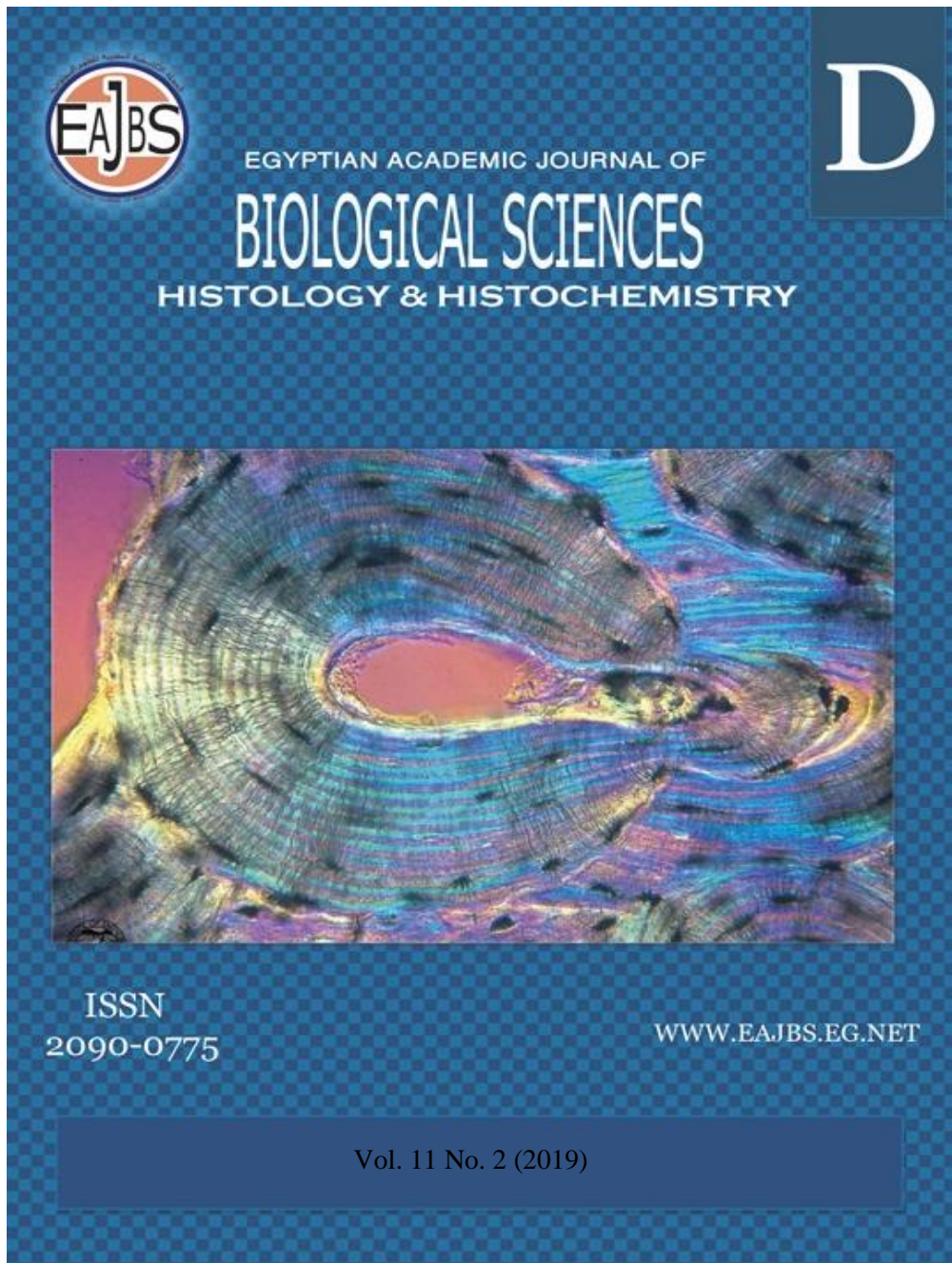


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Age Related Changes in the Cellular Population and Lymphoid Tissue in the Harderian Gland of Turkey

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ABSTRACT

The aim of the current investigation was to identify the cellular changes of the cell populations positioned within the connective tissue of the harderian glands in Turkeys at different ages extended from one day hatched offspring till the age of seven months. To conduct such research, 50 specimens of harderian glands from the above-dated ages of turkey were collected. Post euthanasia and dissection of the birds, specimens were cut and fixed in 10% neutral buffered formalin and Bouin's solution. The subsequent routine histological technique was made on these specimens such as dehydration, clearing, paraffin embedding, block preparation, and sectioning. Tissue sections were obtained and stained with different stains such as hematoxylin-eosin, Masson's trichrome, periodic acid-Schiff (PAS) and Alcian blue (AB).

