its beginning, then thinned gradually through its course inside the gland. The septa subdivided the gland into lobes and lobules. It contained blood vessels, collagenous fibers, and nerves also positive reaction for pas and not stain with alcian blue due to present of neutral polysaccharide (Fig.2,3,4, 5, 6). It was also rich in connective tissue cells specially plasma cells, lymphocytes and macrophages Russell bodies, (Fig. 7, 8).

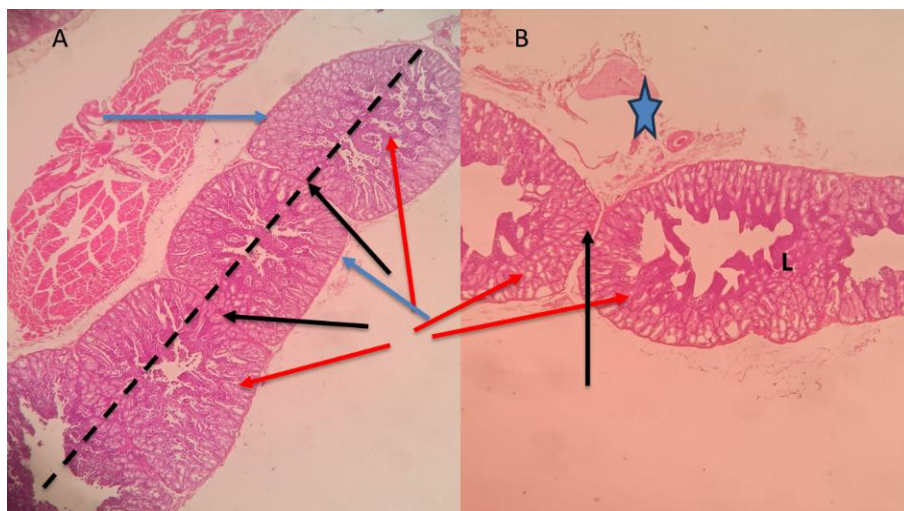


Fig 5:A,B left and right lobe of HD in rooster showed the capsule (blue arrows) contain blood vessels (blue star) send connective tissue inside HD and divided into lobes(interrupted line) and lobules (L) by inter lobular septa (black arrows) & intralobular septa(red arrows) : A&B:X40

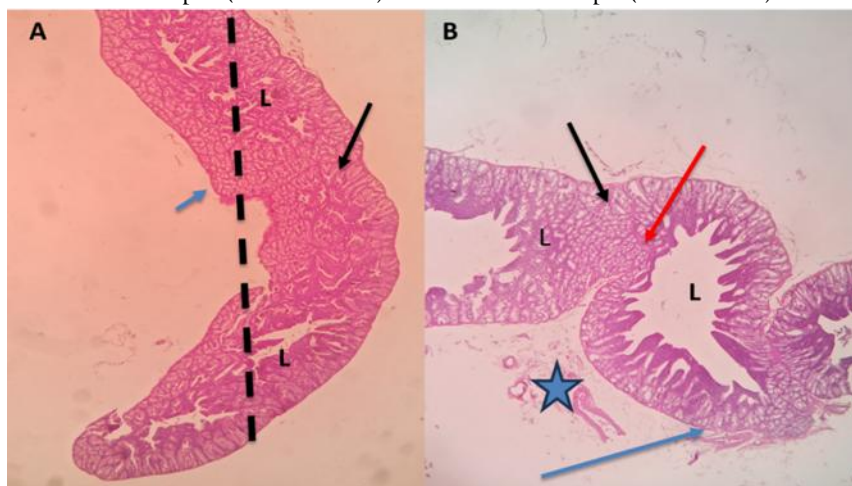


Fig 6:A,B left and right lobe of HD in dove showed the capsule (blue arrows) contain blood vessels (blue star) send connective tissue inside HD and divided into lobes(interrupted line) and lobules (L) by inter lobular septa (black arrows) & intralobular septa(red arrows) : A&B:X40

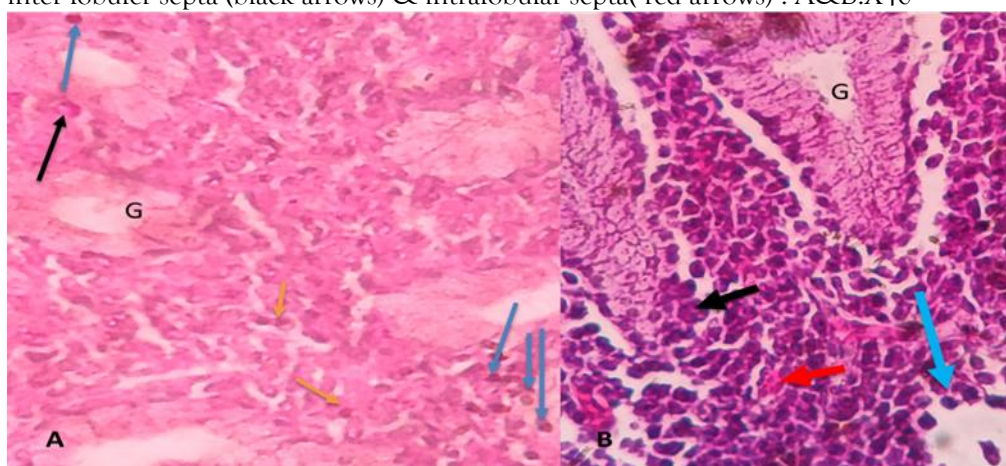


Fig.7A&B: microscopic image of the right lobe of HD in rooster showed present of plasma cells (blue arrows), macrophage (black arrow) and lymphocytes (orang arrows) Russell bodies (red arrow) corpus gland(G). H&E stains; A&BX400

