

Comparative Histological and Histochemical Study of The Ileum and Paneth Cells in Dogs and Sheep

Abdul-mahdy H. Mahdy¹, Firas A. Alhasson¹, Mustafa S. Ghaji¹

¹ Department of anatomy and Histology, Veterinary medicine college, university of Basrah

Abstract:

Referring to the terminal section of the small intestine, the ileum plays a very important role in nutrient absorption and development of the immune system of the body as well as intestinal homeostasis. The study was conducted to evaluate the histological and histochemical features of ileum of different animal species including dogs and sheep. The collected tissue samples from nearby animal markets were examined histologically using five types of staining techniques including hematoxylin and eosin, periodic acid–Schiff, and immunohistochemical techniques. The study results indicated that the ileum of dogs and sheep has four main layers, including mucosa, submucosa, muscularis externa and serosa. However, the study showed significant differences among these species in thickness of these layers as well as in the morphology of villi and the structure of crypts in the ileum. Regarding Paneth cell presence, dogs were found to have a higher number of the cells at the bottom of the intestinal crypts, which was accompanied by better prepared PAS-positive vesicles in comparison to sheep that had a lower number of Paneth cells and weaker PAS activity. The presence of Peyer patches was higher in case of sheep in the ileum, suggesting differences in intestinal immunity reactions. It seems that the structure of the ileum is dependent on the eating habits of the animals and the developed immunity.

Keyword: Histology, Histochemistry, Ileum, Paneth, Dog, Sheep.

Introduction

The small intestines, which is responsible for digestion and absorption of nutrients in mammals, is divided into three sections: the duodenum, jejunum, and ileum (Gartner & Hiatt, 2015; Kierszenbaum & Tres, 2020). Each section is defined by its own tissue structure, which serves to optimize digestion and ensure maintenance of the mucosa system (Fawcett & Jensh, 2002). The ileum, which is the last part of the intestine, acts as an organ that allows absorption of bile salts, absorption of vitamin B12, absorption of electrolytes as well as absorption of water (Corfield & Myerscough, 2000). Being rich in gut-associated lymphoid tissues significantly contributes to the intestinal immunity (Porter et al., 2002). In structure, the wall of the ileum can be said to consist of four layers, or coats: mucosa, submucosa, muscularis externa, and serosa (Kierszenbaum & Tres, 2020). The mucosa is characterized by the presence of numerous finger-like villi along with intestinal crypts that help in increasing the surface area for absorption while providing a suitable environment for the renewal of epithelia (Clevers & Bevins, 2013). The lining of the epithelium comprises the majorly enterocytes for absorbing food, goblet cells, enteroendocrine cells, stem cells, tuft cells and Paneth cells (Bevins & Salzman, 2011). Through these various cell populations, the mucosal epithelium is rendered intact and protected from microbes (Ganz, 2003).

The Paneth cell is one of the key cellular elements of the ileum crypts. These special secretory epithelial cells are situated in the base of the Lieberkühn crypts (Ganz, 2003) and help in the innate immunity function by synthesizing antimicrobial substances like lysozyme, α -defensins, phospholipase A₂, as well as regenerating proteins (Bevins & Salzman, 2011). Apart from functioning as an antimicrobial agent, Paneth cells support intestinal stem cells by controlling the stem cell niche and supporting epithelial regeneration

The occupation and functioning of Paneth cells differ among various animal species depending on dietary style, intestinal microbiota composition, and physiological changes (Ergun et al., 2003; Tadjalli & Saifzadeh, 2012). Paneth cells are abundantly present in herbivore animals, like sheep (Ergun et al., 2003), but are not present in some carnivore species, like dogs (Hovgaard & Brønstad, 2005). Therefore, research about the histology of carnivores like cats and herbivores like sheep would provide vital information about the relation between gut structure, immune defense, and digestive processes (Ahmed & Jassim, 2020; Varasteh & Kiani, 2019).

Materials and Methods

Experimental Animals

This investigation involved ten healthy adult animals, which included five dogs (*Canis lupus familiaris*) and five sheep (*Ovis aries*). Samples of intestinal tissue were acquired from local slaughterhouses in Basrah, Iraq, for the sheep and from the local market for the dogs. Animals were not included in the study if they had visible gastrointestinal disorders.

