



Gut Integrity of Neonatal Piglets: A Histomorphological Analysis

Arup Kalita^{1*}, Kabita Sarma², M. Talukdar², A. Deka² and P.C. Kalita¹

¹*Department of Veterinary Anatomy and Histology, College of Veterinary Sciences and Animal Husbandry, Central Agricultural University, Selesih, Aizawl, Mizoram, INDIA*

²*Department of Veterinary Anatomy and Histology, College of Veterinary Science, Assam Agricultural University, Khanapara, Guwahati, Assam, INDIA*

*Corresponding author: A Kalita; Email: arup.kalita@gmail.com

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ABSTRACT

The present study was undertaken to elaborate histomorphological status of different segments of small intestine of eight apparently healthy Hampshire piglets, irrespective of sex. They were divided into two age groups of 0 day and 7 days, consisting of four animals in each group. After sacrifice at the respective age the different segments of the small intestine were studied for histomorphological structures. The small intestinal wall is composed of four layers viz. tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa from inside to outside. The shape, size, length and width of the villi present at the mucosal surface varied in different regions of the small intestine. The Peyer's patches were composed of four distinct compartments, viz., follicle/lymphatic nodules, a zone of small lymphocytes, an internodular region and the dome area. The Brunner's glands were present in the tunica sub mucosa layer of duodenum. The collagen fibres were prominent in the basement membrane of the lining epithelium and connective tissue covering the lymphoid nodule. The reticular fibres were recorded in the basement membrane of the lining epithelium, periphery of the intestinal glands and lymphoid nodules. The elastic fibres were found in negligible amount in the different segments of the small intestine except at the inner side of the blood vessels. The nerve fibres were seen mostly in the dome and interfollicular area.

Keywords: Gut integrity, neonatal piglet, histomorphology

Pre-weaning piglet mortality continues to be a major economic loss to the farmers. On an average 25% of the piglet mortality is due to various enteric diseases of infectious and non-infectious origin (Kirkden *et al.*, 2013). Piglets during the suckling period, exposed to a variety of stresses. Stress factors can affect the growth and development of the new borne piglets. Intestine is the major organ responsible for the growth and development. The main function of the intestinal tract is the digestion and absorption of nutrients. The small intestine is the principal organ for this purpose. The goal is to first convert the nutrients into an absorbable form and secondly transport them from the gut lumen across the epithelial membrane into the blood or lymphatic system (Zur, 2016). The epithelial surface increases the contact area between nutrients and absorptive cells and thereby multiplies

digestive efficiency. Therefore, the length of the villi and crypt depth of the intestine plays a vital role in the growth and development of the piglets.

MATERIALS AND METHODS

The present investigation was conducted on eight numbers of apparently healthy Hampshire piglets irrespective of sex. The piglets were collected from the ICAR-AICRP/MSP on pig, College of Veterinary Science, AAU, Khanapara, Guwahati. They were divided into two age groups of 0 day and 7 days, consisting of four animals in each group. The experimental animals were first anaesthetized using diazepam @ 2mg/kg body weight followed by ketamine @ 10 mg/kg body weight intravenously and then exsanguinated the animals. Subsequent to sacrifice,

the abdominal cavity of the animals was exposed and the parts of the small intestine were then dissected out as per the method of Habel (1964). These organs were then cleaned in running tap water after evacuating the contents. The tissues were collected from different parts of the small intestine of each animal for histomorphological and histochemical studies as shown in Table 1.

Table 1: Site of collection of tissue for histomorphology

Sl. No.	Parts of small intestine	Site of tissue collection
1	Duodenum	5 cm caudal to the pylorus
2	Cranial Jejunum	Proximal 25% of the jejunum
	Middle Jejunum	At the middle of the jejunum
	Distal Jejunum	Distal 75% of the jejunum
3	Ileum	5 cm cranial to the ileocaecal valve

For histomorphological study, the tissue samples were fixed in 10% neutral buffer formalin solution (Luna, 1968). After proper fixation the tissues were dehydrated, cleared and embedded in paraffin wax as per Luna (1968). Then paraffin blocks were sectioned in Shandon Finesse microtome in 5 µm thickness and the sections were stained by following techniques as described by Luna (1968):

1. Mayer's haematoxylin and eosin stain
2. Van Gieson's method for collagen fibre
3. Hart's method for elastic fibre
4. Gomori's method for reticular fibre
5. Bielschowsky's method for axis cylinder and dendrites

Different parameters on histomorphology and histomorphometry were recorded on haematoxylin and eosin stained sections by means of standard method (Culling, 1974). The data of the present study were analyzed by routine statistical analysis (Snedecor and Cochran, 1994).