Microscopic findings showed connective tissue of the harderian gland was populated by three types of cells that were heterophils, lymphocytes and plasma cells. The incidence of each of these cells was changed with advance of the age. During the growth of the birds, a gradual shift was taking place in the domination of these three cell types. The lymphoid nodules were detected only in 40 days old poults, whilst in adult birds the harderian glands were contained the largest number of mature plasma cells. Some plasma cells enclosed distinctly Russell bodies with different sizes and shapes. In conclusion, 4 weeks aged turkeys developed lymphoid tissue in the harderian gland which is a critical age for vaccination in this avian species.

INTRODUCTION

The harderian gland was discovered and nominated for the first time in 1694 by Johann Jacob Harder in deer. Subsequently, the gland was recorded in approximately all animals' species except fishes and some mammals (Rajkhowa *et al.*, 2018; Klećkowska-Nawrot *et al.*, 2015; Dimitrov, 2012; Payne, 1994).

Harderian gland is usually located posterior to the eyeball in close to the bottom of the orbit supplied with one single duct which opens on the surface of the nictitating membrane. Numerous and various functions were attributed to this gland such as eye protection against the bright light and photodynamic process (Funasaka *et al.*, 2010; Reis *et al.*, 2005; Payne, 1994; Sakai, 1981). In fact, the gland may act also as an endocrine gland (Pradidarcheep *et al.*, 2003).

In the past century, the harderian gland was considered one of the head associated lymphoid tissues (Olah *et al.*, 1992). It plays a great role in the local innate immunity to the eye. In avian species, the gland has participated greatly in response against infections and complications of vaccination (Deist and Lamont, 2018; Zakeri and Kashefi, 2011; Dimitrov and Nikiforov, 2005; Salam *et al.*, 2003). Harderian gland takes different shapes such as irregular in chickens, rodents, and mice, horseshoe-shaped in the rats and rabbits (Sohair and Eltony, 2009) and finally tear drop-like in quails (Kozlu and Altunay, 2011).

Histologically, harderian gland is considered a compound tubulo-acinar or tubulo-alveolar in type and is usually provided with a central duct. Their secretory units are lined with simple columnar epithelium (Bejdić *et al.*, 2014; Mobini, 2012; Boydak and Aydin, 2009). Previously several records were indicated that in domestic birds the interstitial connective tissue of the gland was colonized by different cellular types i.e. lymphocytes and large number of plasma cells which were recorded in adult birds (Klećkowska-Nawrot *et al.*, 2016; Reshag *et al.*, 2016; Jahan *et al.*, 2006; Olá *et al.*, 1995). Actually, plasma cells found very important in immunity so that previous immunohistochemical investigations documented their capacity to synthesize immunoglobulin (Ig) such as IgA, IgM, and IgG (Deist and Lamont, 2018; Sohair and Eltony, 2009; Khan *et al.*, 2007; Ohshima and Hiramatsu, 2002; Olá *et al.*, 1995; Olah *et al.*, 1992).

There is a paucity of works focused on the cellular changes of the cells population that could take place within the connective tissue of harderian gland of birds specifically in the growing turkey. Thus, this research aimed to investigate such facts with advance of the age of the turkey because of the importance of harderian

gland as one of the secondary lymphatic organs. The obtained findings will provide informative data for the pathologists interested in avian diseases.