Tissue Collection

The ileum was excised in segments of 2 - 3 cm from the mid-portion. The contents of the intestine were washed gently with saline solution in order to eliminate the intestinal contents.

Histopathological Process

The tissue was preserved in 10% neutral buffered formalin for 48 h and dehydrated in increasing concentrations of ethanol (i.e., from 50% to 100%), cleared in xylene, embedded in paraffin wax, and sectioned in 5 μ m using a rotary microtome.

Hematoxylin and Eosin (H&E)

The sections were stained using Hematoxylin and Eosin to assess the general histological structure such as villi, intestinal crypts, Peyer's patches, and Paneth cells.

PAS Staining

Periodic Acid-Schiff (PAS staining) was used to assess the carbohydrate-rich secretory granules in Paneth and goblet cells.

Immunohistochemistry

This section discusses the procedure used in conducting immunohistochemical analysis according to (alhasan et al 2017). During this process, the tissue sections were fixed in formalin and then implanted in paraffin before their sectioning into 4 μ m-thick segments. The sections were then subjected to deparaffinization before being rehydrated using a graded ethanol series. Antigen retrieval was done by heating the segments using sodium citrate buffer for 20 minutes. The activity of the endogenous peroxidase was then blocked by treating the segments with hydrogen peroxide and methanol for 10 minutes before incubation with the normal goat serum for an hour. In the next step, the sections were incubated with the primary antibody targeting lysozyme (Elabscience, China) overnight at 4 degrees Celsius. The sections were then rinsed using phosphate-buffered saline three times and treated to a biotinylated goat anti-rabbit secondary antibody for half an hour. Using Avidin-biotin peroxidase kit and DAB, the antigen-antibody complex was formed. Finally, the sections were counterstained using Mayer's hematoxylin before clearing, dehydrating, and mounting.

Results

Gross Histological Structure of the Ileum: The ileum of both dogs and sheep consisted of four distinct layers: mucosa, submucosa, muscularis externa, and serosa (figure 1). Although the general organization was similar, marked species-specific differences were observed. The canine ileum showed the significant thickness in mucosa, submucosa, and muscularis externa than sheep, while serosa was greater in thickness in the sheep compared with dogs. (table 1). The canine ileum possessed relatively taller villi and deeper intestinal crypts than the ovine ileum. The lamina propria was highly vascularized and contained numerous immune cells. In sheep, villi were comparatively shorter and broader, while the submucosa contained extensive lymphoid tissue associated with Peyer's patches. (Figure 2). The mucosal surface in both species was lined by simple columnar epithelium composed mainly of enterocytes and goblet cells. Goblet cells appeared more numerous in sheep than in dogs, indicating greater mucus production. (Figure 3) The intestinal crypts extended deeply into the lamina propria and contained stem cells, enteroendocrine cells, Paneth cells, and supporting cells.

Paneth cells were identified exclusively at the base of the crypts of lieberkühn. In dogs, Paneth cells were larger and more numerous (50 cell/ mm²), displaying abundant eosinophilic secretory granules. In sheep, Paneth cells were fewer in number (32 cell/ mm²)and exhibited less prominent cytoplasmic Granules. Their nuclei were rounded and

basally located in both species. PAS Histochemistry PAS staining demonstrated intense magenta staining within the secretory granules of canine Paneth cells, indicating abundant glycoprotein-containing secretions. Goblet cells in both species showed strong PAS positivity. Sheep displayed weaker PAS reactivity in Paneth cells compared with dogs. (Figure 4)

Immunohistochemistry Immunohistochemical staining revealed positive lysozyme immunoreactivity in Paneth cells of both species. Dogs demonstrated stronger immunostaining intensity throughout the ileal crypts than sheep. Positive staining was localized mainly within the cytoplasmic secretory granules. (Figure 5)

