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Histological and Histochemical Study of the Duodenum structure and cellular distribution of paneth cells in Dogs and Sheep

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I. Abstract:

One of the most important parts of the small intestine is the duodenum where digestion, absorption and mucosal immunity occur. The goal of this research was to determine the histological and histochemical characteristics of the duodenum in dogs and sheep; as well as the cellular localisation of Paneth cells in both animal species. Tissue samples were obtained from clinically healthy adult dogs and sheep. Samples were processed using standard histological techniques. Sections were stained with hematoxylin and eosin (H&E) for routine histological evaluation and also stained with PAS histochemistry to demonstrate mucopolysaccharides and other cells types present. The duodena of both dogs and sheep each have four layers: mucosa; submucosa; muscularis externa; and serosa; The duodenal villi of dogs were tall and leaf shaped, while those of sheep were short and thick. The second difference was in the thickness of the muscularis mucosae of dogs and sheep, where that of dogs was thicker than that of sheep. The muscularis externa was larger in sheep than in dogs. Also, the results of this study found that Paneth cells were more reactive in duodenum of dogs than in the duodenum of sheep according to PAS stain reactivity. In conclusion, these differences illustrate that sheep require prolonged mixing of coarse plant material with mucus for secretion, while dogs require rapid enzymatic digestion of proteins and fat.

Keywords: Duodenum, PAS stain, Small intestine, villi.

II. Introduction

In veterinary medicine, it is important to know the intestinal structure; this helps to diagnose and treat a variety of digestive problems. The intestine is an extremely important part of an animal's digestive system because it helps digest food & absorb nutrients; it is also a major part of the animal's immune system's defence.**(Kadhim et al 2018; van der Flier and Clevers 2009; Bevins and Salzman . 2011; Wehkamp et al. 2004; and Hooper et al . 2003).** A good example of two different animals with different diet types and/or needs are dogs and sheep; therefore, they are used in comparative anatomy and histology studies. By comparing the histological and histochemical properties of the duodenum of these two types of animals, we can better understand how their digestive systems work and also get baseline data that can be used clinically and in research. **(Ruhmaet al 2003; Simawy et al.2024)** The small intestine is a specialised organ that is designed to optimise digestion and nutrient absorption. There are three parts to the small

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intestine: the duodenum, jejunum, and ileum. The walls of the small intestine are composed of four layers: mucosa, submucosa, muscularis externa & serosa. The mucosa is made up of villi that are finger-like projections that provide a greater surface area for absorption. The villi are covered with a layer of cells called simple columnar epithelium that includes enterocytes (adsorbing cells) and goblet cells (cells that secrete mucous).. The lamina propria contains connective tissue, blood vessels, lymphatics, and immune cells, while the intestinal glands (crypts of Lieberkühn) extend into the mucosa and contain various cell types involved in secretion and defense. **. (Wehkamp J. 2004; Hooper LVet al 2003; Wilson CL et al. 1999).**

The submucosa provides support to the mucosa and may contain glands. The muscularis externa of the small intestine is responsible for motility. The duodenum is the first segment of the small intestine and receives the partially digested food from the stomach and facilitates further digestion. Brunner's Glands are the distinguishing histological feature of the duodenum; they are found in the submucosa, secrete an alkaline mucus, and help to neutralise gastric acid and protect the intestinal wal**l. Singh et al.2020; Nowak et al.2023; Boman 1995; Ganz T.2003; Dudás B.2023.** `

The structure of the duodenal mucosa has well-developed villi and intestinal glands; it has many different epithelial cell types including: absorptive cells, goblet cells and specialised secretory cells. The unique coordination of these structures works to allow for efficient digestion and absorption while at the same time assisting in the defence of the mucosa. The histological and histochemical properties of the duodenum provide important functional insight and adaptability to different animal forms. **(Simawy et al.2024)(Al-Arubay2025)(Imeer et al.2023)**

