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Histomorphological studies on Gut Associated Lymphoid Tissue of pig (*Sus scrofa*)

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Abstract

The submucosa of small intestine, showed Peyer's patches of different sizes and shapes. The lymphoid follicles in the submucosa of duodenum were arranged in single row but in jejunum and ileum, lymphoid follicles were arranged in two irregular rows and separated by distinct inter follicular regions. Each lymphoid follicle showed outer darkly stained area called cortex and inner lightly stained region called medulla, distinct inter follicular area, dome region and follicle associated epithelium. Some of the jejunum and ileal lymphoid follicles showed raised areas called domes which were composed of a broad basal portion and a narrow apical part. These domes were lined by a distinct layer of epithelium called follicle associated epithelium which consisted of columnar enterocytes and few "M" cells but devoid of goblet cells. In large intestine, the number of lymphoid follicles were less and most of the submucosa was occupied by the adipose tissue. The lymph glandular complexes were noted in the lamina propria and submucosa of the colon and in the lamina propria of rectum.

Keywords: Histomorphological, Lymphoid Tissue, *Sus scrofa*

1. Introduction

The pigs are highly adaptable and rapidly growing species that may be attractive for small and marginal farmers for rearing. The majority of pigs are using for meat but also using for skin, fat and other materials. The pigs are biologically very similar to the human being. Because of this biological similarity between pigs and humans, pigs are being used medical research (Lind et al., 2007) [10]. In pigs, the major component of gut associated lymphoid tissue is Peyer's patches (Nickel et al., 1979) [12]. These Peyer's patches contain secondary lymphoid tissue that mounts the immune responses against the ingested antigens (Binns and Licence, 1985) [3]. Peyer's patches are covered with follicle-associated epithelium, which contains morphologically distinct cells ("M" cells) specialized in the uptake of antigens from the lumen to the mucosal lymphoid tissue in which the processing and initiation of immune responses occurs (Valpotic et al., 2010) [17]. The present study was planned to gain the comprehensive knowledge of gut associated lymphoid tissue (GALT) which is essential to understand the defence mechanism of pigs.

2. Materials and methods

The present study was conducted on gut associated lymphoid tissue of 18 exotic and 15 desi pigs. The tissue samples were collected from pigs which were slaughtered at AICRP on pigs, Tirupati and also from local slaughter houses in Tirupati. The tissue samples were fixed in 10% Neutral Buffered Formalin and Bouin's fluid. Later these samples were processed for paraffin sections (Singh and Sulochana, 1997) [16]. About 5-6 µm thick paraffin sections were obtained and subjected to routine and special histological staining methods like Haematoxylin and Eosin for routine histomorphology, Wilder's method for reticular fibres, Verhoeff's method for elastic fibres, Masson's trichome for collagen fibers (Singh and Sulochana, 1997) [16].

3. Results and discussion

In the present study, the four distinct layers were noted in intestinal wall of pig i.e., mucosa, submucosa, tunica muscularis and serosa. It is in accordance with the Dellmann and Eurell (1998) [4] in domestic animals. The submucosa of small intestine showed Peyer's patches of different sizes and shapes. Further, each follicle showed a germinal centre, distinct inter follicular area, dome region and follicle associated epithelium. Similarly, Abe and Ito (1977)

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also reported four different portions in lymphoid follicles of pig viz.; germinal centre, follicular area and parafollicular and dome areas in the lymphoid follicles of mouse which is similar to the present finding in pig.

