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# Comparative enzyme histochemistry of different histo compartments of GALT of porcine

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**Abstract**
Gut-associated lymphoid tissue (GALT) in pig comprises of Peyer's patches (PP) in the small intestine and solitary lymphoid nodules (SLN) in the large intestine. GALT is responsible for protecting the gut mucosa from invasion of pathogenic microbes, toxins etc. thereby playing an important role in maintaining gut health. Different enzymes are involved in the process of antigen uptake, sampling and subsequent processing of gut luminal antigen by different components of the GALT and detection of the same may be indicative of the status of proper functioning of the GALT. The general organization pattern of GALT of the small and large intestine is different in terms of histocompartments; hence the presence of Histoenzymes might also differ. Sufficient literature is not available on the difference in the histoenzyme profile of GALT of small and large intestine. Therefore, the present investigation was attempted to elucidate the difference in histoenzyme profile of GALT in small and large intestine.

**Keywords:** Peyer's patch; solitary lymphoid nodule; lympho-glandular complex; pig; histochemistry

## Introduction
Gut-associated lymphoid tissue (GALT), *i.e.*, the Peyer's patches of small intestine and the solitary lymphoid nodules (SLN) of large intestine are responsible for the overall mucosal immunity of the animals. Different components of the GALT used to sense the antigenic exposure of the gut mucosa and thereby initiate the protective response or homeostasis or vice-versa. The mucosal immune system in both health and disease is under investigation worldwide by scientists and researchers with diverse expertise and interests (McGhee and Fujihashi[1]). Different histocompartments of the GALT are intricately involved in the process of antigen uptake and sampling from the gut lumen. The antigen uptake and subsequent cascade of immunological events have direct influence on the histoenzyme profile of the GALT. The comparative assessment of the histoenzymes in different histocompartments of GALT of small intestine and large intestine may be indicative of the difference in the immune-anatomy of the organization of the GALT. The Peyer's patches of the small intestine and the solitary lymphoid nodules of the large intestine; which comprise the GALT in pig have been studied in detail with respect to the histology and immunological aspect. Several workers have worked on the activity of alkaline and acid phosphatase, ATPases and nonspecific esterase in the small and large intestinal epithelium. however, literature regarding the comparative histoenzymic activity of different histocompartments of GALT of small and large intestine is scant. Therefore, the present investigation was attempted to study the difference of histoenzyme profile in different histocompartments of PP and SLN of small and large intestine respectively.

## Materials and Methods
The present study was conducted on 12 (twelve) numbers of growing, apparently healthy piglets irrespective of breed, age and sex. The animals were procured from areas in and around Guwahati city. The animals were dewormed and were maintained on standard ration.
The experiment was conducted in the Department of Anatomy & Histology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-22. The animal experimentation was ethically approved by the Institutional Animal Ethics Committee, Faculty of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati-22. The animals were utilized at first for the *in vivo* antigen uptake study; thereafter the animals were sacrificed humanely and the tissue sections having PP and SLN were collected from small and large

intestine respectively and were preserved in liquid nitrogen kept at -196 $^0$C. The frozen tissues were sectioned (6-8 micron thickness) in a cryotome maintained at -22 $^0$C.

The sections were stained for the following histoenzymes:

a. Gomori's alkaline phosphatase cobalt method (Singh and Sulochana [2]).
b. Gomori's method for acid phosphatase (Singh and Sulochana [2]).
c. Lead method for ATPase (Bancroft[3]).
d. Gomori's method for non-specific esterase (Bancroft[3]).

## Results

The cryosections from small and large intestine were stained for alkaline phosphatase, acid phosphatase, ATPase and non-specific esterase activity by respective methods. The results were tabulated in Table-1 and 2.