Paneth cells are specialized secretory epithelial cells found in the basal region of the crypts of Lieberkühn in the mammalian small intestine. They are named after Joseph Paneth, who first described them in the late nineteenth century. Structurally, Paneth cells can be distinguished from neighboring secretory cell lineages on the basis of their columnar shape, basolaterally oriented nucleus, and apical secretory granules. Paneth cells arise from the intestinal stem cell compartment, are among the first lineages to differentiate, and generally maintain a stable, differentiated state throughout adulthood **(Behnke and Moe, 1964 Nowak B et al 2023; Boman HG 1995; Ganz T.2003).**

III. Materials and Methods

Tissue Collection and Preparation

Duodenal tissue samples were collected from clinically healthy adult dogs and sheep immediately after slaughter. The specimens were rinsed with normal saline to remove intestinal contents and fixed in 10% neutral buffered formalin for 24–48 hours. Following fixation, tissues were dehydrated in ascending grades of ethanol, cleared in xylene, and embedded in paraffin wax. Paraffin sections of 5–7 µm thickness were prepared using a rotary microtome and mounted on clean glass slides for histological and histochemical analysis.

Routine Histological Staining (Hematoxylin and Eosin, H&E)

Sections were deparaffinized in xylene and rehydrated through descending grades of ethanol to distilled water. The slides were stained with hematoxylin for 5–7 minutes and rinsed in running tap water. Differentiation was carried out using acid alcohol, followed by bluing in alkaline water. Subsequently, sections were counterstained with eosin for 1–2 minutes, dehydrated through ascending grades of ethanol, cleared in xylene, and mounted using DPX. This staining method was used to evaluate the general histological architecture of the duodenum, including mucosal layers, villi, and intestinal glands.

Histochemical Staining (Periodic Acid–Schiff, PAS)

For the demonstration of mucopolysaccharides, sections were deparaffinized and rehydrated as described previously. The slides were then oxidized in 1% periodic acid solution for 5–10 minutes and rinsed with distilled water. Sections were treated with Schiff's reagent for 10–15 minutes, followed by thorough washing in running tap water to develop the characteristic magenta color. Counterstaining was performed using hematoxylin, after which the sections were dehydrated, cleared, and mounted. The PAS reaction was used to identify neutral mucins, glycogen, and other carbohydrate-rich substances in goblet cells and intestinal glands.**(Alhasson et al 2022)**

IV. RESULTS

The duodenum had four layers. The duodenal mucosa was composed of tall and slender villi that had approximately a 3:1 ratio of villus length to crypt length. The surface epithelium of the duodenum was comprised of columnar absorptive enterocytes with an extensive brush border. Occasional goblet cells were observed within the surface epithelium but do not predominate the epithelial surface. The lamina propria of the duodenal villi contained loose connective tissue, capillaries and few inflammatory cells. Submucosal: A thick band of dense irregular connective tissue was the location of the Brunner's glands, which are branched, mucin-producing acinar structures lined by very low columnar to cuboidal cells with light, vacuolated cytoplasm. The Brunner glands were found only occasionally invading into the deep-lying mucosa but were almost completely situated within the submucosa. Muscularis propria: There were 2 distinct layers of smooth muscle identified: 1 outer longitudinal layer that was thinner than inner (and also thicker) layer and an inner circular muscularis layer. Evidence of muscular hypertrophy, atrophy, or intermyenteric muscular variations were absent.