3.1 Lymphoid follicles

In the present study, the lymphoid follicles in the submucosa of duodenum were arranged in single row, but in jejunum and ileum, lymphoid follicles were arranged into two irregular rows. These follicles were appeared in different sizes and shapes. Kapoor and Singh (2015) [7] also noted lymphoid follicles in multiple layers in ileum of buffalo calves which is similar to the present study in the pig. Whereas Po *et al.* (2005) have noted 3-4 rows of follicles in the jejunum and ileum of calves. In the present study, the submucosa of duodenum showed oval shaped lymphoid follicles of different sizes and the mean height and width of lymphoid follicles was $369.09 \pm 14.26 \mu\text{m}$ and $397.66 \pm 16.57 \mu\text{m}$ in duodenum respectively (Table 1). The presence of lymphoid follicles in duodenum is characteristic feature of pig. Similarly, Abe and Ito (1977) [1] have noted lymphoid follicles in duodenum of mouse. The presence of lymphoid follicles in duodenum indicate its role in defence mechanism of the body. The shape of the lymphoid follicles were oval, elliptical and pear shaped in jejunum (Fig.1) and their height was approximately $366.92 \pm 23.32 \mu\text{m}$ and width $399.23 \pm 31.11 \mu\text{m}$ (Table 1). However, Reynolds and Morris (1983) [14] in sheep noted pear shaped follicles in the jejunum as observed in the present study. In ileum, the Peyer's patches, were distinct and oval to elliptical in shape and separated from each other by fibrous inter follicular tissue. The mean height and width of follicles in ileum was $440.90 \pm 36.72 \mu\text{m}$ and $417.72 \pm 32.37 \mu\text{m}$ respectively (Table 1). These findings suggested that in ileum the follicle size was greater than the size of the follicles present in the duodenum and jejunum and these follicles were also tightly packed. Further, the density of follicles were more in ileum than the other parts of small intestine. In view of the above, in ileum the Peyer's patches appeared as a continuous band like structure. Medina (1981) [11] reported cylindrical to egg shaped lymphoid follicles in gnotobiotic calves and pigs. Whereas, Reynolds and Morris (1983) [14] reported long cylindrical follicles and they also stated that these follicles were tightly packed in ileum of sheep as observed in the present study. In the present study, the lymphoid follicles in duodenum, jejunum and ileum showed outer darkly stained area called cortex and inner lightly stained region called medulla (Fig.1). Each lymphoid follicle showed central pale area called germinal centre. The presence of germinal centres also reported in the lymphoid follicles of the buffalo calves by Kapoor and Singh (2015) [7]. The germinal centre consisted of proliferating lymphoblasts and few mast and plasma cells. These findings were in accordance with the Lalitha (1991) [9] in buffalo calves. However, noted two distinct zones in germinal centre viz.; central light zone and basal dark zone in calves. Central light zone composed of lymphoblasts, reticular cells and mast cells and basal dark zone consists of small lymphocytes and lymphoblasts. But, in the present study there is no clear cut demarcation within the germinal centres. In the present study, some of the jejunum and ileal lymphoid follicles showed raised areas called domes which were composed of a broad basal portion and a narrow apical part (Fig. 2). These findings were in accordance with the Medina (1981) [11] in gnotobiotic calves and pigs. The epithelium over the dome region was devoid of villi and goblet cells. The