Discussion

The data reveal a profound species-specific dichotomy in intestinal immune architecture, centered on the relative prominence of Paneth cells versus organized lymphoid tissue. In the dog, the ileal mucosa is characterized by a robust complement of Paneth cells (Kleinschmidt et al., 2008). These cells are larger, more numerous, and demonstrate intense PAS positivity, indicating abundant secretory granules rich in glycoproteins, as well as strong lysozyme immunoreactivity. Lysozyme is a key bactericidal enzyme that plays a crucial role in the innate immune defense of the intestinal crypts by degrading the cell walls of Gram-positive bacteria. The strong presence of these cells in the canine ileum suggests that the dog relies heavily on a Paneth cell-mediated, crypt-autonomous antimicrobial strategy to control the local microbial population (Chandra et al., 2018). In contrast, the ovine ileum represents a striking immunological paradigm. While it possesses Paneth cells, they are fewer, have less prominent granules, and show weaker PAS and lysozyme reactivity (Bautista et al., 2024). This is consistent with the observation of a highly developed and specialized GALT: the continuous ileal Peyer's patch. This massive aggregation of lymphoid tissue is a defining feature of the ruminant ileum, extending up to 150 cm in length. The ileal Peyer's patch in sheep functions as a primary lymphoid organ for the generation of B lymphocytes and undergoes a unique developmental cycle, involuting after puberty. This developmental pattern, which occurs largely independent of exogenous antigenic stimulation, suggests a programmed role in establishing the B-cell repertoire early in life (Cui et al., 2023b). Therefore, the data suggest a fundamental shift in immunological strategy along the intestinal tract of these species. The dog appears to prioritize a local, decentralized defense at the crypt level through an abundance of Paneth cells. The sheep, conversely, demonstrates a highly centralized, organized mucosal immune system through its extensive ileal Peyer's patch, perhaps compensating for a less robust Paneth cell-mediated defense in this particular segment (Özbek & Bayraktaroğlu, 2019). It is noteworthy that the reported absence of Paneth cells in dogs is not universally accepted, with some sources describing their presence. The current findings of large, active Paneth cells in the canine ileum underscore the importance of species-specific and potentially region-specific studies (Prochera et al., 2025).

Table1: shows the average thickness of the main layers in the small intestine (ileum). Unit of measurement: micrometer.

	Mucosa	Submucosa	Muscular Externa	Serosa	Total wall thickness
Dog	1300 µm	350 µm	600 µm	1 µm	2251 µm
Sheep	850 µm	200 µm	400 µm	2 µm	1452 µm

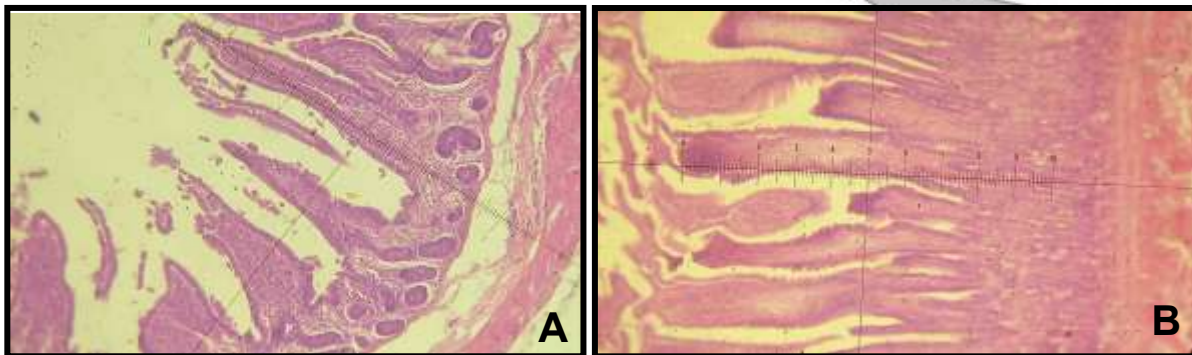


Figure (1): show the histological section of Ilium in dog (A) and sheep(B): Ilium consist of four layers the mucosa yellow line, the submucosa red line, the smooth muscle layer green line, and the serosa black line. (H&E 10X)