In the sheep, the villi are elongated and thin, resembling fingers, with a small diameter or "lumen". The epithelial layer of the villi is composed of tall columnar cells (enterocytes). The dog villi are leaf-shaped (or spatulate) and wider than those in the sheep. The enterocytes in dog villi are taller than those in sheep villi and give a more columnar appearance. The lamina propra in sheep contains scattered (or sparse) immune cells and a meshwork of reticular fibers. There are

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few lymphoid aggregates and they are scattered throughout. However, in dogs, the lamina proprra contains numerous large aggregations (or clusters) of lymphoid nodules called Peyer's patches that are found within the lamina proprra as well as in the submucosa. The center (core) of the villi contains a lymphatic capillary (lacteal) that is larger than that of sheep. In the submucosa of the sheep, there are also Brunner's glands (duodenal glands) that are present but limited in number and located only at the pyloroduenal junction. They are simple (simple bifurcated tubular) glands that secrete alkaline mucus to neutralise the gastric acid. The submucosa of the dog is extensively, highly developed and contains many lobed, branched tubuloalvolar Brunner's glands that fill most of the submucosa in the duodenum. They protect the mucosa from the highly acidic chime from the stomach. (Figure 1A and B)

Paneth Cell Distribution and Morphology

The Paneth cells were exclusively located at the bottom of the Lieberkühn crypts (Figures 2A-B, descriptive). In longitudinal sections of crypts, they were positioned in the lowest third of the crypt epithelium directly next to the crypt lumen. No Paneth cells were seen on the villous surfaces, the intervillous space above the neck of the crypt, or the acini of Brunner's glands. The frequency of crypts containing Paneth cells occurred at such frequency that nearly every crypt contained Paneth cells in this area of the duodenum. Morphologically, Paneth cells exhibited a pyramidal shape to a columnar shape and had round nuclei located toward the base of the cells. The cytoplasm located at the top (luminal) of the cells contained many coarse bright eosinophilic granules that were all fairly consistent in size (1-2 µm in diameter). The cytoplasm at the base of the cell was less eosinophilic than that of the top of the cell.

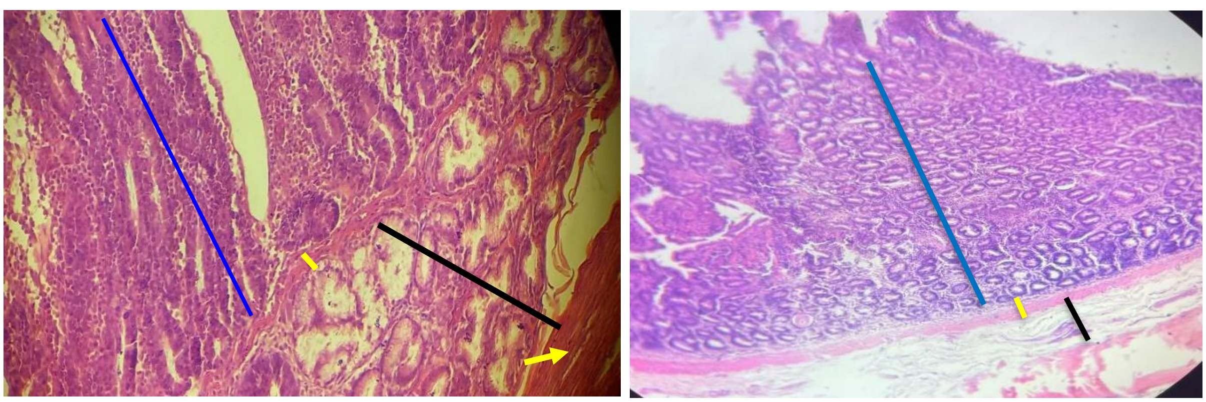

Figure1 A Duodenum in sheep (B) duodenum in dog showed the mucosa (blue line) musclaris mucosa (yellow line) Submucosa (black line), and musclaris externa (yellow arrow)