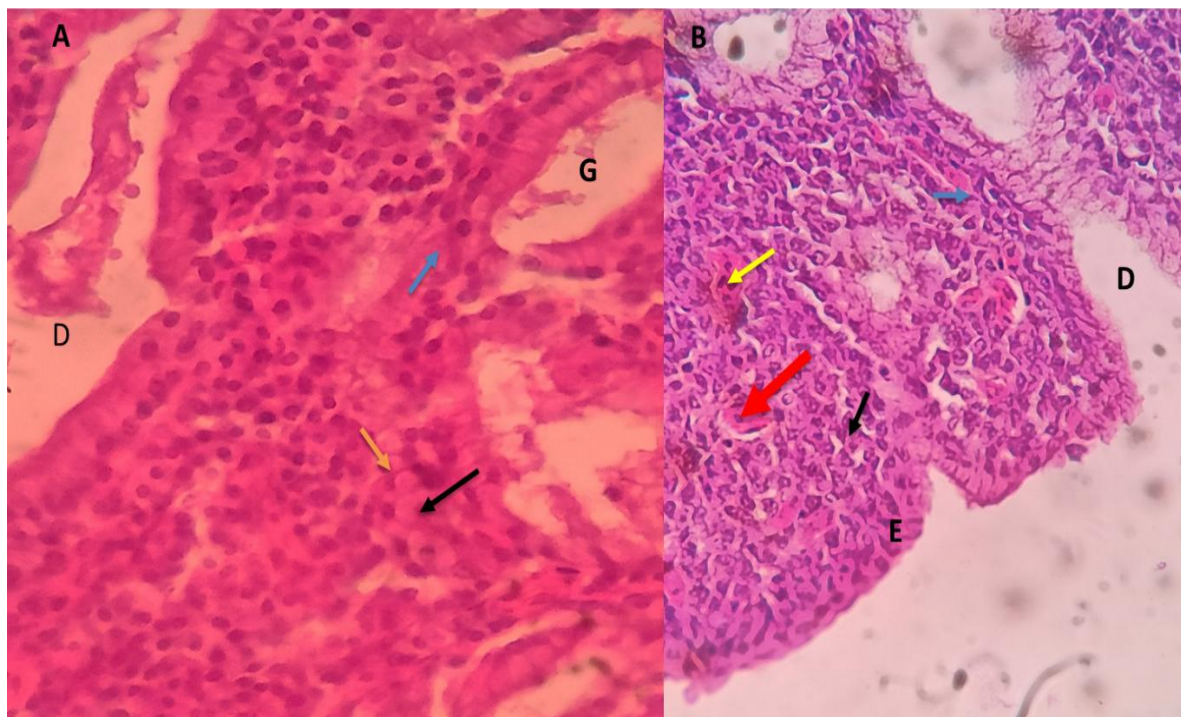


Fig.8A&B: microscopic image of the left lobe of HD in dove showed present of plasma cells (blue arrows), macrophage (black arrow) and lymphocytes (orange arrows), Russell bodies (red arrow) corpus gland(G), duct(D), epithelium (E). H&E stains; X400

The interlobular, intralobular and the main excretory ducts (Fig.9,10) (table.4) were also detected difference between two species. The interstitial connective tissue represented by periacinar, intralobular connective tissue and the connective tissue with collagen fiber (Fig.11,12) of the gland which connect the gland to the eyeball from the anterior aspect. It was also consisted of connective tissue fibers, connective tissue cells, plasma cells, small and large blood vessels and lymphocytes (Fig. 7,8). The interstitial connective tissue showed AB- PAS positive reaction (Fig.2,4)

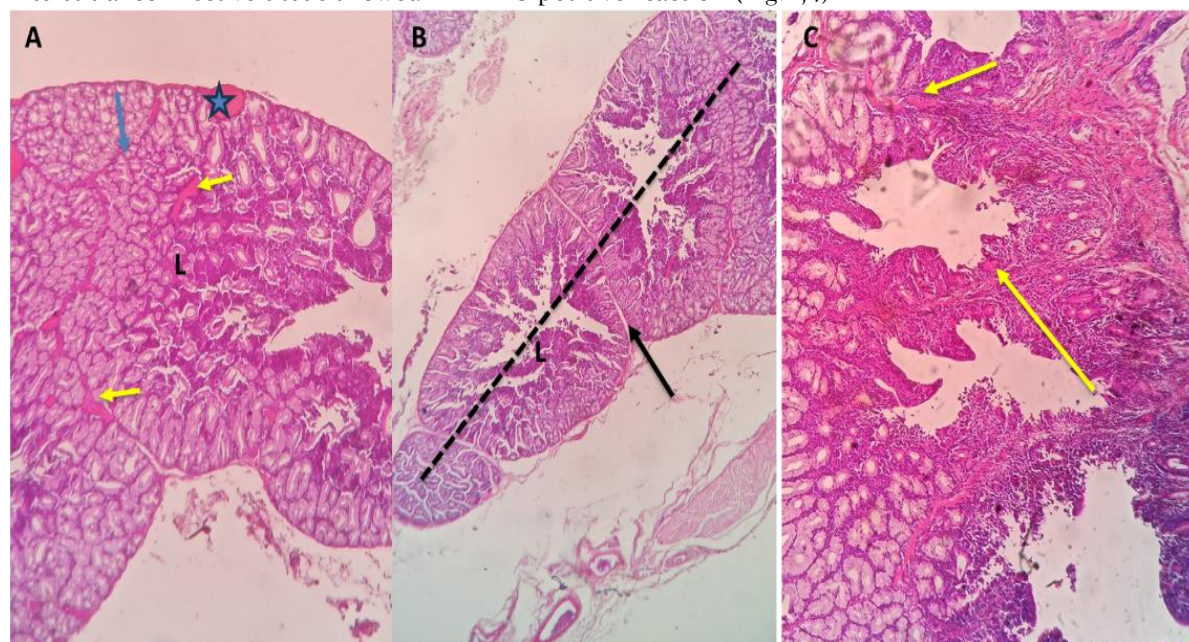


Fig 9:A,B &C; left (A)and right(B) lobe and duct(C) of HD in rooster showed the parasympathetic ganglia in capsule (blue star), blood vessels (yellow arrows) send connective tissue inside HD and divided into lobes(interrupted line) and lobules (L) by inter lobular septa (Black arrows) & Intralobular septa(blue arrows): A&C:X200; B; X40

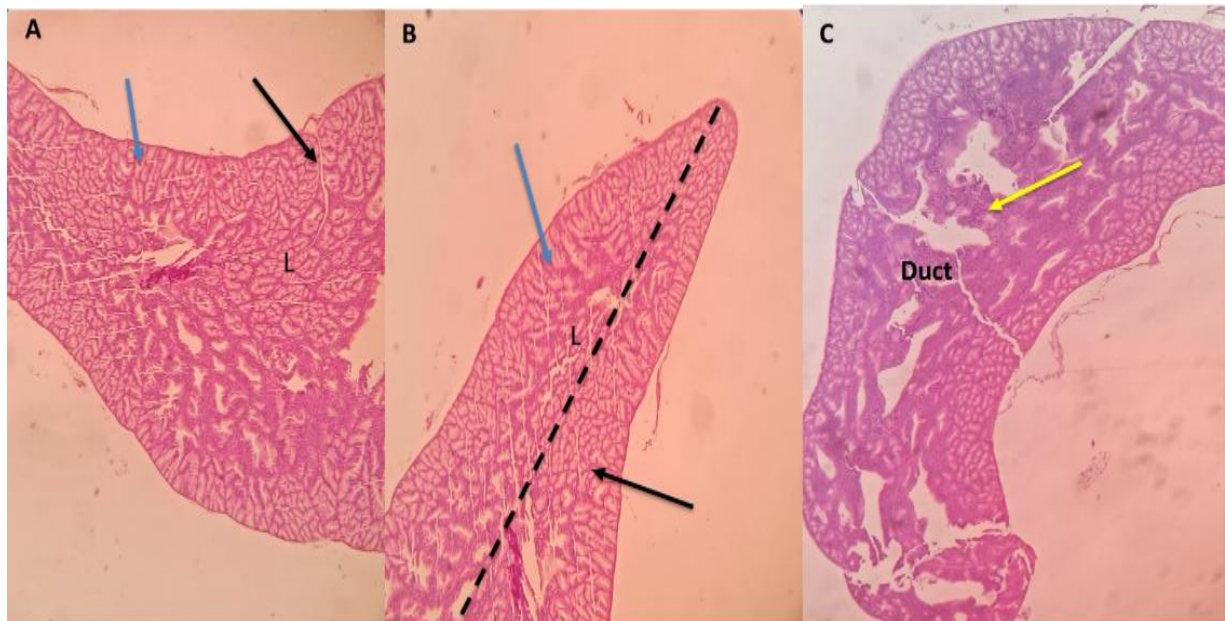


Fig 10:A,B &C; left (A) and right (B) lobe and duct (C) of HD in dove showed the blood vessels (yellow arrows) send connective tissue inside HD and divided into lobes (interrupted line) and lobules (L) by inter lobular septa (Black arrows) & Intralobular septa (blue arrows) : A & C: X100; B; X100