absence of goblet cells in the domes is in accordance with the findings of Dellmann and Eurell (1998) [4] in domestic animals, Medina (1981) [11] in gnotobiotic calves and pigs, Shuchi and Singh (1996) [14] in dog. The lymphoid follicles of the submucosa of duodenum, jejunum and ileum were encapsulated by thick connective tissue capsule which was made up of collagen and reticular fibers. The collagen and reticular fibers were present around the follicles and in the inter follicular region (Fig. 3). The number of collagen fibers were more in jejunum and ileum than in the duodenum. Similarly, Medina (1981) [11] in gnotobiotic calves and pigs and Suchi and Singh (1996) in dog also reported encapsulated lymphoid follicles with reticular fibers. Further, few wavy elastic fibers were also noted around the follicles in pig. In the present study, the aggregation of the spherical lymphoid follicles were observed in the submucosa of the ileocaecal junction. The height and width of lymphoid follicles was $329.71 \pm 18.39 \mu\text{m}$ and $311.71 \pm 18 \mu\text{m}$ respectively (Table 2). The follicles were separated by the small inter follicular tissue. The follicles of ileocaecal junction did not show separate zones i.e., cortex and medulla. In middle and distal colon and rectum, the lymphoid element was composed of one or more elliptical submucosal follicles with sharply delimited inter nodular lymphoid tissue (Fig. 5). In colon, the height of lymphoid follicles was $247.50 \pm 20.42 \mu\text{m}$ and the width of the follicles was $349.37 \pm 32.2 \mu\text{m}$ (Table 2). In rectum, the height of the follicles was $238.18 \pm 28.62 \mu\text{m}$ and the width was $261.81 \pm 44.98 \mu\text{m}$ (Table 2). The follicles of colon and rectum showed two distinct zones i.e., peripheral cortex and inner medulla in the present study. In the present study, the lymphoglandular complexes were noted in the lamina propria and submucosa of the colon (Fig.5) and in the lamina propria of rectum. These lymphoglandular complexes were mainly consisted of lymphatic tissue with numerous lymphocytes and glands of lamina propria and submucosa. Lymphoglandular complexes were two types i.e., superficial and deep type. The superficial type was present in lamina propria whereas deep type was present in submucosa of the colon. But, submucosal lymphoglandular complexes were not observed in the rectum in the present study. It is in accordance with the findings of Kapoor and Singh (2016). In the lymphoglandular complexes regions, the lamina muscularis was interrupted distinctly because of the invasion of the mucosal glands of the crypts towards lymphoid nodules in the submucosa (Fig.5). Lymphoglandular complexes may also involved in defence mechanism of the gut in addition to their normal secretory activity. Similarly, Kapoor and Singh (2016) [7] noted that there was an extensive invasion of mucosal glands of the crypt towards lymphoid nodules present in submucosa resulting in disintegration of lamina muscularis as noted in the present study. They also noted some of the deeply seated lymphoglandular complexes opening into lumen in proximal colon of buffalo calves. Dev Choudhury *et al.* (2017) [15] noted lymphoglandular complexes in the tunica submucosa of distal caecum, colon and rectum of pigs. In ileocecal junction, the collagen and reticular fibers were present in the crypts of the lamina propria in the present study. In the colon and rectum, the collagen fibers were present around the follicles in the submucosa. The number of the collagen fibers was comparatively less in colon and rectum. In colon and rectum, the density of reticular fibers was more around the crypts in the lamina propria of pig. Few elastic fibers were present in the submucosa around the follicles.

3.2 Follicle Associated Epithelium

In the present study, some of the lymphoid follicles in the submucosa of jejunum and ileum showed domes. These domes were lined by a distinct layer of epithelium called follicle associated epithelium. This follicle associated epithelium consisted of columnar enterocytes and few “M” cells but devoid of goblet cells (Fig. 6). Similarly, Medina (1981) [11] noted that follicle associated epithelium consisted of a single layer of columnar epithelial cells and absence of goblet cells in follicle associated epithelium in gnotobiotic calves and pigs and Dellmann and Eurell (1998) [4] in domestic animals, Shuchi and Singh (1996) [14] in dog. Contrary to the present study, Chu *et al.* (1979) [13] reported cuboidal cells and few goblet cells in dome epithelium of young swine. The “M” cells were lightly stained with more eosinophilic cytoplasm with centrally placed nucleus as opined by Lalitha (1991) [9] in buffalo. In the present study, the more “M” cells were noted at the base of the follicular

associated epithelium of the domes. But their number decreased gradually towards the free surface of the domes in the jejunum and ileum of the small intestine of the pig. Contrary to this Medina (1981) [11] noted that “M” cells uniformly cover follicle domes in Peyer’s patches of gnotobiotic calves and pigs.

