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Macro and micro anatomical study of Harderian gland of pig

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Abstract
In the present investigation, 6 numbers of each apparently healthy adult Pigs were utilized for detailed anatomical study on gross, histomorphological, histochemical and ultrastructural analysis. In the present study, the Harderian gland was observed with the naked eye in the medioventral region of the orbital cavity attached along with the eye ball,a large glandular structure present in dorso ventral direction. The mean size of the gland was recorded as length 29.29±0.88mm. width (vental part) 13.16±0.24mm, width (dorsal part) 10.41±0.22 mm and thickness 7.80±0.22 mm. In the present study, the Harderian gland of pig was a multilobar tubuloalveolar gland with abundances of alveoli and acini. It was covered by a thick connective tissue capsule and contained collagen, elastic, reticular and nerve fibers. The connective tissue capsule also contained blood vessels. The duct of this gland was lined by simple cuboidal epithelium. The connective tissue penetrated from the capsule into the glandular tissue and formed numerous thick and sparse thin septa, and it divided the gland into small and big lobes. The histochemical sections were found positive for PAS Alcian blue 2.5 in Pig but showing very weak reaction glycogen. In the current study, scanning electron microphotograph of harderian gland showed the acini, secretory granules and connective tissue fibers.

Keywords: Harderian gland, pig

Introduction
Three hundred years ago, a scientist named Johann Jacob Harder (1694) reported his discovery of a 'glandula nova lachrymalis', suggesting that the large structure he had identified in deer server to moisten the surface of the eye. Later on, this gland is named as Harderian gland. The Harderian gland forms a unitary structure which is firmly attached to the medial part of the orbit and whose duct normally opens on the surface of the nictitating membrane. The functions attributed to the gland are many and diverse. The harderian gland is act as a part of Head associated Lymphoid tissue (HALT) Olah *et al*. (1992) [1] and serve local innate immunity to the upper respiratory system, to the Eye, and oral cavity. It has been held to be a site of immune responses, a source of thermoregulatory lipids, a source of pheromones, a photoprotective organ and part of a retinal-pineal axis. In north east region the farmers usually keep fowl and ducks as farm livestock along with their routine agricultural activities. Most of the village it is a common culture that the livestock are kept in free range system and take food in scavengering system. They are very prone to infectious diseases as well as zoonotic point of view. Apart from the immunity it has other many functions such as a source of thermoregulatory lipids, a source of pheromones, a photoprotective organ and part of a retinal-pineal axis. So considering these points, an effort has been given to give the basic and primitive information about the gross and microscopical studyof harderian gland in the local Pigs of Assam. By keeping these points in view, the present study was done to study the gross anatomical characteristics, histomorphology, certain histochemical and ultra-structural characteristics of Harderian gland in Pigs

Materials and Methods
In the present investigation, 6 numbers of heads of Pig were utilized. The head of each animals were procured from in an around the market of Guwahati area. The Harderian glands were dissected out from the orbital cavity very gently and the biometry of the gland recorded with the help of vernier calipers (Mc Cance, 1974) [2]. For histological study the tissue samples were fixed in 10% neutral buffered formalin and were processed to prepare paraffin blocks as per the procedure followed by Luna (1968) [3]. The paraffin blocks were sectioned in Shandon

Finesse microtome in 5µm thickness and the sections were stained by different methods as described Luna (1968) [3]. The following staining methods were carried out in the present study:

a. Mayer's Haematoxylin and Eosin stain.
b. Van Gieson's method for collagen fibres.
c. Gomori's method for reticular fibres.
d. Hart's method for elastic fibres.

Different micrometrical parameters were recorded on Hematoxylin and Eosin stained sections by means of standard method of micrometry using Nikon E 200 camera mounted microscope and Image Pro Express Ver-2.0 Software.