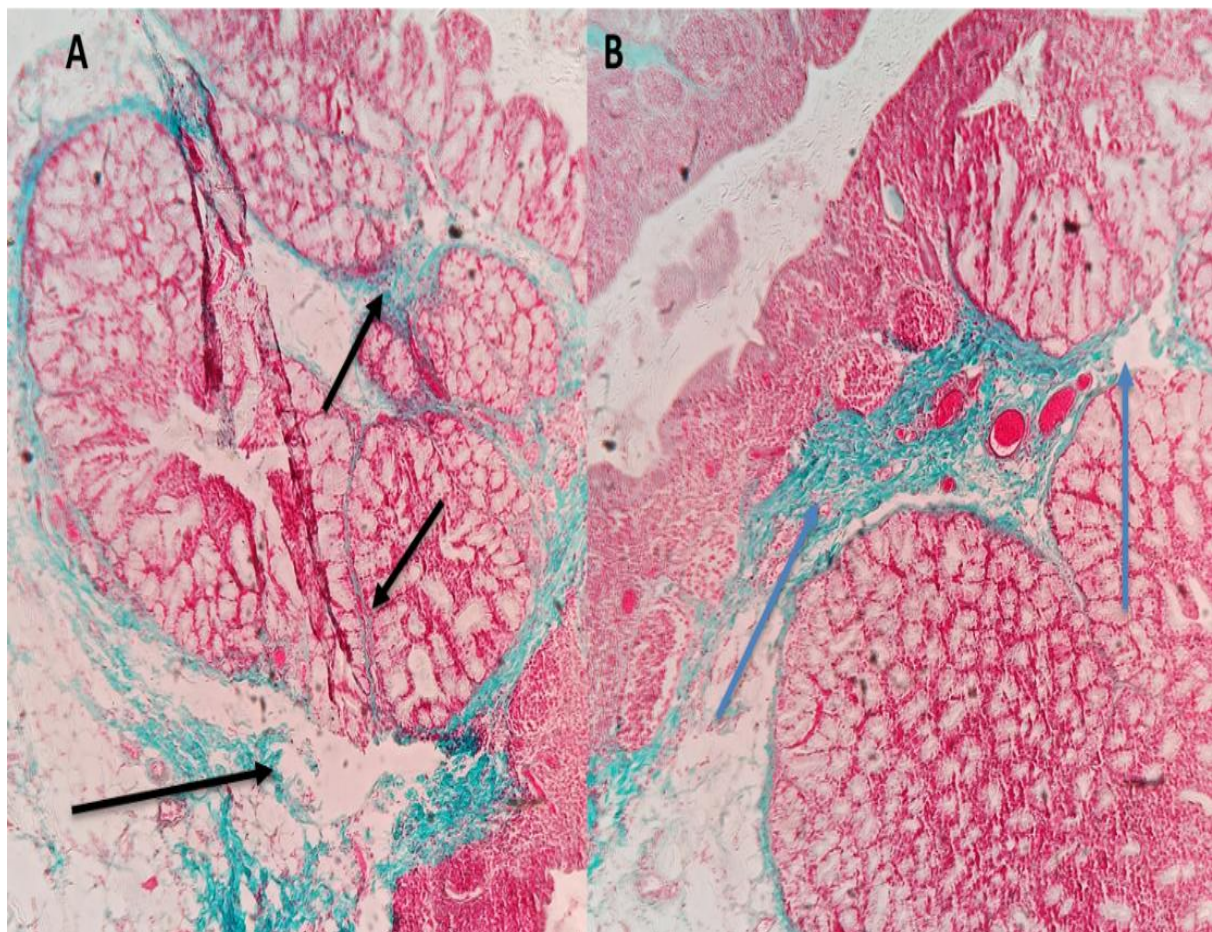


Fig 11 A & B: right (A) lobe and duct (B) of HD in rooster showed collagen fiber (black arrows) (yellow arrows) in lobe and (blue arrows) in duct from capsule send connective tissue inside HD and divided into lobes and lobules by inter lobular septa (Black arrows) & Intralobular septa: A & B; X100



Fig 12A&B: left(A) lobe and duct(B) of HD in dove showed slight present of collagen fiber (black arrows) in lobe and (blue arrows) in duct from capsule send connective tissue inside HD and divided into lobes and lobules by inter lobular septa & Intralobular septa;;A& B; X100

(2) Parenchyma:

In rooster the histological structure of parenchyma the gland was large, with distinct tubuloalveolar lobes. Simple columnar or cuboidal, secretory cells containing lipid droplets. Oil Red O have Strong Positive (Fat), The presence of lymphoid tissue within the parenchyma in left lobe larger than right lobe. The overall shape of the lobes is less compact, clearly segmented & lined by simple cuboidal epithelium (Fig.13,14,15).