RESULTS AND DISCUSSION

General Histomorphology

In the present study, the wall of the small intestine of piglets consisted of four layers viz., tunica mucosa, tunica submucosa, tunica musculosa and tunica serosa from inside to outside (Fig. 1).

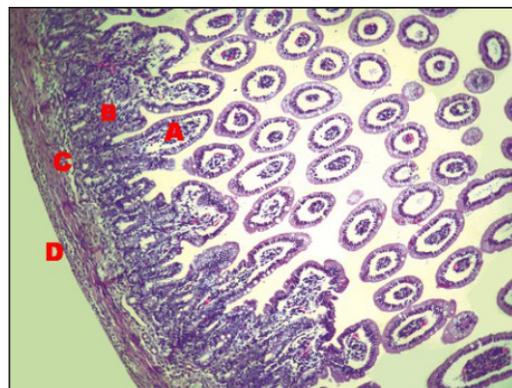


Fig. 1: Photomicrograph showing tunica mucosa (A), submucosa (B), musculosa (C) and serosa (D) in the duodenum of 7 day old Hampshire piglet. H&E, x100

Internally the longitudinal folds running intermittently throughout the intestinal tract and the small intestinal villi (finger like projections) present at the mucosal surface which varied in shape, size, length and width depending on the regions of the small intestine. Similar findings were recorded by Cormack (1987). The villi were covered by simple columnar epithelium comprising numerous goblet cells interspersed amongst them. The number of goblet cells decreased at the tip of the villi and increased towards the base. The epithelium consisted of columnar lining cells and goblet cells. The simple tubular intestinal crypts opened at the base of the villi as simple, tubular invaginations (Fig. 2). These findings were in accordance with the findings of Talukdar (1999) in adult crossbred pigs.



Fig. 2: Photomicrograph showing intestinal crypts (A) open at the base of the villi (B) as simple tubular invaginations in the duodenum of 0 day old Hampshire piglet. H&E, x100

Histomorphologically, Peyer's patches were distributed into four distinct compartments, viz. follicle/lymphatic nodules, a zone of small lymphocytes that capped the lymphatic nodules, an internodular region rich in T-lymphocytes and post capillary venules (HEV) and the elevated region overlying lymphatic nodules, the dome area (Fig. 3).

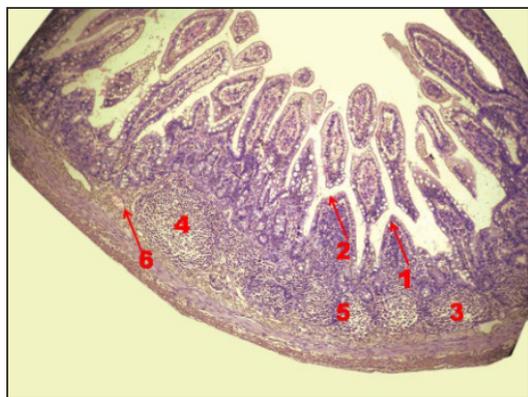


Fig. 3: Photomicrograph showing follicle associated epithelium (1), dome (2), follicle (3), germinal centre (4), interfollicular area (5) and HEV (6) in the jejunum of 7 day old Hampshire piglet. H&E, x100

The lymphoid follicles of the Peyer's patches were extended from the lamina propria to the submucosa of small intestine (Fig. 4).