3.3 Inter follicular region

In the present study, between the follicles there was a distinct inter follicular regions. These inter follicular regions consisted of large number of collagen and reticular fibers, connective tissue cells, blood vessels and nerve fibers. In duodenum and jejunum, the inter follicular areas were wide whereas in ileum, the inter follicular regions were narrow in size. These findings were in accordance with the reports of Barman (1996) in pig and in conventional animals, Kapoor and Singh (2015) [7] in buffalo calves.

Table 1: Average height and width of the lymphoid follicles in small intestine (Mean ± SE μm) in pig.

S.No	Intestine segment	Height (μm)	Width (μm)
1.	Duodenum	369.09 ± 14.26	397.66 ± 16.57
2.	Jejunum	366.92 ± 23.32	399.23 ± 31.11
3.	Ileum	440.90 ± 36.72	417.72 ± 32.37

μm: micrometer
SE: standard error

Table 2: Average height and width of the lymphoid follicles in large intestine (Mean ± SE μm) in pig.

S.No	Intestine segment	Height (μm)	Width (μm)
1.	Ileocaecal junction	329.71 ± 18.39	311.71 ± 18
2.	Colon	247.50 ± 20.42	349.37 ± 32.2
3.	Rectum	238.18 ± 28.62	261.81 ± 44.98

μm: micrometer
SE: standard error

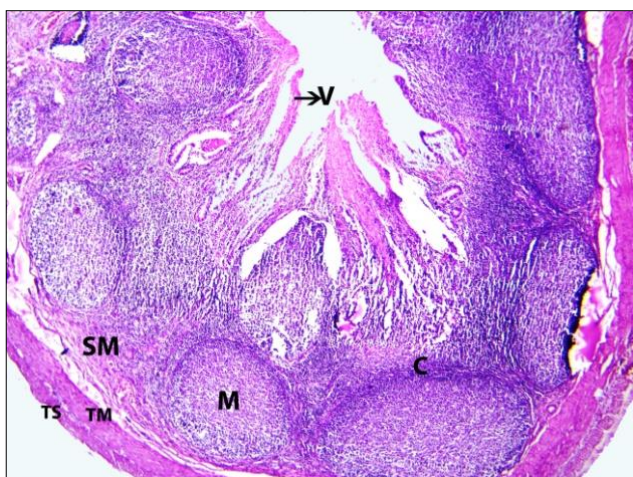


Fig 1: Photograph of jejunum showing oval to elliptical shaped lymphoid follicle with outer cortex, inner medulla and inter follicular region. Cortex (C) Medulla (M) Villi (V) Tunica muscularis(TM) Submucosa (SM) and Tunica serosa (TS) (Haematoxylin and Eosin X 40)

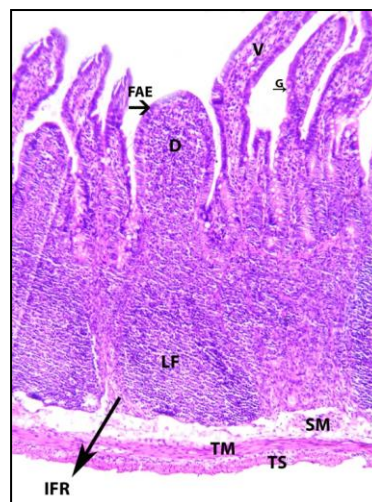


Fig 2: Photomicrograph of jejunum showing lymphoid follicles with dome region containing follicle associated epithelium. Villi (V) Follicle associated epithelium (FAE) Goblet cell (G) Inter follicular region (IFR) Dome (D) Submucosa (SM) Tunica muscularis (TM) Tunica serosa (TS) and Lymphoid follicle (LF) (Haematoxylin and Eosin X 100)