For ultrastructral study (Scanning Electron Microscopy) the thyroid glands of seven different groups of *Pati* ducks were utilized. The tissue samples were processed as per techniques of Parsons *et al*., (1991) [4] which was slightly modified by SAIF, NEHU, Shillong. The samples were cut into small pieces of 2 mm size and fixed in 2% glutaraldehyde solution for 4 hours at 4 ºC. The tissue sections were washed in 0.1M sodium cacodylate buffer.3 changes of 15 minutes each at 4 ^{0}C. The tissues were post-fixed in 1% osmium tetroxide in 0.1M sodium cacodylate buffer at 4 ^{0}C. The tissue samples were washed in 0.1M sodium cacodylate buffer 3 changes of 15 minutes each at 4 ^{0}C. Dehydration of the sections was done by ascending grades of acetone. The drying was performed by tetra methyl saline method. The dry specimens were then mounted on aluminium stubs. Gold coating was applied in the tissue samples in a JFC-1100(Joel) ion sputter coater. The stubs with the tissue samples were loaded in the JMS-35CF (Joel) scanning electron microscope operated at 20KV.

For histochemical parameters, tissue samples were preserved in deep freeze maintained at -80^0 C (except for PAS-alcian blue, where paraffin sections were utilized) immediately after collection. They were then shifted directly to cryostat microtome (Shandon Finesse) which was maintained at -22 ^{0}C. The frozen sections were cut at 10 µm thickness and were collected on clean slides. They were temporarily stored at -22 ^{0}C and were then treated as per the method of Singh and Sulochana (1991) [5] for histochemical demonstration of following parameters:

1. Alkaline phosphatase.
2. Acid phosphatase.

Representative tissue samples from the same birds were also processed as per procedure followed by Luna, (1968) [3] and paraffin sections were cut at 8 µm thickness with the Shandon Finesse microtome and were stained for mucopolysaccharides by Periodic Acid Schiff –alcian blue method (Luna, 1968) [3].

Results and Discussion

In the gross study, the Harderian gland was observed with the naked eye in the medioventral region of the orbital cavity attached along with the eye ball (Fig.1), a large glandular structure present in dorso ventral direction. The gland was not having any grossly demercable lobulation but still there was dorsally slightly narrow part whereas the ventral part was somewhat more wider. The mean size of the gland was recorded as length 29.29±0.88mm. Width (vental part) 13.16±0.24mm, width (dorsal part) 10.41±0.22 mm and thickness 7.80±0.22 mm. The mean size of the Harderian gland in Europian Bison was reported by Nawrot *et al*. (2015) [6] and which is almost similar that of the present study.

Histologically, the Harderian gland of pig was a multilobar tubuloalveolar gland with abundances of alveoli and acini (Fig.2). It was covered by a thick connective tissue capsule and contained collagen, elastic, reticular and nerve fibers. The connective tissue capsule also contained blood vessels (Fig.3). The duct of this gland was lined by simple cuboidal epithelium (Fig.4). The connective tissue penetrated from the capsule into the glandular tissue and formed numerous thick and sparse thin septa, and it divided the gland into small and big lobes. These septa contain numerous collagen, elastic, reticular and nerve fibers (Fig.5, 6). The glandular parenchyma contained the alveoli and acini with irregular, wide lumen, which were composed of tall conical cells with round nuclei. Similar observations were recorded by Nawrot *et al.*, (2015) [6] in Bison.

The histochemical sections were found positive for PAS Alcian blue 2.5 in Pig but showing very weak reaction glycogen which were matching with findings of Mobini (2012) [7] in Native chicken of Shahrekord, Iran. The reaction for acid phosphates and alkaline phosphatase were also moderate to intense in the harderian gland. However, the literatures for acid and alkaline phosphates reactions in harderian gland were found nil.

In scanning electron microphotograph of harderian gland showed the acini, secretory granules and connective tissue fibers (Fig.7). The secretions of the gland were accommodated inside the acini and looks like a woolen ball (Fig.7). The present findings were reflected the same findings of Payne, 1994 [8]; McGadey *et al*. 1992 [9] Brownscheidle & Niewenhuis, (1978) [10] in rat.