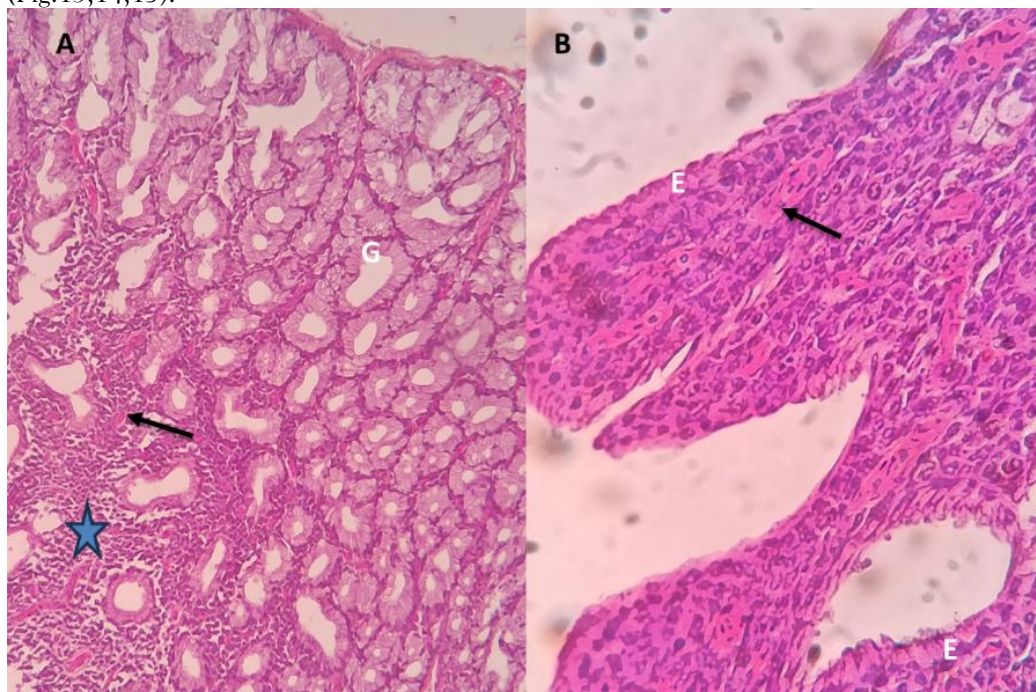


Fig 13:A&B: left(A) lobe and duct(B) of HD in rooster showed lobule lined by simple cuboidal epithelium (E), parenchyma contain lymphoid tissue (Blue star), tubuloalveolar gland (G) lined with simple cuboidal epithelium (E), Russell bodies (black arrows) , A;X100& B; X400

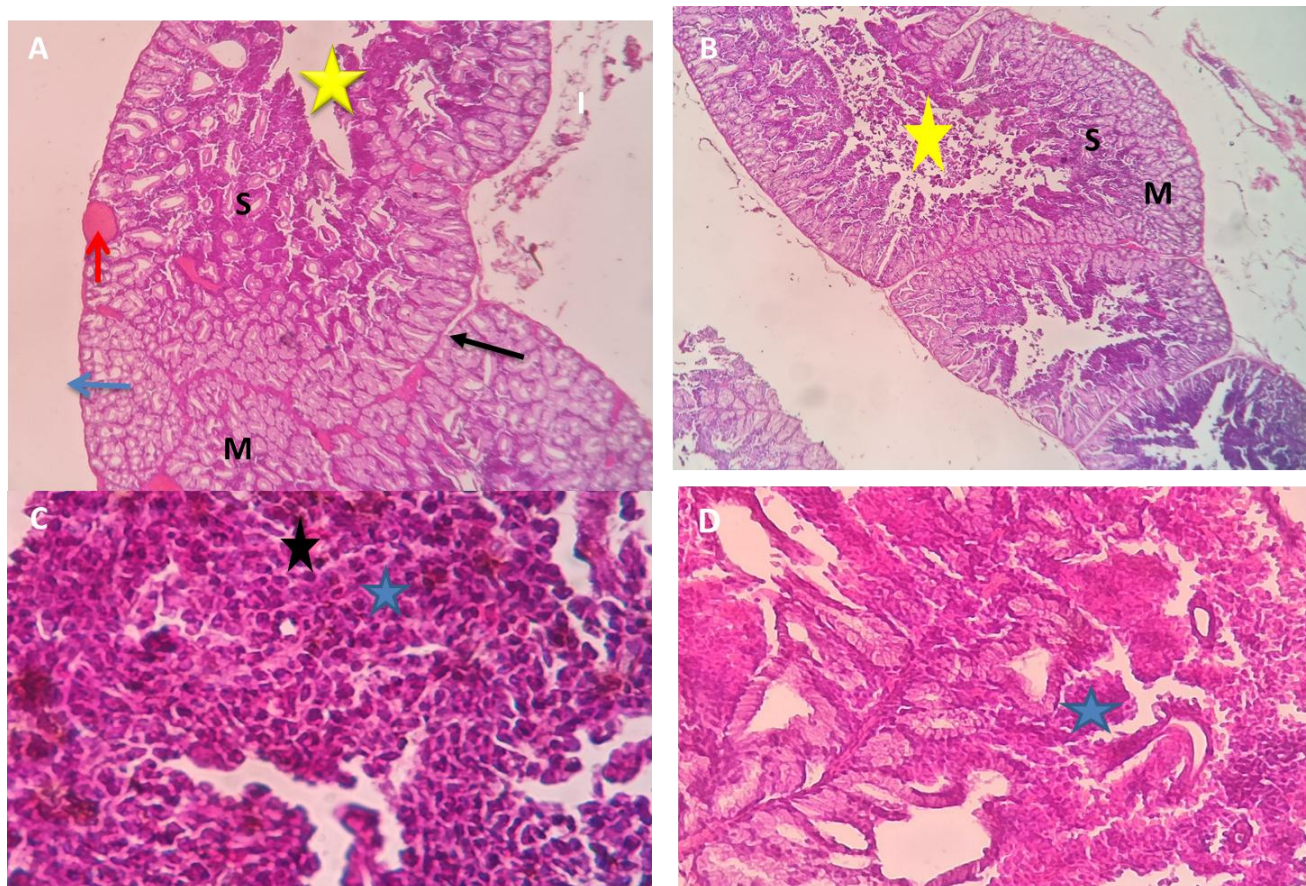


Fig. 14: (A&C left HD) & (B&D right HD) Histological section of exocrine tubuloalveolar. The Harderian gland of local male chicken was covered with a thin capsule (blue arrow) which consisted of adipose tissue (I) duct (Yellow stars) interlobular septa (black arrow) with parasympathetic ganglia (red arrows), mucous acini (M), serous acini (S), aggregation of lymphocyte (blue stars), Russell bodies (black star) (H and E stain; & B'X40; C:X400; D:X200). The left glands more compact, more dense of lymphocyte compare with right HD.

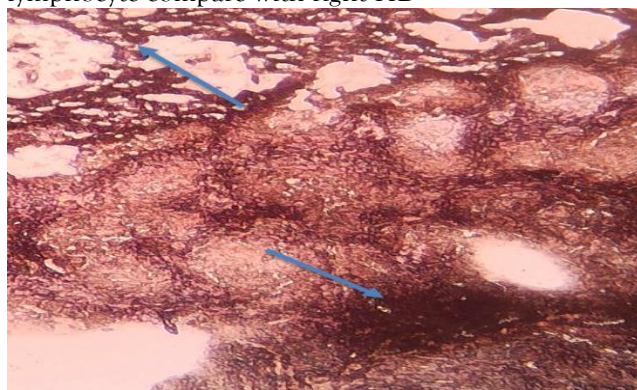


Fig 15: left of HD in rooster showed lobule and capsule contain Fat. Oil red stained, X400
(In figure 16, 17, 18, 19) In dove, the gland is relatively small, the lobes more compact and tubular in shape. Often simple cuboidal epithelium, with mucin vacuoles within the cytoplasm. Mucous secretion – with sticky, PAS-positive secretions, The cells are smaller, mucin vacuoles are clearly visible within the cytoplasm. PAS: Very positive (glycogen-rich mucus).- Alcian Blue: Positive (acidic mucus). The presence of lymphoid tissue within the parenchyma is clear and extends between the lobes, indicating immune activity. The overall shape of the lobes is denser and closer together, with small rounded lobes.