MATERIALS AND METHODS

The research was conducted on fifty healthy Turkeys. The harderian glands were collected from five different groups of turkeys each of ten birds selected according to age that was 1, 2, 3, 4 and 25 weeks post-hatching ages. Birds were hatched and later on reared at one of the commercial turkey farms locally constructed in Diyala city in the east of Iraq. Water *ad libitum* and diets were provided for all birds. Birds of each age were randomly selected from the above farm and transported to the Department of Anatomy /Veterinary Medicine Collage/ Baghdad University where they were decapitated in accordance with the ethics guidelines. The heads of birds were cautiously dissected and the whole harderian glands were detached and washed with normal saline and then immersed in 10% neutral buffered formalin for 48 hrs. For future staining with histochemical stains, some specimens were fixed by Bouin's solution for 16 hr. In the next step, specimens were dehydrated through ascending series of ethyl alcohol (70%, 80%, 90%, and 100%) each for 2 hrs, then cleared with xylene for ½ hr. Specimens were infiltrated with paraffin wax (58 °C) then embedded with new paraffin wax to obtain blocks of paraffin. Paraffin sections of 6 µm were prepared by using rotary microtome. Tissue sections were stained with hematoxylin-eosin for general histological examination, periodic acid Schiff (PAS) and Alcian blue to detect the type of mucopolysaccharides and to recognize Russell bodies in the plasma cells. In addition to that, Masson's Trichrome stain to identify the collagenous connective tissue in

the stroma of the gland (Vacca, 1985). Histological slides were photographed using the color USB 2.0 digital image system (Scope Image 9.0) which was provided with image processing software.

RESULTS AND DISCUSSION

The Harderian gland of turkey was tear-like in shape with pinkish to reddish color changed to pale white in formalin-fixed organs (Fig.1). The gland adhered to the floor of the orbital cavity by connective tissue just behind the eyeball. It took the ventromedial side and was ended with narrow head that was connected to a single duct. The morphological description and location were similar to those observed in the same gland in the quails (Kozlu and Altunay, 2011) and in osprey (Kozlu *et al.*, 2010). Morphologically the gland's shape in turkey was different than what recently recorded in pheasant. The gland in the latter bird was characterized by wide extremity and narrow middle portion (Klećkowska-Nawrot *et al.*, 2016). Grossly, the turkey's harderian gland was also different from the same gland in broilers and native chickens where they have possessed a bilobed gland instead of one lobed gland as in the currently studied turkeys (Jahan *et al.*, 2006).

Microscopic examination of hematoxylin and eosin-stained tissue sections of turkey's harderian gland revealed a compound tubular-alveolar glandular parenchyma. The gland was covered with a thin fibrous connective tissue capsule from which fine septae were originated and entered into the interior of the gland dividing it into many irregulars and unequal sized lobules. Each lobule was constructed from many tubulo-acinar secretory units provided by one central duct. The latter duct was lined with columnar cells characterized with lightly stained cytoplasm and darkly stained rounded or oval-shaped basally located nuclei (Fig. 2). The lumen of the central duct

was irregular in shape and certainly in the diameter. This histological organization of the gland in turkey was similarly recorded recently in the harderian glands of geese (Boydak and Aydin, 2009) and in that of the native chickens (Mobini, 2012). This histological picture was constantly encountered in all studied post-hatching aged turkeys. Actually, the differences between the studied ages were noticed only in the cell types and their distribution within the stroma. These cells were observed in the interstitial connective tissue present between the secretory units and those occupied the central parts of the lobules of the gland. In the first week after hatching, the connective tissue of each lobule was colonized by heterophils granulocytes and only few lymphocytes were recognized among them (Fig. 3). This result was in agreement with those records of Survashe and Aitken (1978) in avian species and Bejdić *et al.* (2014) in the chicks of laying hens. The latter reference recognized heterophils in the connective tissue of the studied gland in the birds aged few days post-hatching.

In the harderian glands of turkey's poults aged 2 to 3 weeks, the interstitial connective tissue was infiltrated by lymphocytes and in contrary heterophils was diminished in number or even disappeared (Fig. 4). These cellular changes were similarly encountered in the harderian gland of chickens where marked decrease in the heterophils granular cells in the interstitial connective tissue after the first week of age (Bejdić *et al.*, 2014).