Samples containing PP and LGC of growing piglets were processed for alkaline phosphatase activity. Alkaline phosphatase activity was intense in absorptive epithelium, dome epithelium of the small intestine (Figure 1-2). The T-cell area and the neck portion of the lymphoid follicle showed a positive reaction. This activity was moderate in dome and follicular area of PP whereas weak to negative activity was observed in interfollicular area and glandular epithelium throughout all segments of small intestine (Figure 4). Alkaline phosphatase activity was moderate in the surface epithelium of duodenum and ileum; while the activity was strong in the jejunum indicating the preferred nutrient absorption site. The reactivity of alkaline phosphatase in the FAE and dome area of PP might be indicative of the presence of energy dependent active transport mechanism at these sites. In large intestine, the activity of alkaline phosphatase was strong to intense in absorptive and dome epithelium and interfollicular area (Figure 3). This activity was moderate in dome and follicular area of PP whereas negative to weak activity was observed in glandular epithelium throughout all segments of large intestine (Figure 1-3).

In the present study; acid phosphatase, being a lysozomal enzyme, activity was intense in glandular epithelium; strong in dome area of PP; moderate in FAE, absorptive epithelium, follicular and interfollicular areas of PP of the small intestine (Figure 5-7). The intense reaction of this enzyme in the glandular epithelium might indicate the presence of lysozomal activity in the respective site. The dome area which is important for antigen capturing after the FAE, might be having lysozyme rich dendritic cells for presentation of particulate antigen and subsequent transport to other immune organs of the body. Moreover, some cells of the lamina propria and some in the epithelial lining were found to be positive for this enzyme activity. In large intestine acid phosphatase activity pattern was similar to that of small intestine (Figure 8-10). But the activity in the glandular epithelium was more pronounced in case of distal colon and rectum. Specifically, the glandular structures in the LGC of rectum showed intense acid phosphatase activity (Figure 9-10.). The presence of lysozyme activity in these regions might be necessary to protect the delicate mucous membrane from antigenic exposure.

The lymphoid follicles of both PP and LGC of growing piglets were found to show intense reaction for adenosine tri-phosphatase activity. ATPase activity was strong in glandular epithelium and interfollicular areas in both small and large intestine, whereas this activity was moderate in FAE, absorptive epithelium and dome in small intestine (Figure 11-14). It was observed that the ATPase enzyme activity can be utilized for compartmentalization of B cell area of PP. This enzyme activity could be proportional to the number of B cell present in the respective compartment. The use of monoclonal antibody for detection of B cell subsets is costly and a time-consuming procedure. It might be conceived that ATPase enzyme histochemistry might give a rough idea on the localization of this type of cell. In the present study, the lymphoid follicles of LGC showed intense reaction for ATPase, strong in interfollicular area and glandular epithelium (Fig.11, 12). These compartments could be assumed to be B cell rich.

In the present study, the lymphoid follicles of the PP and LGC, especially in the neck portion and the inter-follicular area were intense for non-specific esterase activity and reticular reaction was observed. The lympho-reticular tissues were found to take moderate to strong staining throughout the PP and LGC area (Figure 15-20). In the small intestine, NSE activity was strong in glandular epithelium; moderate in absorptive epithelium, dome area and follicular area. In large intestine, this activity was moderate in gland and follicular area; weak in dome and absorptive epithelium and dome area. The sites of non-specific esterase activity were found to be T cell rich. NSE enzyme histochemistry might be useful in marking T cell area which would have otherwise been costly and time consuming by immunohistochemistry.

**Table 1:** Histochemical characterization of histo compartments of Peyer's patch of crossbred growing piglets.

| Histoenzymes | Peyer's patches | | | | | |
|---|---|---|---|---|---|---|
| | FAE/DE | Absorptive epithelium | Gland | Dome Area | Follicle | Inter follicular area |
| Alkaline phosphatase | ++++ | ++++ | -/+ | ++ | ++ | +/++ |
| Acid phosphatase | ++ | ++ | ++++ | +++ | ++ | + + |
| Adenosine tri-phosphatase | ++ | ++ | ++ + | ++ | ++++ | +++/++ |
| Non-specific esterase | ++ | ++ | +++ | ++ | ++ | ++++ |

Gradation for intensity of histochemical reaction:

- = Negative
+ = weak
++ = Moderate
+++ = Strong
++++ = Intense

**Table 2:** Histochemical characterization of histocompartments of LGC of crossbred growing piglets.

| Histoenzymes | Solitary Lymphoid Nodule | | | | | |
|---|---|---|---|---|---|---|
| | FAE | Absorptive epithelium | Gland | Dome | Follicle | Interfollicular area |
| Alkaline phosphatase | +++/++++ | +++/++++ | -/+ | ++ | + + | -/+ |
| Acid phosphatase | +++ | +++ | ++++ | ++ | + + | ++ |

| | | | | | | |
|---|---|---|---|---|---|---|
| Adenosine tri-phosphatase | ++ | ++ | ++ + | ++ | ++++ | +++ |
| Non-specific esterase | + | + | ++ | + | ++ | ++++ |

Gradation for intensity of histochemical reaction

- = Negative
+ = weak
++ = Moderate
+++ = Strong
++++ = Intense

**Fig 1:** Alkaline phosphatase activity in villous epithelium and dome epithelium. Gomori's, x100.

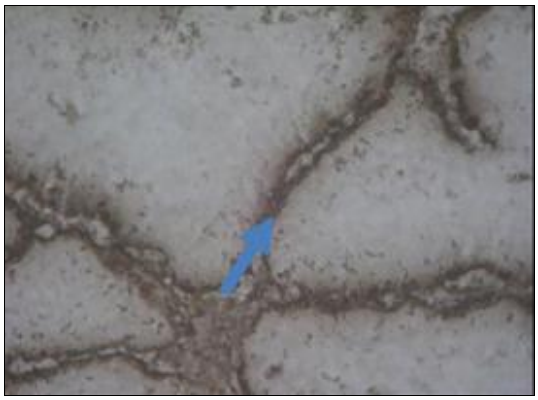

**Fig 2:** Alkaline phosphatase activity in villous epithelium and dome epithelium. Gomori's, x400.

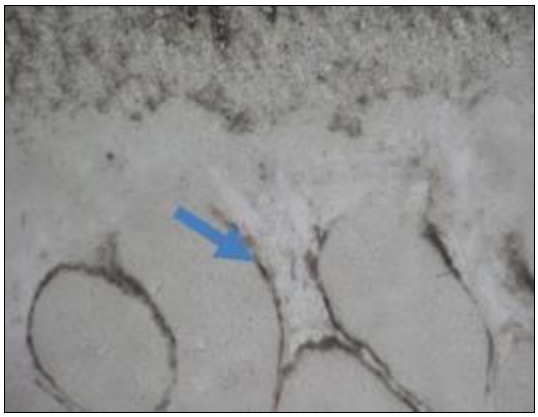

**Fig 3:** Alkaline phosphatase activity in crypt epithelium and LGC follicles. Gomori's, x100.

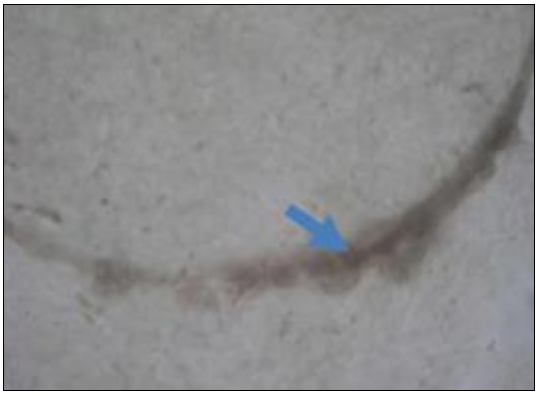

**Fig 4:** Alkaline phosphatase activity in interfollicular area. Gomori's, x400.

**Fig 5:** Acid phosphatase activity in dome and absorptive epithelium and follicle. Gomori's, x100.

**Fig 6:** Acid phosphatase activity in dome and absorptive epithelium and follicle. Gomori's, x100.

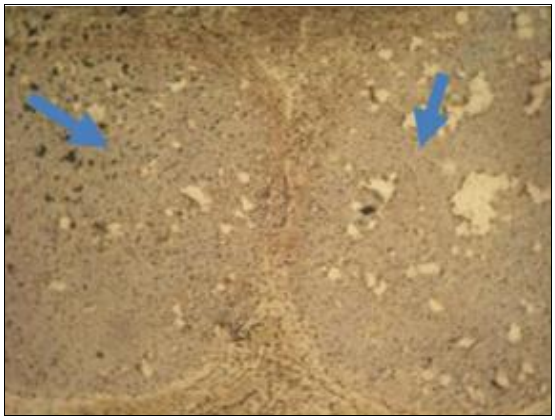