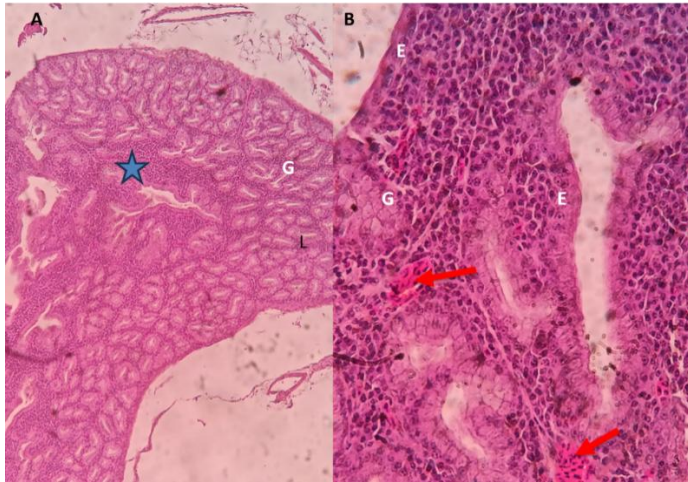


Fig 16:A&B: left(A) lobe and duct(B) of HD in dove showed lobule lined by simple cuboidal epithelium (E), parenchyma contain lymphoid tissue (Blue star), tubuloalveolar gland (G) lined with simple cuboidal epithelium (E), Russell bodies (black arrows), A;X100& B; X400

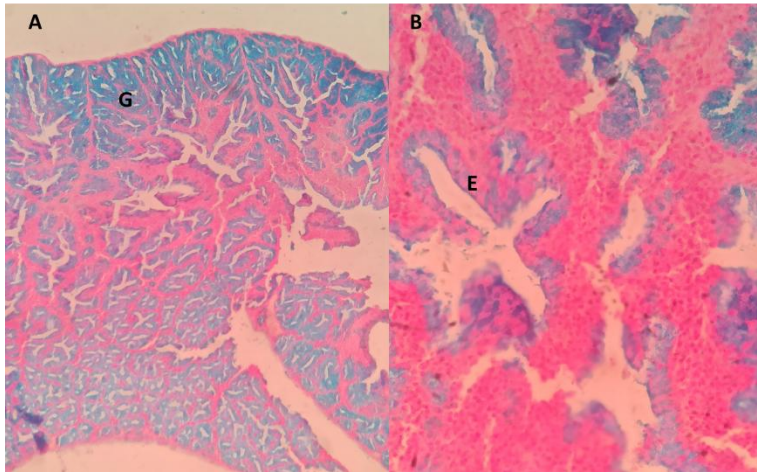


Fig 17:A&B: left(A) lobe and duct(B) of HD in dove showed lobule lined by simple cuboidal epithelium (E), parenchyma contain tubuloalveolar gland (G) lined with simple cuboidal epithelium (E), strong reaction for combined AB-PAS stains, A;X100& B; X400

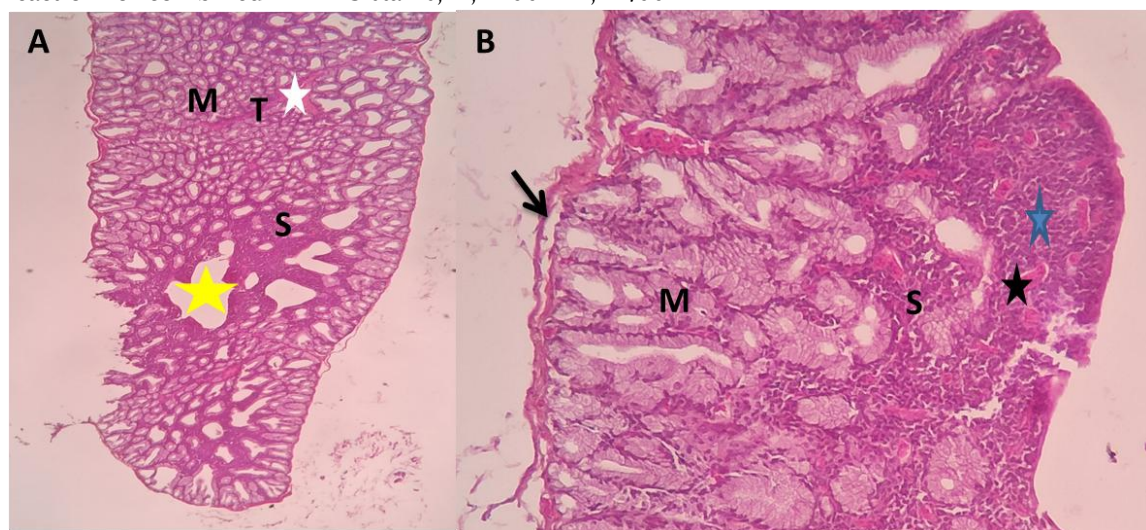


Fig. 18: (A left HD)&(B right HD)Histological section of exocrine tubuloalveolar The Harderian gland of dove was covered with a thick capsule (black arrow)which consisted of duct (Yellow stars) interlobular septa(T)with blood vessel(white star), mucous acini(M), serous acini (S), aggregation of

lymphocyte (blue arrows), Russell bodies (black star) (H and E stain).A;X40; B;X100 . The left glands compact , dense of lymphocyte similar to the right HD

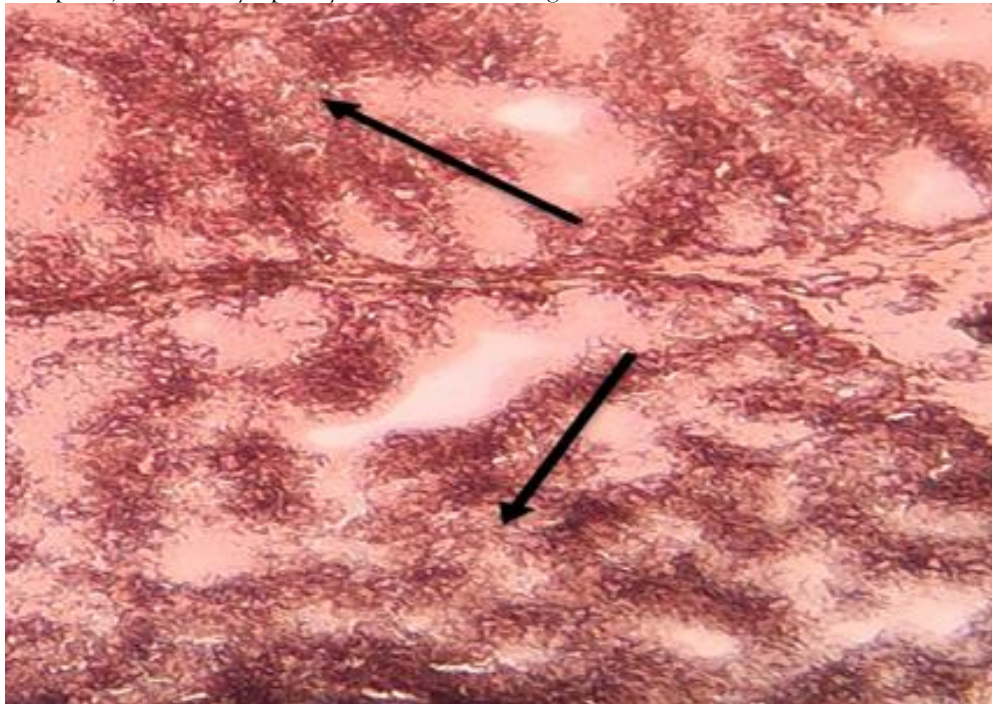


Fig19: right of HD in dove showed lobule contain Fat. Oil red stain X400

In two species. the myoepithelial cells were arranged in discontinuous manner surrounded the secretory units of both lobes, they were enclosed between the basal surface of the glandular cells and the basement membrane .The presence of mixed lobe was more frequent at any level of the gland(Fig.20, 21).