In turkey's poults aged four weeks, lymphoid nodules were noticed in the subepithelial region of the central lobular ducts. Far from the duct connective tissue, it showed many plasma cells and lymphocytes. The plasma cells possessed Russell bodies that were continuously increased with advanced age (Fig. 5). The cellular changes concerned plasma cells came

in accordance with Scott *et al.* (1996) postulation. The reference supposed that proliferation and differentiation of plasma cells may reflect the effects of some factors secreted from the surrounding gland stroma.

Turkeys that aged up to 28 weeks and more, plasma cells were numerous and formed the majority of the cellular population in the interstitial stroma of the harderian gland (Fig. 6). Such characterization of cellular organization was in agreement with the findings of Jahan *et al.* (2006) in the harderian glands of native chickens; Klećkowska-Nawrot *et al.* (2016) in Capercaillie and Reshag *et al.* (2016) in pigeons.

Histochemical staining with the combined PAS-AB (pH 2.5) showed that the cytoplasm of the columnar cells in the lining epithelium of the secretory units and the central duct gave a positive reaction with AB stain only (Fig. 7). This positive reaction indicated acidic mucopolysaccharides contents in the secretory columnar cells, whereas, negative reaction with PAS indicated absence of neutral mucopolysaccharides. These reactions were also documented in pigeon's harderian glandular parenchyma (Reshag *et al.*, 2016) and in osprey (Kozlu *et al.*, 2010).

Partially different to the current findings, some previous references such as Klećkowska-Nawrot *et al.*

(2016), Boydak and Aydin (2009) and Mobini (2012) recorded positive reaction toward both PAS and AB stains in the columnar secretory cells which lined the glandular acini of the harderian glands of pheasant, geese and native chickens, respectively.

In animal species such as pig, the gland was totally different from that of the turkey's gland by their positive reaction toward the PAS stain (Rajkhowa *et al.*, 2019).

Currently, the turkey's glands at 4 weeks of age and older showed plasma cells with Russell's body gave a positive reaction toward PAS stain. The plasma cells contain cytoplasmic granules of the pink to red color in different sizes (Fig. 5). This reaction was also recognized in harderian glands of other avian species such as laying hens (Bejdić *et al.*, 2014); pheasant (Klećkowska-Nawrot *et al.*, 2016) and pigeon (Reshag *et al.*, 2016).

Conclusions: The study findings supposed that in turkeys, the harderian gland can be considered an important lymphoid organ at 4 weeks of age and such period is very critical to perform vaccination in this species.

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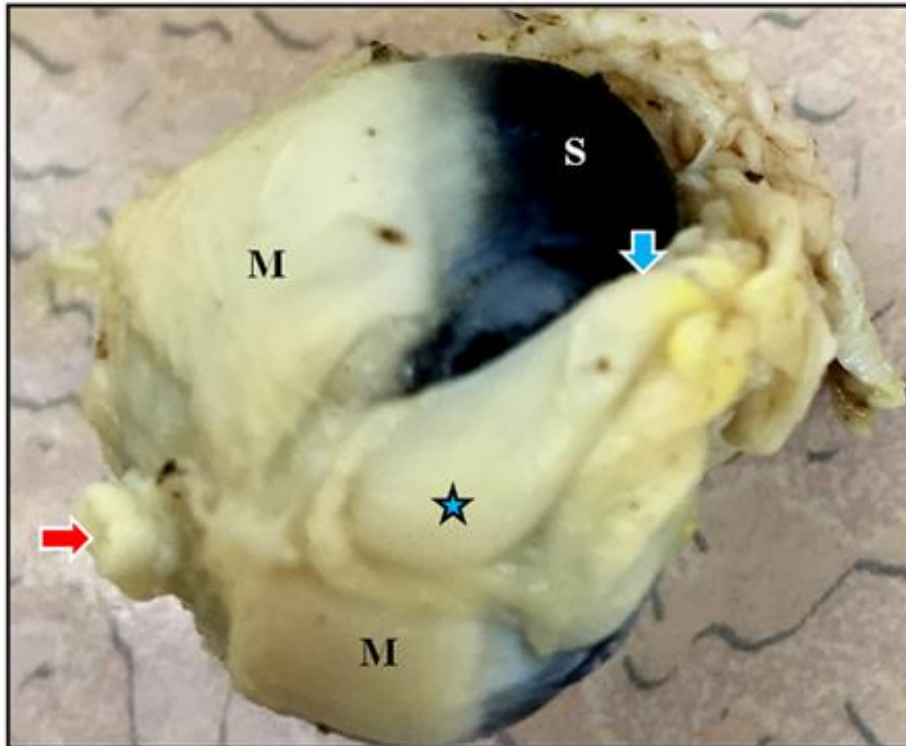