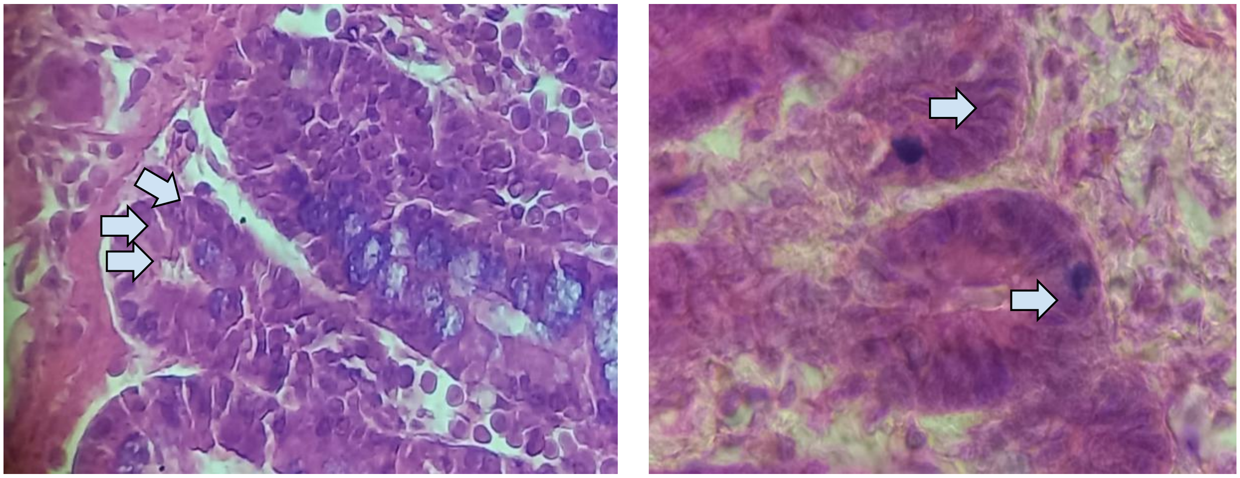

Figure2 (A) Duodenum in sheep (B) duodenum in dog : showed Lieberkühn crypts and Paneth cells location (white arrow) (H&E) (400X)

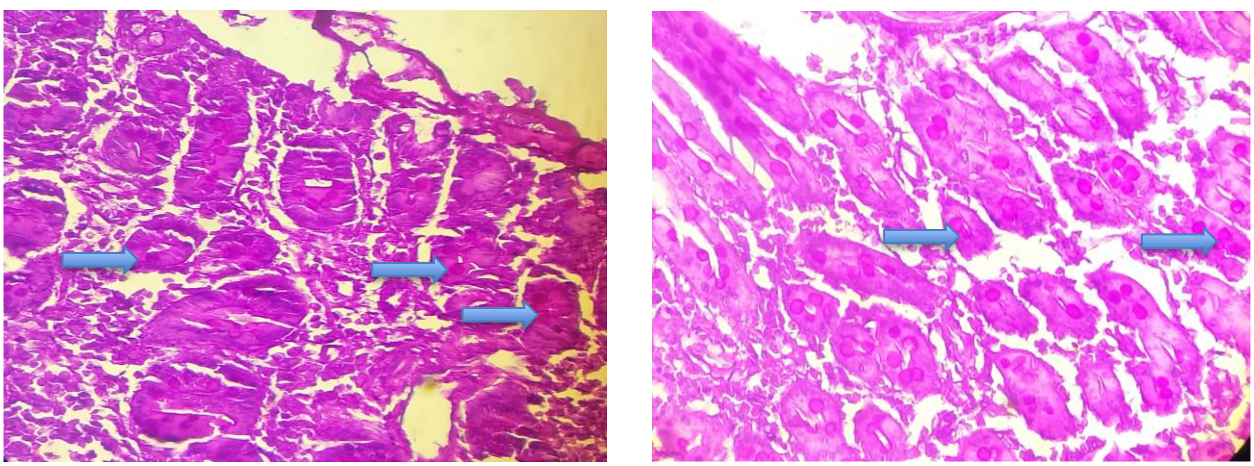

Figure 3: Show the Duodenum in dogs (A) , Duodenum in sheep (B), The paneth cells number in the duodenum sections (blue arrows) . PAS stsin (40X)

Discussion

The differences in histology between the dog and sheep/in, are evolutionary adaptations of both species to the kinds of food they eat.The results of this study appealed that the food types of species is matter in duodenal structure.