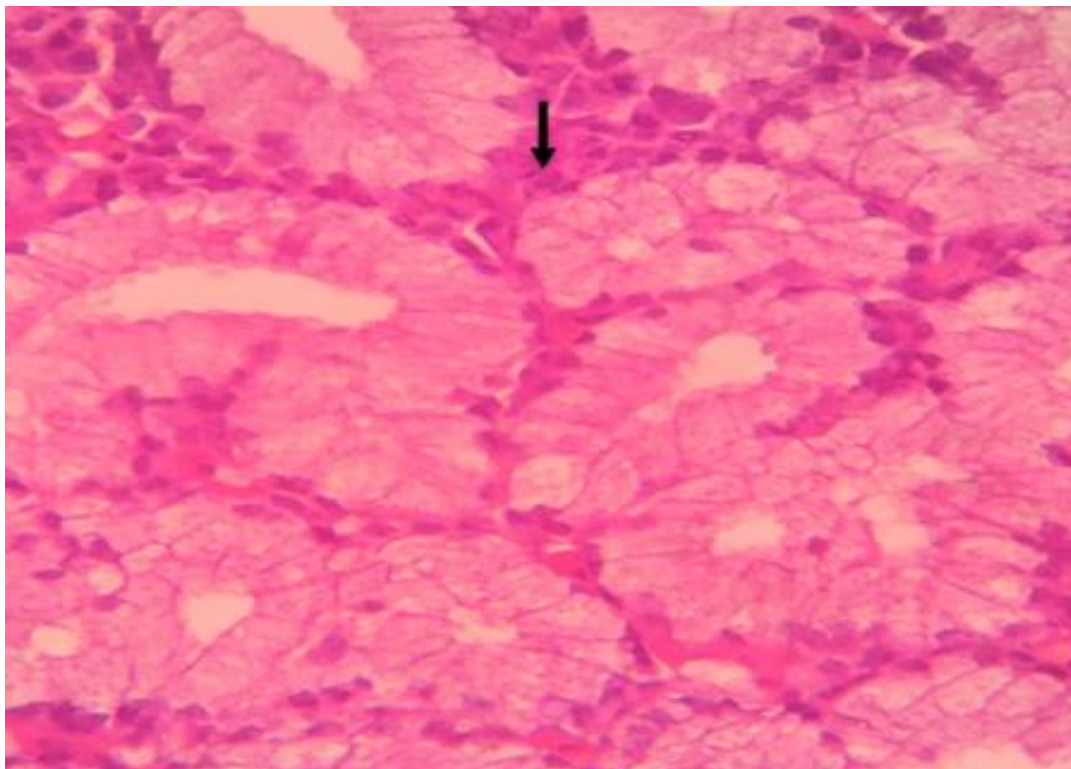


Fig 20: right of HD in rooster showed the myoepithelial cells (black arrow) were arranged in discontinuous manner surrounded the secretory units of lobule. H&E stain X400

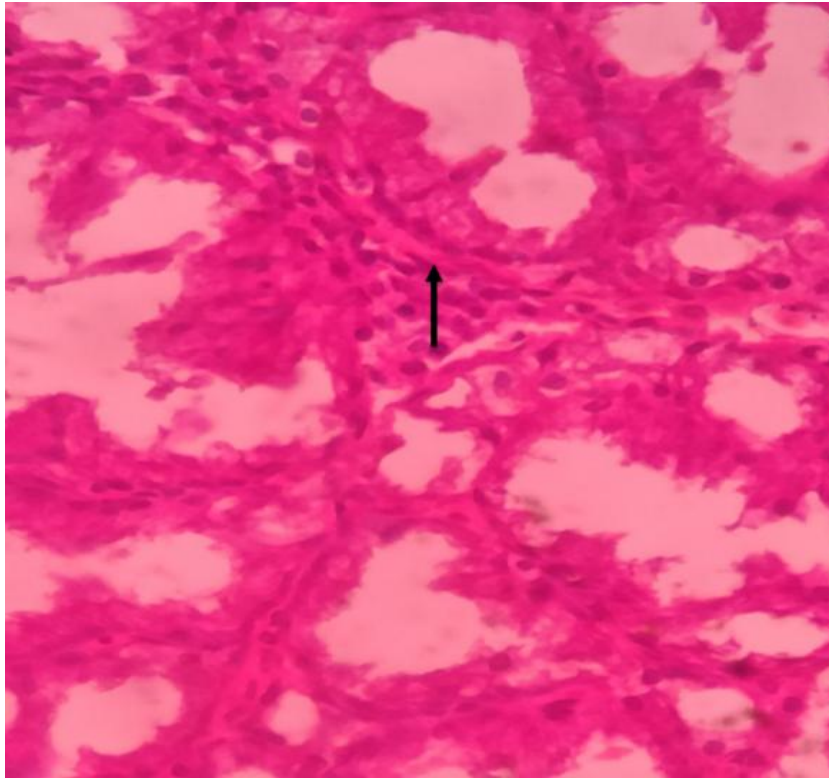


Fig 21: right of HD in dove showed the myoepithelial cells (black arrow) were arranged in discontinuous manner surrounded the secretory units of lobule. H&Estain X400

In the Harderian gland of rooster and dove , the duct system (Fig.22,23)was consisted of:

- a. Intralobular ducts,
- b- Interlobular ducts,
- c- Large ducts
- d- The main excretory duct.