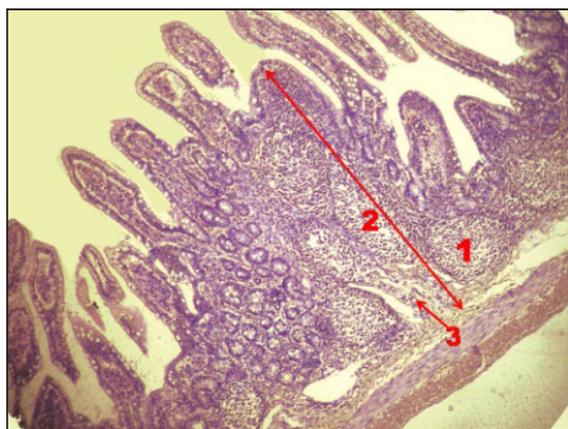


Fig. 4: Photomicrograph showing Peyer's patches (1), extended from lamina propria to submucosa (2) and HEV (3) in the jejunum of 7 day old Hampshire piglet. H&E, x100

Each follicle showed a peripheral cortex and inner medulla, the germinal centre (Fig. 3). The cortical area

appeared as a thickly populated dark zone packed with mature lymphocytes, while the germinal centre was a lightly stained area consisting of large lymphoblasts, a few small lymphocytes and mast cells, supported by a reticular framework. The follicles of duodenal and jejunal PP were rounded to ovoid whereas ileal PP were elongated, round, ovoid, elliptical and leaf shaped. The lymphoid tissues were found to be located mainly in three areas of the gut, viz.

1. intra and inter epithelial lymphocytes (IEL) – located within the epithelial cells of villi and between these cells;
2. diffusely distributed lamina propria lymphocytes (LPL) and Peyer's patches in the nodular form at mucosa; and
3. specialized nodules of lymphoid cells in the submucosa.

The present findings were closely similar to the findings of Talukdar (1999) in adult pig and Gautam (2015) in growing piglets.

Duodenum

The duodenal villi of 0 and 7 days piglets had different shape and size. Most of the villi were long and slender, although, spatula, club, conical and leaf shaped villi were also present. The average height and width of the villi of the duodenum were 295 μm and 85 μm , respectively in 0 day old pig. The average crypt depth of duodenum was 97 μm in 0 day old pig. However, Masri *et al.* (2015) recorded the height of the villi of the duodenum 350 μm in 3 day old piglet. These might be due to age differences. In the present study the average height and width of the villi of the duodenum were recorded 313 μm and 92 μm , respectively in 7 day old pig. The average crypt depth of duodenum was 102 μm in 7 day old pig. However, Marion *et al.* (2002) reported that the height and width of the villi of the duodenum were 975 μm and 127 μm , respectively in 7 day old pig. They also revealed that the crypt depth of small intestine was 116 μm in 7 day old pig. These might be due to species differences.

The Brunner's glands were present in the tunica submucosa layer of both the age groups (Fig. 5). Peyer's patches along with the dome epithelium were observed

in both the age groups; however these gut associated lymphoid tissue were in more organized form in the 7 day old piglets. The present finding was might be due to external exposure of antigens in 7 day old piglet which stimulated the immune system.



Fig. 5: Photomicrograph showing long, slender villi (A) and brunner's gland (B) in the duodenum of 7 day old Hampshire piglet. H&E, x100

Jejunum

In the present investigation the surface of the mucosa in the jejunum was folded and covered by finger-shaped villi. The villi were thinner at birth compared to 7 day old piglets. These villi were not uniform in length as there were shorter villi in between the taller ones in both the group. Similar types of observation were also recorded by Skrzypek *et al.* (2005) in day old piglets. The average height and width of the villi of the jejunum were 288 μm and 95 μm , respectively in 0 day old pig. The average crypt depth of jejunum was 109 μm in 0 day old pig. The average height and width of the villi of the jejunum were 315 μm and 105 μm , respectively in 7 day old pig. The average crypt depth of jejunum was 110 μm in 7 day old pig. The morphometry of villi of jejunum recorded in the present investigation was closely similar to the findings of Talukdar (1999) in pig and Masri *et al.* (2015) in 3 day old pig. However, the present findings were lower than that observed by Skrzypek *et al.* (2005) in pig. This might be due to variation in species. The number of goblet cells was more in the jejunum compared to duodenum in the present study.