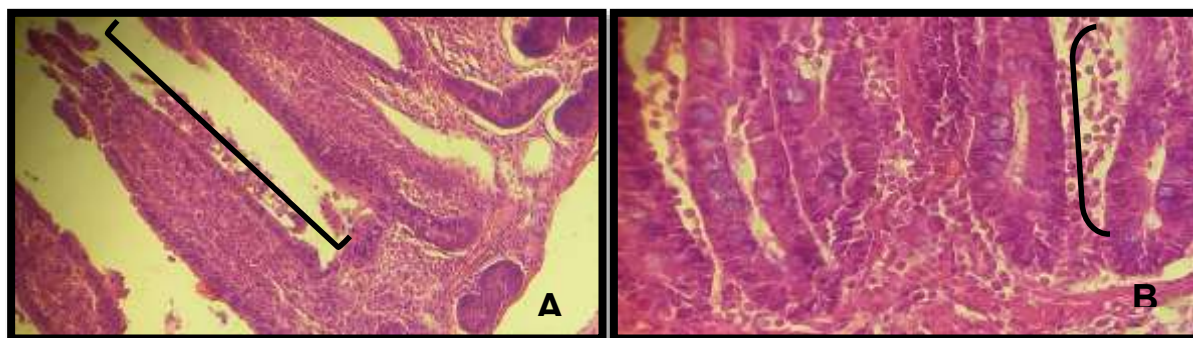


Figure (2): the histological section of Ilium in dog (A) and sheep (B):show the length of villi and depth of crypt of lieberkuhn in ilium (H&E 40X)

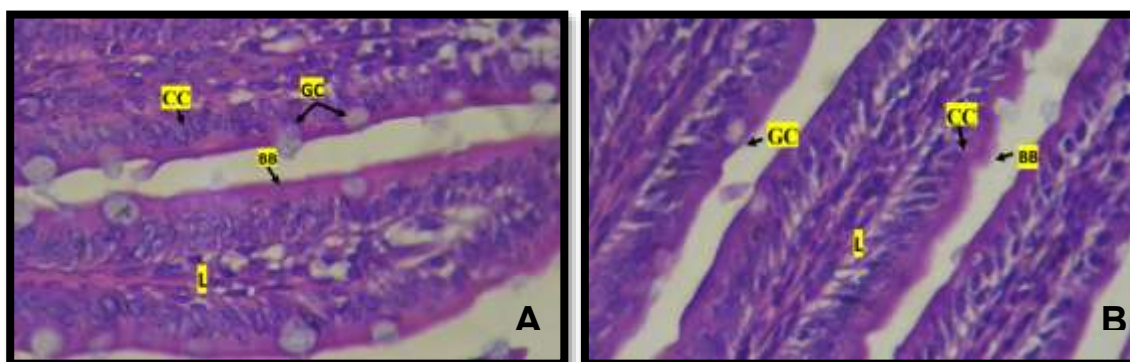


Figure (3): Cross longitudinal for ileum of villi for the dog (A) and sheep (B) show: Columnar cell (CC), lacteal (L) and Goblet cell (GC). H&E 40X

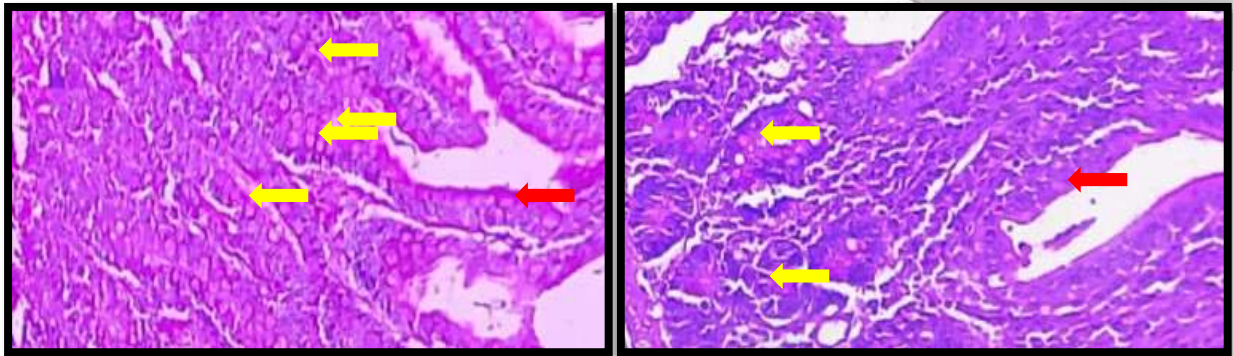


Figure 4: Histological section of Ilium in dog (A) and sheep(B) show the PAS reactions in paneht cells (yellow arrows) and goblet cells (red arrow). The reaction of the PAS was more in dogs compare with the sheep samples. (PAS