Fig. 1. Gross appearance of the Turkey's eye showed Harderian gland (blue star), main excretory duct (blue arrow), muscles (M), optic nerve (red arrow) and sclera (S)

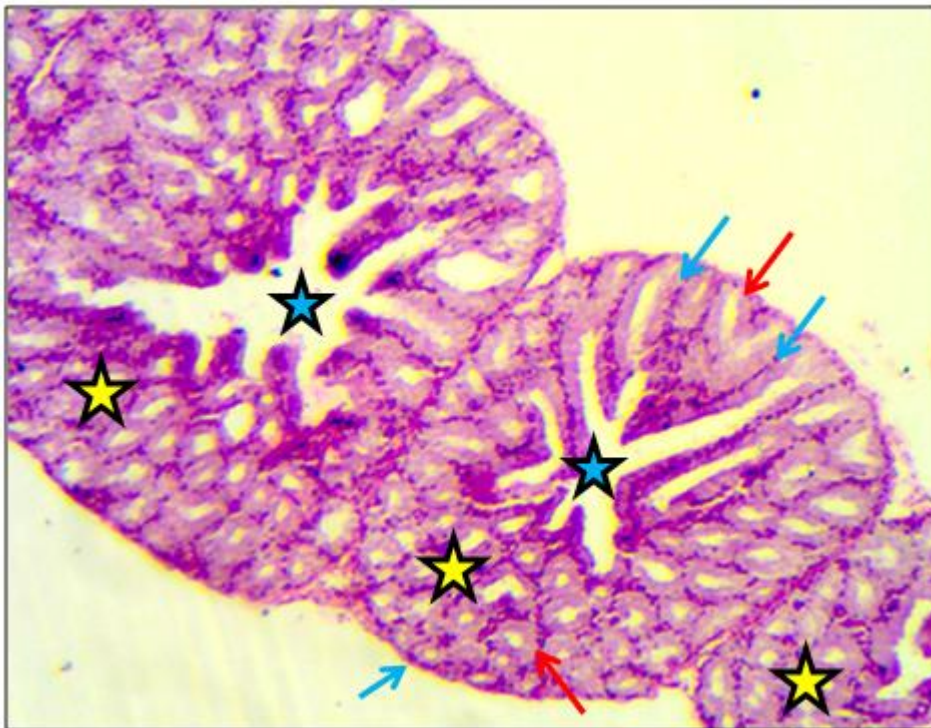


Fig. 2. Cross section in the Harderian gland showed central duct (blue stars), lobules (yellow stars), capsule (red arrows) and septae (blue arrows). H&E stain, X 40