The jejunal Peyer's patches at 7 day old piglet were clearly distinct. They were arranged in two different forms. Some of the follicles were arranged in groups in the tunica submucosa layer connected to a single dome area (Fig. 4). Others were arranged in continuous chain like pattern in the submucosa layer (Fig. 3).

Ileum

The average height and width of the villi of the ileum were 257 μm and 97 μm , respectively in 0 day old pig. The average crypt depth of ileum was 85 μm in 0 day old pig. The average height and width of the villi of the ileum were 305 μm and 92 μm , respectively in 7 day old pig. The average crypt depth of jejunum was 117 μm in 7 day old pig. Masri *et al.* (2015) recorded the height of the villi of the mid ileum was 282 μm in 3 day old piglet which is similar to the present finding. The goblet cells were found more than the jejunum in both the groups of the study. The Peyer's patches were found in the submucosa layer which evaginated to the lamina propria (Fig. 6).

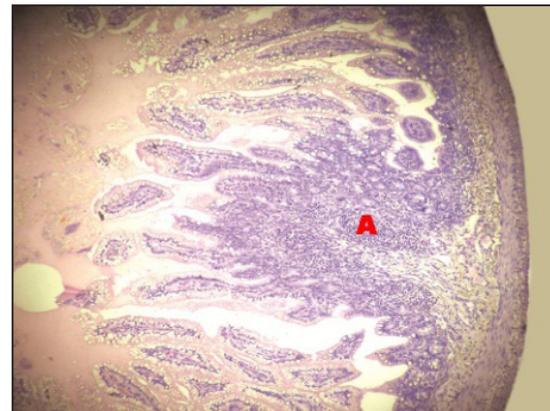


Fig. 6: Photomicrograph showing Peyer's patches (A) in the submucosa layer which evaginated to the lamina propria in the ileum of 0 day old Hampshire piglet. H&E, x100

Connective tissue fibres and Nerve fibres

Collagen fibres

In the current study, the collagen fibres were observed in the basement membrane of the lining epithelium (Fig. 7). The connective tissue covering the lymphoid nodule was prominent and mostly collagen fibres were seen (Fig. 8). These fibres were responsible for distinct

compartmentalization of the Peyer's patches. Collagen fibres were very less in the dome area and in between the follicle associated epithelium and in the lymphoid follicle. This might be showing the possible passage of free and processed antigens from the FAE to other compartments of the Peyer's patches. This finding was in accordance with the finding of Gautam (2015) in growing piglets.



Fig. 7: Photomicrograph showing showing collagen fibres (arrow) in the basement membrane of the lining epithelium in the duodenum of 0 day old Hampshire piglet. Van Gieson, x400



Fig. 8: Photomicrograph showing collagen fibres (arrow) covering the lymphoid nodule (A) in the jejunum of 7 day old Hampshire piglet. Van Gieson, x100

Reticular fibres

The presence of reticular fibres was recorded in the basement membrane of the lining epithelium (Fig. 9), periphery of the intestinal glands and lymphoid nodules along with the collagen fibres (Fig. 10) in the present study as reported by Gautam (2015) in growing piglet.

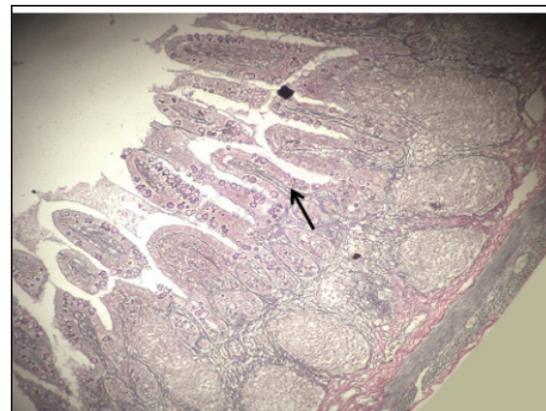


Fig. 9: Photomicrograph showing reticular fibres (arrow) in the basement membrane of the lining epithelium in the jejunum of 7 day old Hampshire piglet. Gomori's, x100