**Fig 7:** Acid phosphatase activity in follicular and interfollicular area of PP. Gomori's, x400.

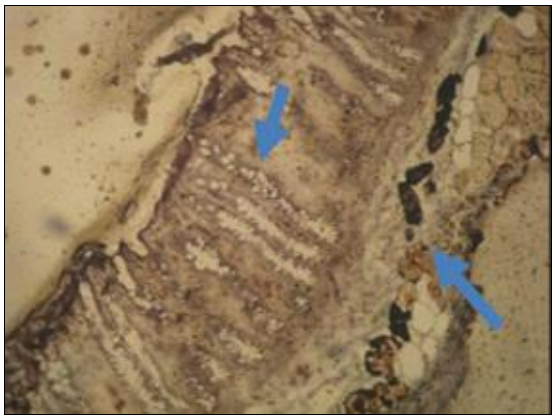

**Fig 8:** Acid phosphatase activity in crypt epithelium, follicular area of LGC, Gomori's, x400.

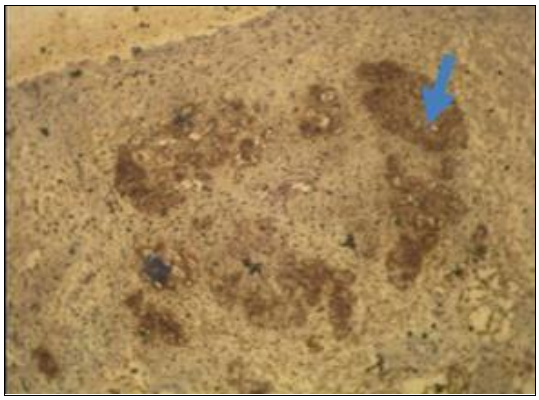

**Fig 9:** Acid phosphatase activity in crypt epithelium, follicular area of rectal LGC, Gomori's, x400.

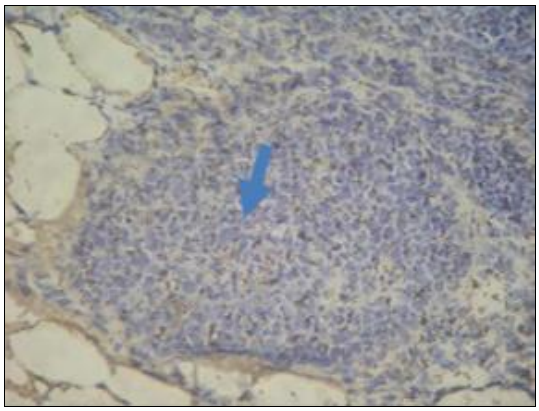

**Fig 10:** Acid phosphatase activity in crypt epithelium, follicular area of colon LGC, Gomori's, x400.

**Fig 11:** ATPase activity in normal and dome epithelium, follicular and interfollicular area of PP, Lead method, x100.

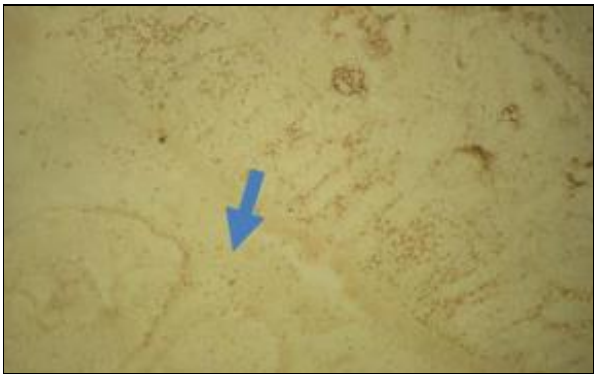

**Fig 12:** ATPase activity in crypt epithelium, follicular and interfollicular area of LGC, Lead method, x100.

**Fig 13:** ATPase activity in follicular and interfollicular area of PP, Lead method, x400.

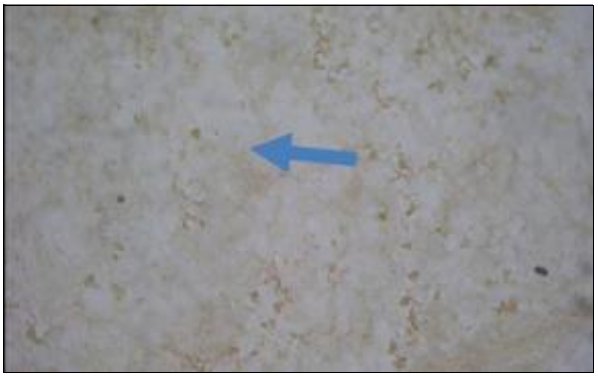

**Fig 14:** ATPase activity in follicular and interfollicular area of LGC, Lead method, x400.

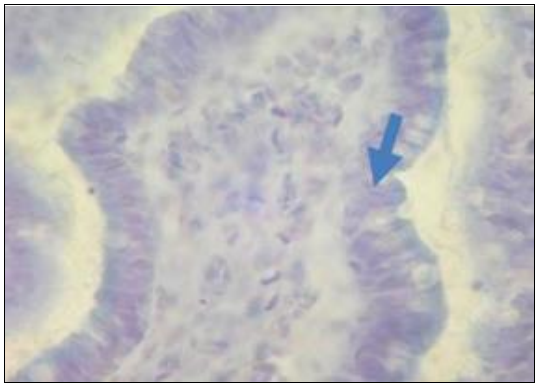

**Fig 15:** NSE activity in dome epithelium and dome area, Gomori's method, x100.

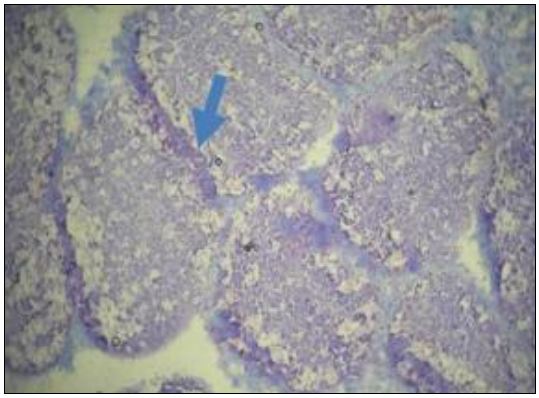