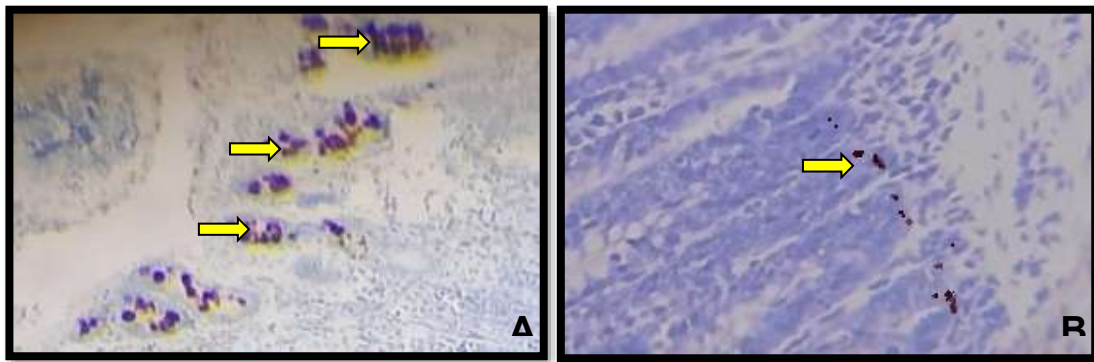


Figure 5: show the Lysozyme reactivity (yellow arrows) in Ilium of dogs(A) and Sheep(B). (IHC of anti lysozyme 10X)

References

1. Bautista, G. M., Cera, A. J., Schoenauer, R. J., Persiani, M., Lakshminrusimha, S., Chandrasekharan, P., Gugino, S. F., Underwood, M. A., & McElroy, S. J. (2024a). Paneth cell ontogeny in term and preterm ovine models. *Frontiers in Veterinary Science*, 11, 1275293.
2. Bautista, G. M., Cera, A. J., Schoenauer, R. J., Persiani, M., Lakshminrusimha, S., Chandrasekharan, P., Gugino, S. F., Underwood, M. A., & McElroy, S. J. (2024b). Paneth cell ontogeny in term and preterm ovine models. *Frontiers in Veterinary Science*, 11, 1275293.
3. Bildstein, T., Charbit-Henrion, F., Azabdaftari, A., Cerf-Bensussan, N., & Uhlig, H. H. (2024). Cellular and molecular basis of proximal small intestine disorders. *Nature Reviews Gastroenterology & Hepatology*, 21(10), 687–709.
4. Chandra, L., Borchering, D. C., Kingsbury, D., Atherly, T., Ambrosini, Y. M., Bourgois-Mochel, A., Yuan, W., Kimber, M., Qi, Y., & Wang, Q. (2018). Adult canine intestinal derived organoids: a novel in vitro system for translational research in comparative gastroenterology. *BioRxiv*, 466409.
5. Cui, C., Li, L., Wu, L., Wang, X., Zheng, Y., Wang, F., Wei, H., & Peng, J. (2023a). Paneth cells in farm animals: current status and future direction. *Journal of Animal Science and Biotechnology*, 14(1), 118.
6. Cui, C., Li, L., Wu, L., Wang, X., Zheng, Y., Wang, F., Wei, H., & Peng, J. (2023b). Paneth cells in farm animals: current status and future direction. *Journal of Animal Science and Biotechnology*, 14(1), 118.