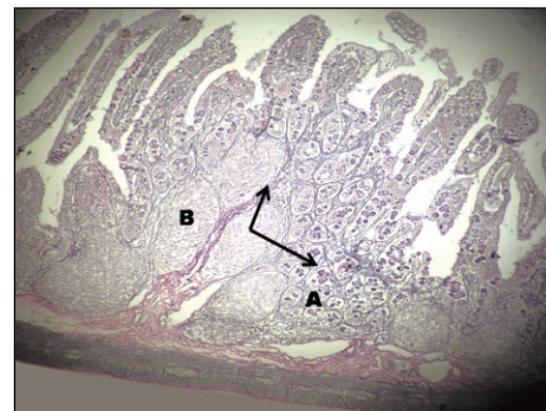


Fig. 10: Photomicrograph showing reticular fibres (arrow) in the periphery of intestinal glands (A) and lymphoid follicles (B) in the jejunum of 7 day old Hampshire piglet. Gomori's, x100

Elastic fibres

The elastic fibres were found in negligible amount in the different segments of the small intestine. However, their presence showed in the inner side of the blood vessels in the tunica muscularis layer (Fig. 11). These findings are in accordance with Gautam (2015) in growing piglet.

Nerve fibres

In the present study, nerve fibres were seen mostly in the dome and inter-follicular area (Fig. 12). These fibres were observed in varying quantities in the epithelium, lamina

propria, submucosa and muscular layer. Submucosal plexuses were seemed to be the root of such innervations in the lymphoid aggregates of small intestine as also described by Gautam (2015) in growing piglet.

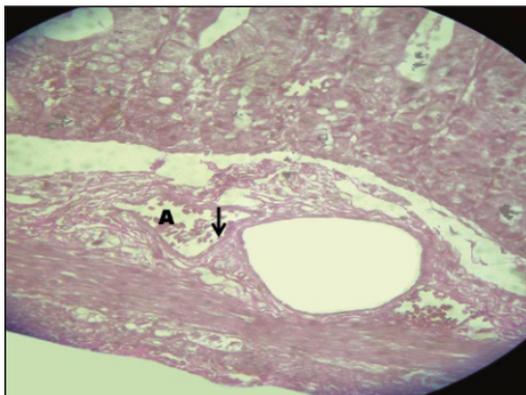


Fig. 11: Photomicrograph showing elastic fibres (arrow) in the inner side of the blood vessel (A) in the jejunum of 7 day old Hampshire piglet. Hart's, x400

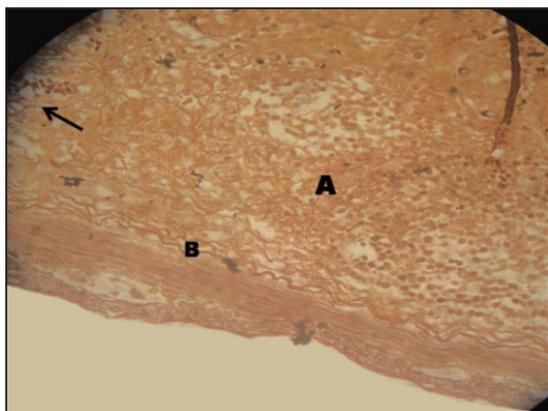


Fig. 12: Photomicrograph showing nerve fibres (arrow) in the interfollicular area (A) and inner side of the tunica muscularis layer (B) of ileum in 7 day old Hampshire piglet. Beilchowsky's, x400

CONCLUSION

The present study was concluded that small intestinal wall of Hampshire piglets composed of tunica mucosa, tunica submucosa, tunica muscularis and tunica serosa from inside to outside. The shape, size, length and width of the villi present at the mucosal surface varied in different regions of the small intestine. The Peyer's patches were

composed of follicle/lymphatic nodules, a zone of small lymphocytes, an internodular region and the dome area. Brunner's glands were present in the tunica sub mucosa layer of duodenum. The collagen fibres were prominent in the basement membrane of the lining epithelium and connective tissue covering the lymphoid nodule. The reticular fibres were recorded in the basement membrane of the lining epithelium, periphery of the intestinal glands and lymphoid nodules. The elastic fibres were found in negligible amount in the different segments of the small intestine except at the inner side of the blood vessels. The nerve fibres were seen mostly in the dome and interfollicular area.

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