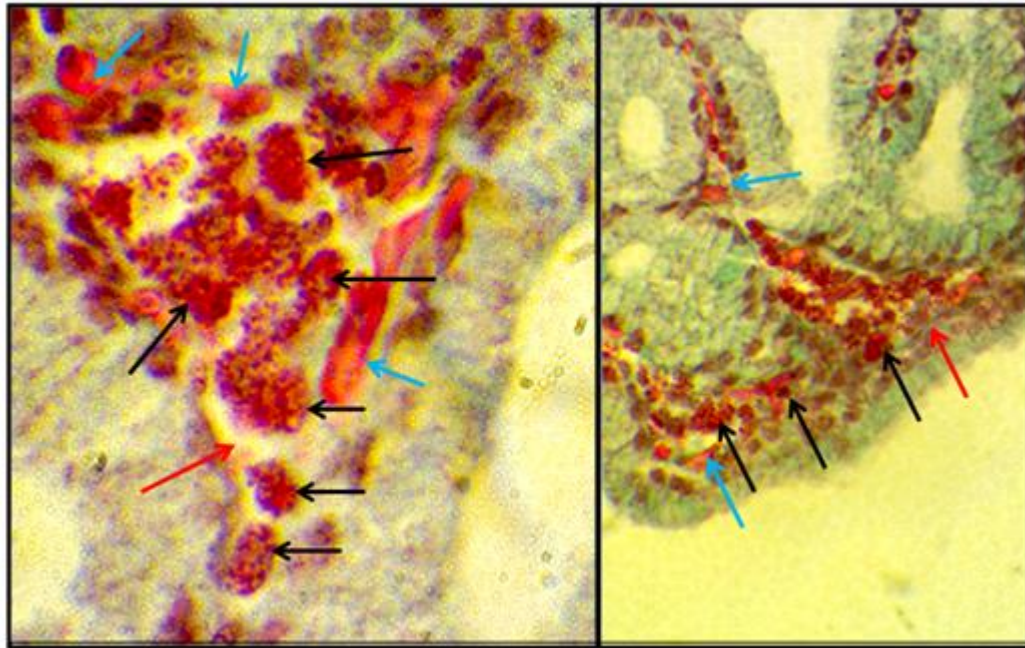


Fig. 3. Cross section of harderian gland showed numerous Heterophils granulocytes (black arrows) populated in the interstitial connective tissue stroma (red arrows) and numerous blood vessels (blue arrows). Masson's Trichrome, X1000 (left), X400 (right)

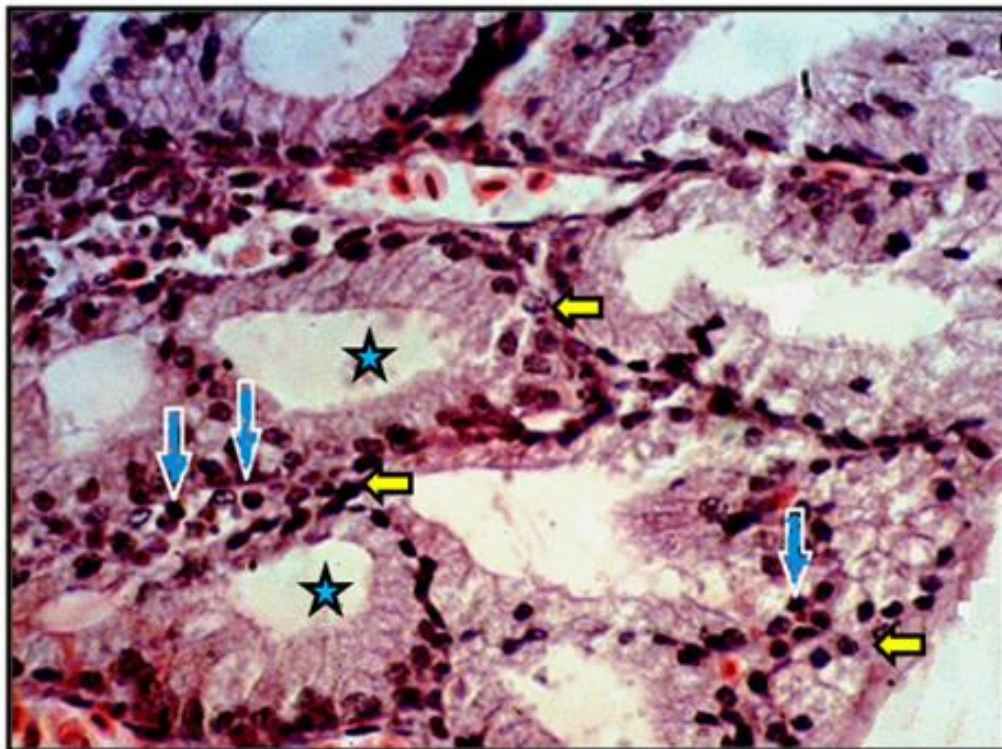


Fig. 4. Cross section of harderian gland showed lymphocytes infiltration (blue arrows) in the interstitial stroma (yellow arrows) around acini (blue stars). H&E, X400