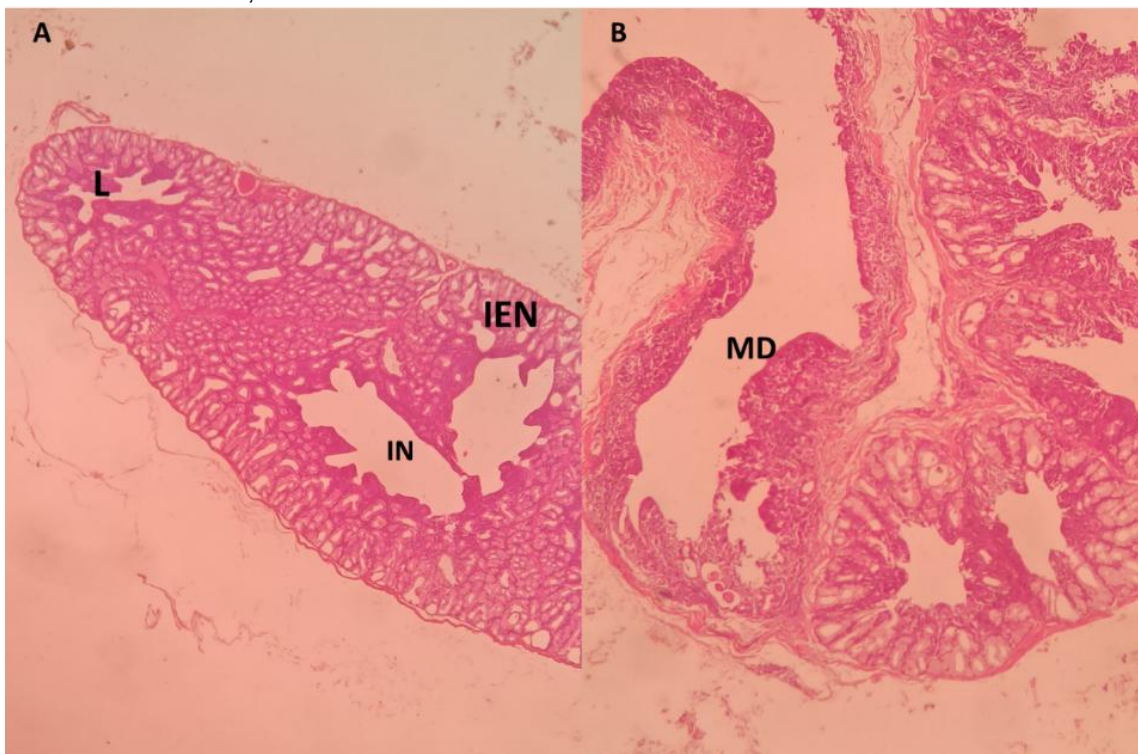


Fig 22: A&B Left of HD in rooster showed. Intralobular ducts(IN), Interlobular duct(IEN) , Large ducts (L), main excretory duct(MD); H&Estain A&B :X100

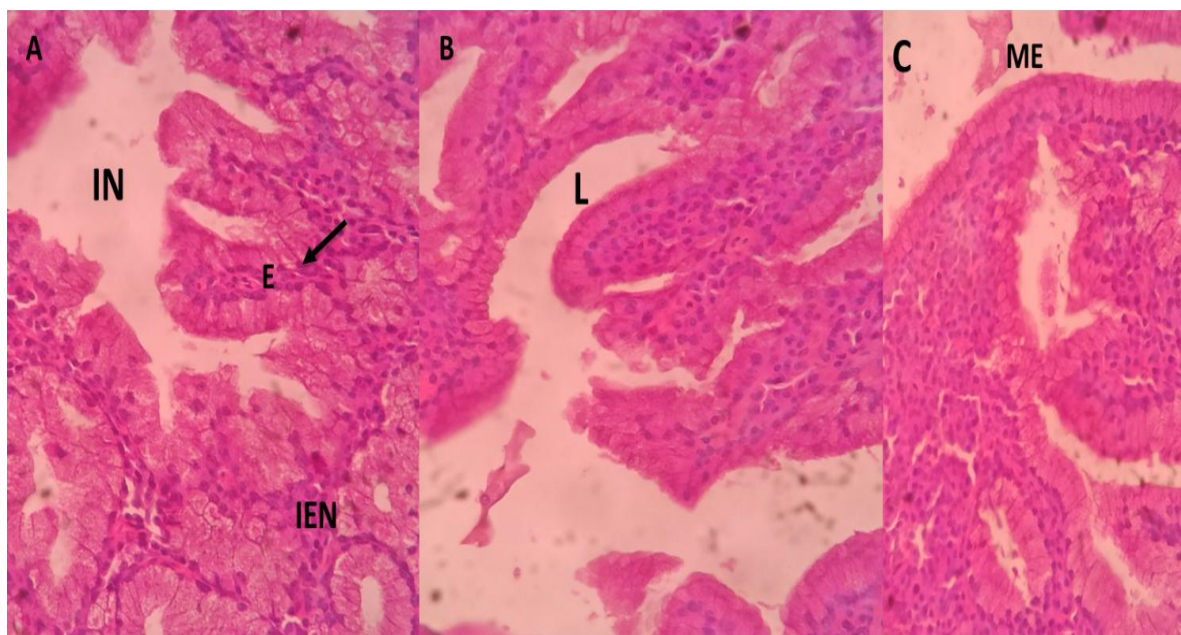


Fig 23: A,B&C; Left of HD in dove showed. Intralobular ducts(IN), Interlobular duct(IEN) , Large ducts (L), main excretory duct(ME); H&Estain A,B&C :X400

The duct system of the left and right lobe of the Harderian gland started with intralobular duct (Fig. 22,23) which was lined with simple cuboidal epithelium that transformed in to low columnar epithelium which reacted positively with AB- PAS stain (Fig.24,25).

These intralobular ducts joined with each others to form the interlobular duct (Fig. 24,25) which was lined by stratified columnar epithelium. Their epithelium reacted positively with PAS stain (Fig. 33,34). These interlobular ducts united to form a large duct called interlobar duct their epithelium reacted positively with AB-PAS stain with the formation of some positive granules (Fig. 24,25). These ducts drained into the main excretory duct (Fig. 24,25). The latter appeared with wide lumen, and their lining, epithelium was low columnar with goblet cells that showed strong AB- PAS positive granules (Fig. 24,25). The main excretory duct opened in the inner surface of the third eyelid which appeared to be formed by a conjunctival fold (Fig. 24,25). Before its opening, their epithelium changed into squamous epithelium.