In sheep, the great number of goblet cells and the strong lamina muscularis mucosae help the sheep push the thickened, fibrous mass of food which comes from the sheep's ram. Actually, there are fewer Brunner's glands in sheep than there

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are in dogs. However, sheep have continuous flow of food from their rumen, and this buffered food is usually less acidic than what comes from the dog's stomach. Therefore, both have the ability to push their food through their intestines.these results ia inagree with **Ismail, S., 2000**. which mentioned that elemintary trunk is help in moving food and the small intestine is working in reduced the acidity of stomach.

Therefore, the ability for dogs to be able to secrete enough acid (pH 1-2) to auto-digest their food is much greater than that of sheep, and therefore dogs rely on Brunner's glands to produce an alkaline mucous. Since the dog eats dead, decaying animals, this produces a high bacterial count in their rectum, and they therefore need extensive gut-associated lymphoid tissue (GALT).This result was inagree with **Ruhma et al 2003, Cleverset al 2013**
The thickness of doudenal layeris was higher in dog espcially in musclaris mucosa compering with sheep one. The result was ingree with **Porter et al. 2002; Wehkamp Jet al 2005; Salzman et al2003; Laubitz D, et al. 2020.** Also. dogs have folded, leaf-like villi that greatly increase the surface area to absorb simple sugars and amino acids from their very digestible protein very quickly.

Conclusion:

In conclusion, the present study demonstrates that the duodenum in both dogs and sheep shares a common fundamental histological organization, while exhibiting notable
histochemical differences that reflect species-specific functional adaptations. These variations, particularly in mucous secretion and glandular activity, may be closely related to dietary habits and digestive physiology. The findings of this study provide a valuable morphological and histochemical baseline that can support further investigations in comparative veterinary anatomy and gastrointestinal research.

References:

Simawy, M. S. H., Mustafa Fadhil, and Khalid Hadi Kadhim. "Study of Brunner's Glands Using Histology and Histochemistry in Various Age Groups of Cats, Dogs, and Goats'." South Asian Research Journal of Biology and Applied Biosciences 6.05 (2024): 184-93. sarpublication.com

Al-Arubay, Naseer Abdulameer. "Histological and Histochemical investigation of the Brunner Glands in Wild Brown Rats." Kerbala Journal of Veterinary Medical Sciences 1.3 (2025): 70-73. uokerbala.edu.iq

Imeer, Ali Thoulfikar A., et al. "Histological and Histochemical Developmental Study of the Duodenal Glands in Rabbit and Mice During Different Ages." Journal of Natural Science, Biology and Medicine 14.2 (2023): 246-252. jnsbm.org

Hyuk Jang, W., Park, A., Wang, T., Johng Kim, C., Chang, H., Yang, B. G., Joon Kim, M., Myung, S. J., Im, S. H., Ho Jang, M., Kim, Y. M., and Hean Kim, K. "Two-photon microscopy of Paneth cells in the small intestine of live mice." 2018. ncbi.nlm.nih.gov

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https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 231.

Kadhim, A.B., Dali, E.I., Sharoot, H.A. and AbdulRida, M.A.H., 2018. Histomorphological study of the duodenum in goose (*Anser anser*). *Al-Qadisiyah Journal of Veterinary Medicine Sciences*, 17(2), pp.43–48.

Ruhma, M.L., Islam, M.R., Asaduzzaman, M. and Khan, M.Z., 2003. Lymphoid tissue in digestive tract of chicken. *Pakistan Journal of Biological Sciences*, 6, pp.1145–1150

Ismail, S., 2000. Comparative macroscopic and microscopic studies on vertebrates. M.Sc. Thesis, Al-Azhar University, Egypt

Clevers HC, Bevins CL. Paneth cells: maestros of the small intestinal crypts. Annu Rev Physiol. 2013;75:289-311.

Ouellette AJ. Paneth cell α-defensins in enteric innate immunity. Cell Mol Life Sci. 2011;68(13):2215-2229.