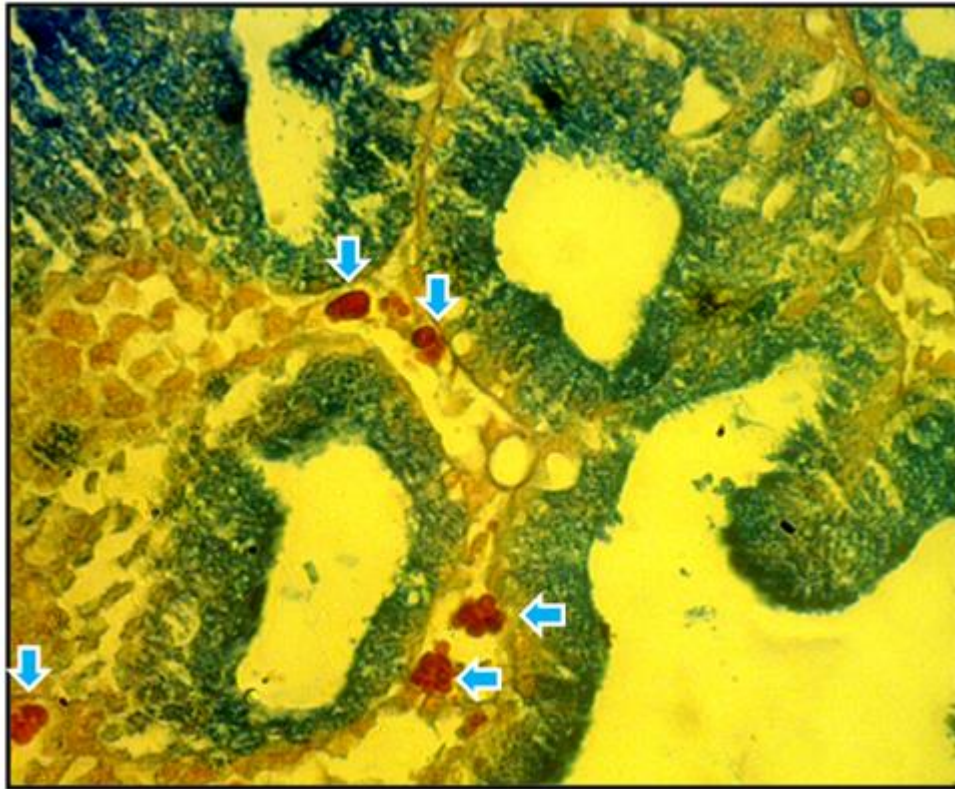


Fig. 5. Cross section of harderian gland showed Russell bodies reacted positively with PAS stain (blue arrows). Combined AB - PAS, X400