**Fig 16:** NSE activity in follicular and interfollicular area of PP, Gomori's method, x100.

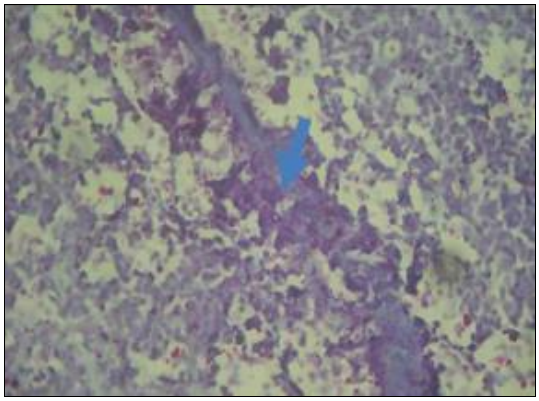

**Fig 17:** NSE activity in follicular and interfollicular area of PP, Gomori's method, x400.

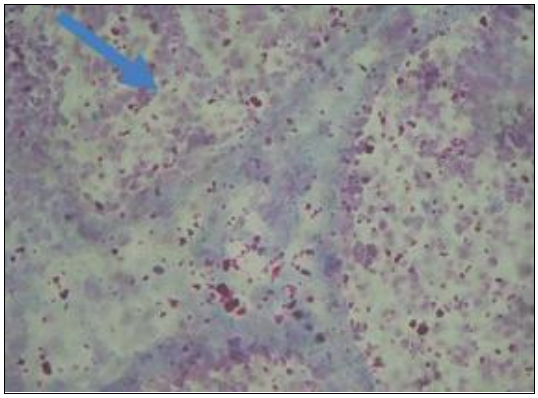

**Fig 18:** NSE activity in follicular and interfollicular area of PP, Gomori's method, x400.

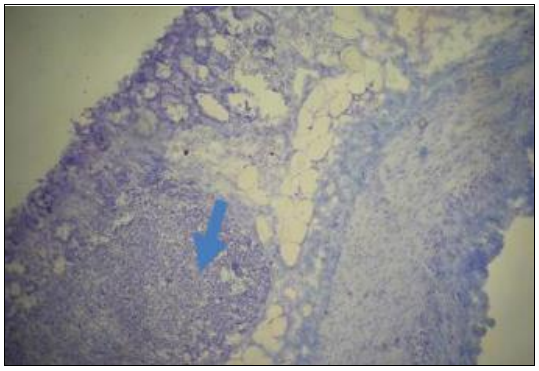

**Fig 19:** NSE activity in crypt, follicular and interfollicular area of LGC, Gomori's method, x100.

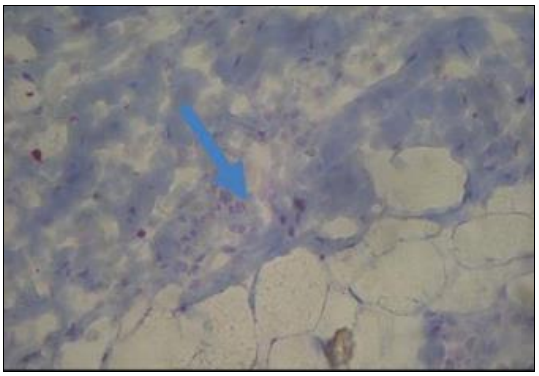

**Fig 20:** NSE activity in follicular and interfollicular area of LGC, Gomori's method, x400

**Discussions**

Alkaline phosphatase activity as observed in the present experiment could be compared with the reports of Halleraker[4] in PP of calf, goat and lamb. Gautam *et al*. [5] found that in the lamina propria of small intestine of adult Assam local goat some areas showed weak enzyme activity. The strong reactivity of alkaline phosphatase in the FAE and dome area of PP might suggest the necessity of energy dependent active transport mechanism. The antigen transport through M cells present in the FAE was transcellular and active as opined by Schulz and Pabst [6]. Similarly, the strong activity of alkaline phosphatase in absorptive and dome epithelium might be indicative of the active transport against this barrier. This finding was in consonance to the findings of Dawson [7] who opined that alkaline phosphatase facilitate transport across intestinal mucosa in human and this was evident in intestinal villi of malnourished children that were devoid of alkaline phosphatase activity. Gautam *et al*. [5] found that the mucosa of cecum, colon and rectum showing weak alkaline phosphatase activity in large intestine of adult goat.

The activity of acid phosphatase, as observed in the present study could be compared with the findings as recorded by Bancroft [3] who opined acid phosphatase enzyme as a lysozomal enzyme. Lelouard *et al*. [8] also reported the function of Lyso-DC in transport of luminal antigen in mice. Halleraker [4] detected acid phosphatase in reticular pattern in interfollicular regions of PP of calf, kid and lamb. In the present study, some cells of the lamina propria and some in the epithelial lining were found to be positive for this enzyme activity and were in consonance to the findings of Gautam *et al*. [5] in goat. This finding was also tuned to the findings of Dawson [7] and Kudweis *et al*. [9]; who reported that acid phosphatases are lysozomal enzymes present within the phagolysosomes of absorptive cells and macrophages within

the lamina propria. Besides Kudweis *et al.* [9] detected acid phosphatase activity in the intestinal crypts in suckling piglets.