Porter EM, Bevins CL, Ghosh D, Ganz T. The multifaceted Paneth cell. Cell Mol Life Sci. 2002;59(1):156-170.

Wehkamp J, Salzman NH, Porter E, et al. Reduced Paneth cell α-defensins in ileal Crohn's disease. Proc Natl Acad Sci USA. 2005;102(50):18129-18134.

Salzman NH, Ghosh D, Huttner KM, Paterson Y, Bevins CL. Protection against enteric salmonellosis in transgenic mice expressing a human intestinal defensin. Nature. 2003;422(6931):522-526.

Yu S, Balasubramanian I, Laubitz D, et al. Paneth cell-derived lysozyme defines the composition of mucolytic microbiota and the inflammatory tone of the intestine. Immunity. 2020;53(2):398-416.e8.

Coutinho HB, Robalinho TI, Coutinho VB, et al. Immunocytochemical demonstration that human duodenal Brunner's glands may participate in intestinal defence. J Anat. 1996;189(Pt 2):393-397.

Lopez-Lewellyn J, Erlandsen SL. Cytodifferentiation of the rat Paneth cell: an immunocytochemical investigation in suckling and weanling animals. Am J Anat. 1980;158(3):285-297.

van der Flier LG, Clevers H. Stem cells, self-renewal, and differentiation in the intestinal epithelium. Annu Rev Physiol. 2009;71:241-260.

Bevins CL, Salzman NH. Paneth cells, antimicrobial peptides and maintenance of intestinal homeostasis. Nat Rev Microbiol. 2011;9(5):356-368.

Wehkamp J, Harder J, Weichenthal M, et al. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. Gut. 2004;53(11):1658-1664.

Hooper LV, Stappenbeck TS, Hong CV, Gordon JI. Angiogenins: a new class of microbicidal proteins involved in innate immunity. Nat Immunol. 2003;4(3):269-273.

Wilson CL, Ouellette AJ, Satchell DP, et al. Regulation of intestinal alpha-defensin activation by the metalloproteinase matrilysin in innate host defense. Science. 1999;286(5437):113-117.

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https://iasj.rdd.edu.iq/journals/journal/issue/20226 https://doi.org/10.54174/utjagr.v13ii 231.

Ciccia F, Bombardieri M, Rizzo A, et al. Over-expression of paneth cell-derived anti-microbial peptides in the gut of patients with ankylosing spondylitis and subclinical intestinal inflammation. Rheumatology. 2010;49(11):2076-2083.

Jayawardena D, Anbazhagan AN, Majumder A, et al. Ion transport basis of diarrhea, Paneth cell metaplasia, and upregulation of mechanosensory pathway in anti-CD40 colitis mice. Inflamm Bowel Dis. 2024;30(5):816-828.

Singh A, Bhardwaj V, Kaur S, et al. Metaplastic Paneth cells in extra-intestinal mucosal niche indicate a link to microbiome and inflammation. Front Physiol. 2020;11:280.

Nowak B, Wanat M, Świątko A, et al. Exploring the microscopic terrain of the small intestinal epithelium: a comprehensive overview of general architecture and the present understanding of intestinal stem cells. Med J Cell Biol. 2023;11(3):87-92.

Boman HG. Peptide antibiotics and their role in innate immunity. Annu Rev Immunol. 1995;13:61-92.

Ganz T. Defensins: antimicrobial peptides of innate immunity. Nat Rev Immunol. 2003;3(9):710-720.

Dudás B. Small intestine. In: Human Histology. Elsevier; 2023.

Alhasson FA, Kareem DA, Shehan NA, Ghaji MS, Abbas BA. Effect of Eggshell Nanoparticles on Healing Bone Fracture. Arch Razi Inst. 2022 Jun 30;77(3):1173-1180. doi: 10.22092/ARI.2022.357392.2032. PMID: 36618278; PMCID: PMC9759247.