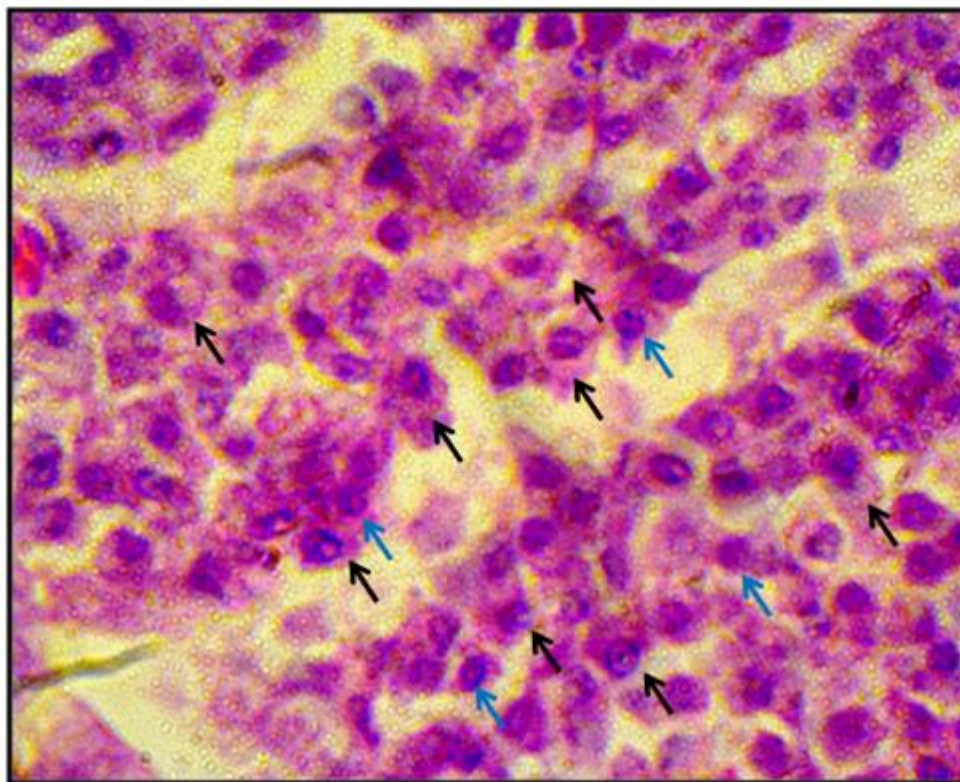


Fig. 6. Cross section of the harderian gland showed lymphocytes (Blue arrows) and plasma cells black arrows). H&E, X1000

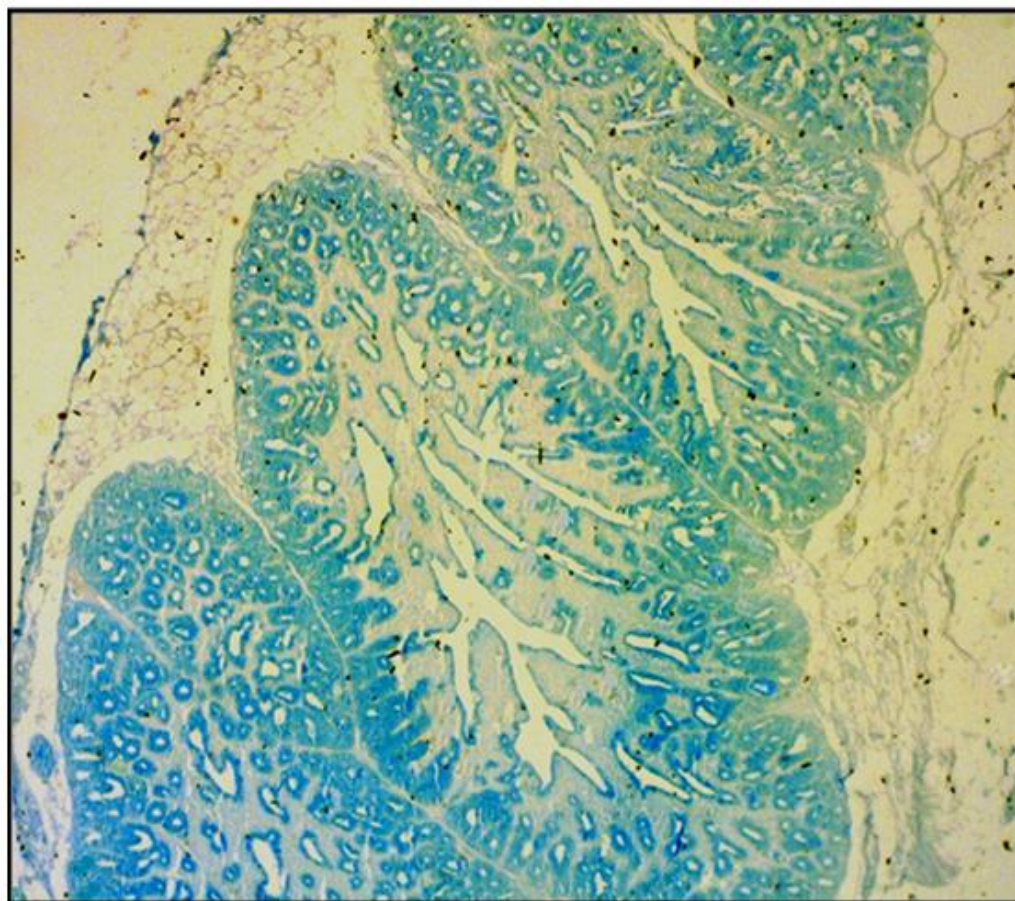


Fig. 7. Cross section of harderian gland showed positive reaction toward Alcian blue stain in the epithelial lining of the acini. AB stain, X40

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