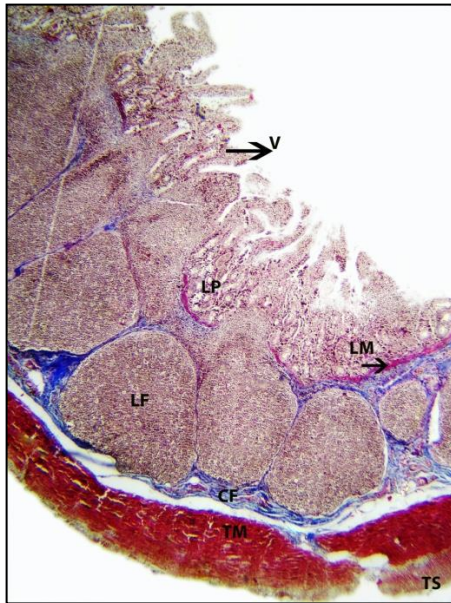


Fig 3: Photomicrograph of ileum showing collagen fibers around the lymphoid follicles in the submucosa. Lymphoid follicle (LF) Lamina propria (LP) Lamina muscularis (LM) Collagen fibers (CF) Tunica muscularis (TM) Tunica serosa (TS). (Masson's Trichrome X 100)

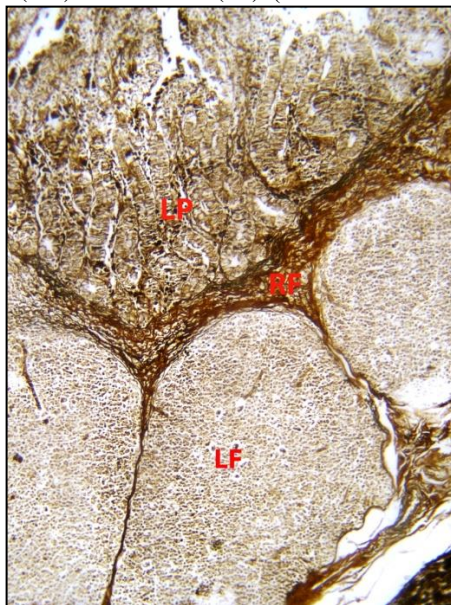


Fig 4: Photomicrograph of ileum showing network of reticular fibers around the follicles. Lamina propria (LP) LF – Lymphoid follicle (LF) Reticular fibers (RF) (Wilder's method X 100)

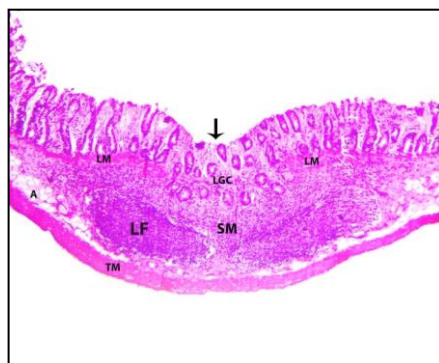


Fig 5: Photomicrograph of colon showing lymphoid follicles in the submucosa and invagination of lymphoglandular complex into submucosa (arrow). Lymphoid follicle (LF) Adipose tissue (A) Lamina muscularis (LM) Tunica muscularis (TM) Lymphoglandular complex (LGC) Submucosa (SM) (Haematoxylin and Eosin X 40)

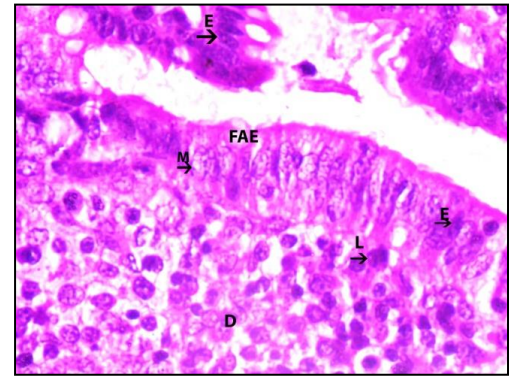


Fig 6: Photomicrograph of dome showing follicle associated epithelium in the jejunal Peyer's patch. Follicle associated epithelium (FAE) Enterocytes (E) "M" cells (M) Lymphocyte (L) Dome (D). (Haematoxylin and Eosin X 400)

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