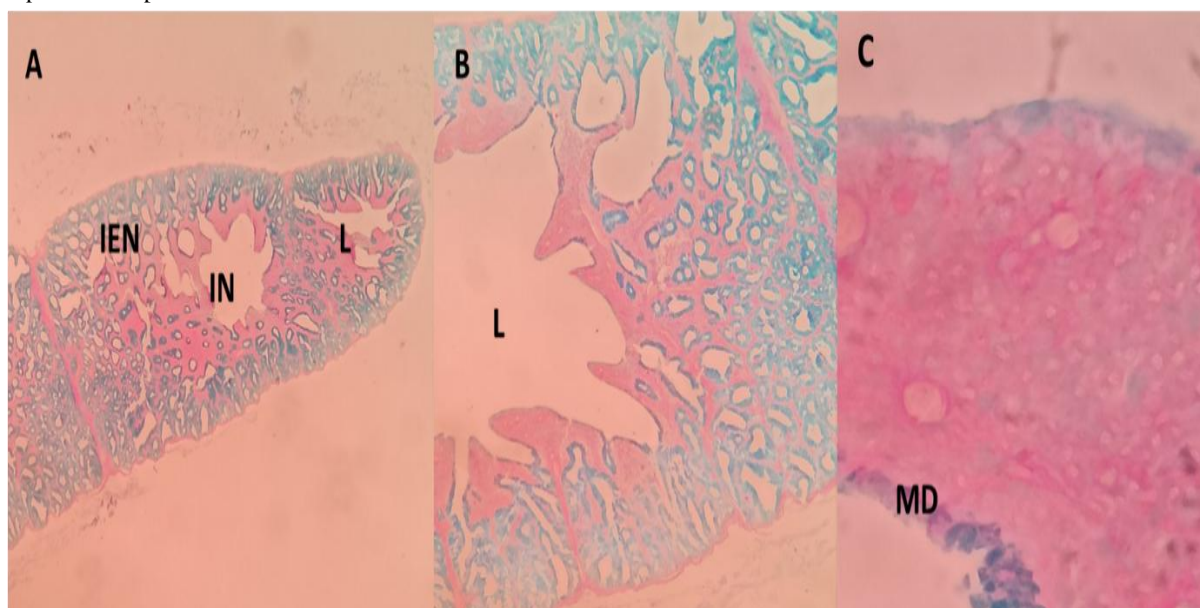


Fig 24: A,B&C; Left of HD in rooster showed. Intralobular ducts(IN), Interlobular duct(IEN) , Large ducts (L), main excretory duct(MD); positive rection for AB-PAS; AB-PAS stain A,X40; B X100 &C :X400

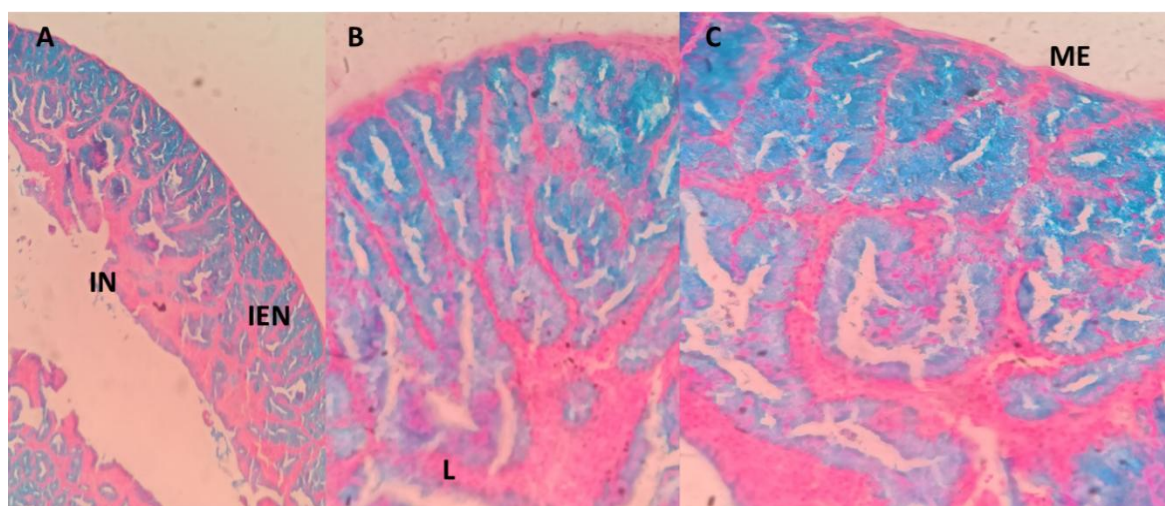


Fig 25: A,B&C; Left of HD in dove showed. Intralobular ducts(IN), Interlobular duct(IEN) , Large ducts (L), main excretory duct(MD); positive reaction for AB-PAS; AB-PAS stain A,X40; B&C :X200

DISCUSSION

The results showed that the Harderian gland in both domestic roosters and male pigeons is a multilobular, tubular-alveolar exocrine gland, consistent with previous studies confirming its complex structure and its immune and secretory functions. Histologically, the rooster gland is surrounded by a thick capsule of connective tissue rich in collagen, blood vessels, and nerves, enhancing its protective and nutritional functions. In pigeons, the capsule is thinner and contains lymphocytes, indicating a more pronounced immune role. Such densely vascular capsules have been previously documented in similar anatomical studies on chickens (14, 15). In contrast, the capsule of the Harderian gland in showed a thinner structure containing the same components but with lymphocytes within the connective tissue, reflecting a clear immune role for the gland. (3 and 16) suggested that the Harderian gland in birds contains a rich lymphoid tissue that may be part of the mucosal immune system (MALT). Histochemical analyses showed negative results with alcian blue staining, indicating the absence of acidic mucopolysaccharides, while they showed a positive reaction with PAS, indicating the presence of glycogen and neutral glycoproteins, reflecting the non-mucosal secretory nature of the surrounding connective tissue. The Harder's Gland Parenchyma of the Domestic Rooster and Dove, The present study revealed clear differences in the histological and histochemical composition of the Harder's gland parenchyma between the domestic rooster and the dove, reflecting differences in functional and immunological composition between the two species, In the domestic rooster, the gland was characterized by a relatively large structure, consisting of distinct tubuloalveolar lobes lined by simple columnar or cuboidal epithelial cells. These cells contained lipid droplets and showed a strong positive reaction with Oil Red O, confirming the presence of a large amount of lipids within the secretory cells. This is consistent with previous studies, such as that described by (3), who suggested that the gland may contain high concentrations of lipids, which are thought to contribute to protecting the eye from environmental factors such as dehydration and infection and (17). In the male dove, the gland was smaller, but it was characterized by the presence of more closely spaced, more striated lobes, with a more regular tubular shape. The lobes were lined with simple cuboidal epithelial cells containing mucin vacuoles within the cytoplasm. These vacuoles showed strong positive reaction with PAS (indicating the presence of glycogen-rich mucus) and Alcian Blue (indicating the presence of acidic mucus), which is consistent with the reports of (18, 19 and 20) on the dual nature of the mucus secreted by the Harderian gland in birds. In the alveolar lumen at the ends of the lobes, lipid droplets, cellular debris (including nuclei), and oval lamellar bodies of porphyrin accumulations were observed, which showed strong positivity with PAS staining, indicating that they contain lipid and complex polysaccharides that are stainable with periodic oxide-Schiff (21). A broad lumen lined by low columnar epithelium containing goblet cells rich in granules that are strongly positive for both AB and PAS stains, indicating a dense mucus secretion. This epithelium transforms to a squamous layer before opening onto the

inner surface of the third eyelid, formed by a conjunctival fold. This epithelial arrangement ensures continuous hydration and mechanical and microbial protection of the ocular surface, and is consistent with the description given by (22) in his comprehensive study of the gland and its lacrimal-immunological role.

CONCLUSION

The histological differences between the dove and the domestic rooster in Hardier's gland reflect variations in secretory and immunological functions depending on the species and their environmental conditions. The gland in the rooster is more specialized for lipid secretion, whereas in the dove, it exhibits more pronounced immune components, especially in the ducts.

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