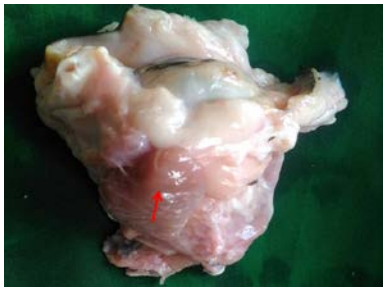

Fig 1: Photograph showing *in situ* position of harderian gland (arrow) of pig.

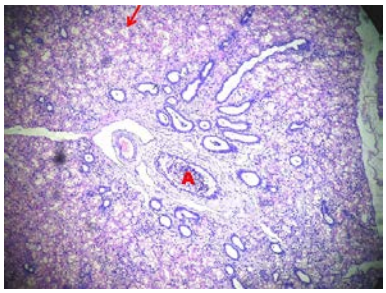

Fig 2: Photomicrograph showing the duct (A) and ACINI (Arrow) of Harderian gland of pig. H&E, 100X

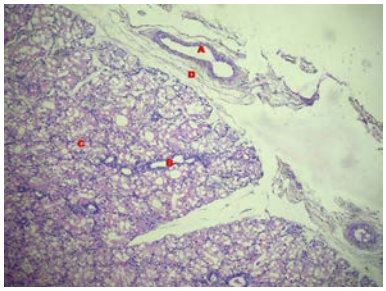

Fig 3: Photomicrograph showing the artery (A) and Capsule (D), DUCT(B) and ACINI(C) of Harderian gland of pig. H&E, 100X

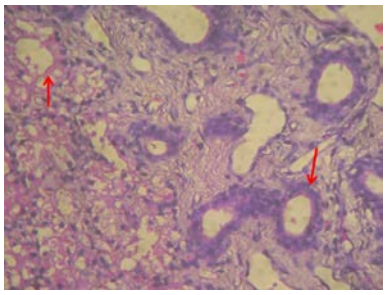

Fig 4: Photomicrograph showing the ACINI (Arrow Top) and DUCT (Arrow Bottom) of Harderian Gland of pig. H&E, 400X

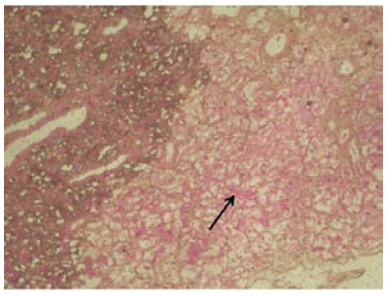

Fig 5: Photomicrograph showing the collagen fibers (Arrow) of Harderian Gland of pig. Van Gieson's Method, 100X

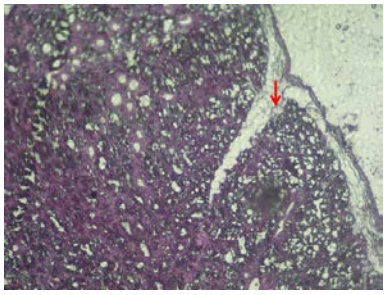

Fig 6: Photomicrograph showing the elastic fibers (arrow) of Harderian Gland of pig. Hart's Method, 100X

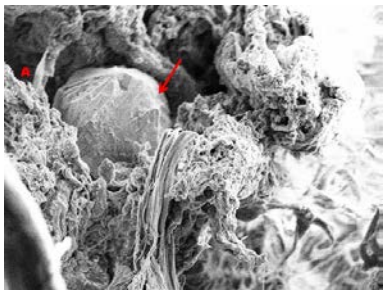

Fig 7: Scanning electron microphotograph showing the ACINI (A) and secretary granules (Arrow) of Harderian Gland of pig. Bar=3µm, 4.99KX.

Conclusion

The present investigation was undertaken to elaborate certain gross anatomical, histomorphological, histochemical and ultrastructural of Harderian gland in Pigs. Since, there is no available literature on the detailed anatomical study on the harderian gland of Pig, the present study was designed to establish some anatomical norms on the Harderian gland. The outcome of this research finding shall help physiologist, pathologist, micro biologist and poultry scientists for carrying out further research work as well as to develop the disease control regime.

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