7. Erkert, L., Haag, L.-M., & Becker, C. (2026). Paneth cells as orchestrators of epithelial barrier defense and emerging therapeutic targets in inflammatory bowel disease. *Seminars in Immunopathology*, 48(1), 1.
8. Erkin, K., Nilufar, O., & Sherali, K. (2020). Morphological features of the immune structures of the thin intestine of laboratory animals with various characters of nutrition. *ACADEMICIA: An International Multidisciplinary Research Journal*, 10(7), 537–540.
9. Firas A Alhasson, Suvathi Das, Varun Chandrashekar, Diptadip Dattaroy, Ratanesh Kumar Seth, Mitzi Nagarkatti, Prakash Nagarkatti, Saurabh Chatterjee, Adipokine Leptin mediated NOX-2 promotes kidney inflammation in nonalcoholic fatty liver disease via miR21-dependent mesangial cell immune activation, *The Journal of Immunology*, Volume 198, Issue Supplement_1, May 2017, Page 197.16, <https://doi.org/10.4049/jimmunol.198.Supp.197.16>
10. Garic, M., Vernekar, R., Martín, D. I. Y., Tanguy, S., de Loubens, C., & Loverdo, C. (2025). Intestinal villi and crypts density maximizing nutrient absorption. *ArXiv Preprint ArXiv:2507.03472*.
11. Ginoski, V., Cortés Sánchez, J. L., Kahlert, S., Schulze Holthausen, J., Grześkowiak, Ł., Zentek, J., & Rothkötter, H.-J. (2025). Goblet cells and mucus composition in jejunum and ileum containing Peyer's patches and in Colon: a study in pigs. *Animals*, 15(19), 2852.
12. Huang, Y., Tu, S., Xu, Z., Xu, L., Wang, X., Tian, H., He, Q., Huang, L., Lei, X., & Wang, S. (2025). Loss of Mist1 alters the characteristics of Paneth cells and impacts the function of intestinal stem cells in physiological conditions and after radiation injury. *The Journal of Pathology*, 265(2), 132–145.
13. Işbilir, F., Işbilir, İ., Yavaş, S. E., & Güzel, B. C. (2025). Ultrastructure of the intestinal canal in healthy turkeys (*Meleagris gallopavo*) using light and scanning electron microscopy. *Research in Veterinary Science*, 193, 105805. <https://doi.org/https://doi.org/10.1016/j.rvsc.2025.105805>
14. Jergens, A. E., & Heilmann, R. M. (2022). Canine chronic enteropathy—Current state-of-the-art and emerging concepts. *Frontiers in Veterinary Science*, 9, 923013.
15. Kleinschmidt, S., Meneses, F., Nolte, I., & Hewicker-Trautwein, M. (2008). Distribution of mast cell subtypes and immune cell populations in canine intestines: evidence for age-related decline in T cells and macrophages and increase of IgA-positive plasma cells. *Research in Veterinary Science*, 84(1), 41–48.
16. O'Mara, M., Zhang, S., Fergus, C., Kelly, V. P., & Knaus, U. G. (2026). Epithelial barrier DUOX2 serves as early immune defense in intestinal pathogen control. *Mucosal Immunology*, 16. Özbek, M., & Bayraktaroğlu, A. G. (2019). Developmental study on the ileal Peyer's patches of sheep, and cytokeratin-18 as a possible marker for M cells in follicle associated epithelium. *Acta Histochemica*, 121(3), 311–322.
17. Prochera, A., Muppirala, A. N., Kuziel, G. A., Soualhi, S., Shepherd, A., Sun, L., Issac, B., Rosenberg, H. J., Karim, F., & Perez, K. (2025). Enteric glia regulate Paneth cell secretion and intestinal microbial ecology. *Elife*, 13, RP97144.
18. Tang, Y., Lu, P., Chowanadisai, W., Smith, B. J., Salak-Johnson, J. L., Lucas, E. A., Clarke, S. L., Conway, T., Tang, M., & Lin, D. (2025). An integrated single- cell atlas of the mouse ileum links nutrient metabolism with epithelial and immune crosstalk. *The Journal of Nutrition*.
19. Yu, L.-E., Yang, W.-C., & Liang, Y.-C. (2024). Crosstalk within the intestinal epithelium: aspects of intestinal absorption, homeostasis, and immunity. *Biomedicines*, 12(12), 2771.
20. Zhou, Y., & Hubscher, C. H. (2025). Biomarker expression level changes within rectal gut-associated lymphoid tissues in spinal cord-injured rats. *ImmunoHorizons*, 9(4), vlaf002.