The adenosine tri-phosphatase activity of lymphoid follicles of both PP and LGC of growing piglets could be compared with the findings of Halleraker [4] who opined that follicular dendritic cells and dendritic cells of dome and B-cell area were positive for magnesium-dependent ATPase activity, contrasting to a negative reaction at the periphery. ATPase activity was strong in glandular epithelium and interfollicular areas in both small and large intestine, whereas this activity was moderate in FAE, absorptive epithelium and dome in small intestine. Rajkhowa [10] found ATPase activity to be strong in surface epithelium than that of glandular epithelium of small intestine in all the age group of growing piglets. By alkaline phosphatase anti-alkaline phosphatase (APAP) immunohistochemistry technique Mishra [11] and Rajkhowa [10] demonstrated the B cell rich zones of PP in piglets. They found that follicular area and dome area were rich in B cells which were confirmed by ATPase enzyme histochemistry in this study.

The non-specific esterase activity, as observed in the present study could be compared with the findings of Halleraker [4], who described a positive reticular reaction in the T- cell area and the neck portion of the follicles and detected macrophages stained positive with non-specific esterase in ruminants. The sites of non-specific esterase activity were found to be T cell rich which was demonstrated by APAP technique by Mishra [11] and Rajkhowa [10] in pig.

## Conclusion

Different histoenzymes present in different histo compartments of GALT are the indicative of their role in the entire process of antigenic stimulation and subsequent cascade of events. The differences in the histo compartmentalization of GALT illustrate the intricate mechanism of innate immune response at the gut mucosal level and its linkage with the adaptive immunity. The further in-depth study on the histoenzymic profile of the different components of GALT will be helpful for assessing suitable vaccine delivery candidate to induce the mucosal immunity; hence will be helpful for development of mucosal vaccines against different pathogens.

## Declaration of interests

In accordance with *Journal of Entomology and Zoology Studies* publication policy and my ethical obligation as a researcher, I am reporting that I do not have any potential conflict of interest with any individual/organization in publishing this article entitled 'Comparative enzyme histochemistry of different histocompartments of GALT of porcine' in the *Journal of Entomology and Zoology Studies*.

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## References

1. McGhee JR, Fujihasi K. Inside the mucosal immune system. PLOS Biology, e1001397. 2012; 10(9):1-5.
2. Singh UB, Sulochana S**.** A laboratory manual of histological and histochemical technique Kothari Medical Publishing House, Bombay, 1978, 1.
3. Bancroft JD. Theory and Practice of Histological Techniques Churchill Livingstone, Elsevier Health Sciences, 2008, 6.
4. Halleraker M, Landsverk T, Nicander L. Organization of ruminant Peyer's patches as seen with enzyme histochemical markers of stromal and accessory cells. Veterinary Immunology Immunopathology. 1990; 26:93-104.
5. Gautam CK, Talukdar M, Sarma K, Baishya G, Goswami S. Histochemical Characterization of Caprine Intestine and Gut-associated lymphoid Tissue. Indian Veterinary Journal. 2014; 91(11):09-11.
6. Schulz O, Pabst O. Antigen sampling in the small intestine. Trends in Immunology. 2013; 34(4):155-161.
7. Dawson IMP. The value of Histochemistry in the Diagnosis and Prognosis of Gastrointestinal Diseases. In: Histochemistry: The Widening Horizons, Stoward PJ, Polak JM, (Eds.) John Wiley and Sons, New York, 1981.
8. Lelouard H, Fallet M, De Bovis B, Meresse S, Gorvel JP. Peyer's patch dendritic cells sample antigens by extending dendrites through M cell-specific transcellular pores. Gastroenterology. 2012; 142:592-601.
9. Kudweis M, Lojda Z, Julis I. Histochemistry of acid phosphatase in small intestine mucosa in experimental coccidiosis in suckling piglets. Veterinary Medicine (Praha). 1991; 36(2):93-105.
10. Rajkhowa J. Development of small intestine of piglets during preweaning period. MVSc. Thesis submitted to Assam Agricultural University, Jorhat, Assam, India, 2003.
11. Mishra S. Immuno histological study of gut associated lymphoid tissues in pigs. MVSc. Thesis submitted to the Assam Agricultural University, Assam, India, 1998.