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Morphological Study of the Jejunal and Ileal Peyer's Patches of Three-month Old Calves

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Abstract: The current study was conducted with the aim to described the light and electron microscopic features of jejunal and ileal Peyer`s patches of three-month old calves. The samples of jejunum and ileum portion of small intestine of three-month old calves were taken and processed for light microscopic, scanning electron microscopy and transmission electron microscopy examinations. Histologically, jejunal Peyer`s patches were characterized by pear-shaped lymphoid follicles with large dome and interfollicular area. Ileal Peyer`s patches were composed of long sac like follicles with poor developed interfollicular area and an inconspicuous corona. The Follicle Associated Epithelium (FAE) is composed of absorptive epithelial cells or enterocytes and intraepithelial lymphocytes but lack of goblet cells and specialized cells or membranous cells (M cell). The jejunal Peyer's patches consist more intraepithelial lymphocytes than that of the ileal peyer's patches. The number of intraepithelial lymphocyte was significantly higher ($p<0.05$) in villi than those of crypts. Most of the intraepithelial lymphocytes were found in the subnuclear position below the nuclear level of the enterocytes. Electron microscopic examination revealed that the FAE of jejunal Peyer`s patches had scattered membranous cells or microfolds (M cells). M cells of jejunal Peyer`s patches were columnar shaped with luminal surface that bulged toward the intestinal lumen. M cells of dome epithelium of small intestine in calves were covered by blunt microvilli that were irregular, short and thick. These microvilli differed from microvilli of absorptive epithelial cells (enterocytes). Membranous bound particles were found in the dome epithelium of jejunal and ileal Peyer`s patches.

Key words: Morphology, Jejunal and ileal Peyer' patches, Intraepithelial lymphocytes, Calves

Introduction

Gut Associated Lymphatic Tissues (GALT) or Peyer`s patches are macroscopically visible structures and situated chiefly along the surface opposite to the mesenteric attachment and begin about one metre from the pylorus. There were two types of Peyer`s patches (jejunal and ileal) in some species, like sheep and pig (Reynolds *et al*., 1985 and Pabst and Rothkotter 1999). Adult bovine small intestine consists about 18 to 40 discrete Peyer`s patches (DPPs). They were located in the duodenum and jejunum. Parsons *et al*. (1989) described that at 40 weeks of gestation, the bovine fetus contained up to 76 discrete Peyer`s patches in the duodenum and jejunum. The number, size and distribution of DPPs are dependent on species and age. Microbial contents of gut influence the size of jejunal Peyer's patches but their number and position remain constant (Pabst and Rothkotter, 1999). The jejunal Peyer's patches are the typical peripheral lymphoid tissues and persist throughout life. The ileal Peyer's patches consist of Continuous Peyer's Patches (CPPs) which may be a primary lymphoid organ (Owen and Jones, 1974). Involution of CPP commenced soon after birth and only isolated follicles remained in adult cattle. The ileal patches constitute the largest single lymphoid organ and the number of lymphocytes is greater than the thymus (Parsons *et al*., 1989). These two types of Peyer`s patches differ in structure, lymphocytes migration and production.

Peyer`s patches consist of different shapes of follicles, interfollicular area, dome region and FAE. The follicles have a typical structure with a distant germinal center and are different in size and shape. The FAE presents between normal absorptive villi and found in several species of mammals but differ from the epithelium of other sections of the small intestine (Owen and Jones, 1974). The follicle-associated epithelium is composed of enterocytes, microfold cells or membranous (M cells), mononuclear cells and intraepithelial lymphocytes. The intraepithelial lymphocytes of follicle-associated epithelium migrated from the lamina propria and were mostly of T cell origin (Guy-Grand *et al*., 1974). M cells are numerously distributed in the follicle-associated epithelium of crypt and the lower epithelium of dome (Bye *et al*., 1984). The follicle associated epithelium of small intestine consists of uniform population of M cells which are covered by densely packed blunt microvilli (Torres-Medina, 1981). Landsverk, (1987) and Parson *et al*. (1989) described that the follicle associated epithelium of CPP had continuous or homogeneous population of specialized cells with a surface of centrally placed, short, sparse microvilli or microfolds and peripherical concentric fold. M cells have a similar role in the uptake and presentation of antigen to the underlying lymphoid tissues and appear to be basic for the establishment of a local secretory immune response.

The intraepithelial lymphocytes occur in villi, crypts and dome epithelium (Ferguson, 1977). They have round, dense nuclei and scanty cytoplasm and a possible component of immune apparatus. The intraepithelial lymphocytes have migrated from intestinal lamina propria and they are T cell origin (Meader and Lander, 1967 and Guy-Grand *et al.*, 1974). The intraepithelial lymphocytes involve in several activities including cytoxicity, lymphokine release and regulation of renewal of epithelium.
The morphological features of M cell and intraepithelial lymphocytes are important for understanding the immune response in calves against intestinal antigens. Thus, this study was conducted with the aim at describing the light and electron microscopic features of jejunal and ileal Peyer`s patches of small intestine of calves. In addition, the amount of intraepithelial lymphocytes of the villi and crypts of the jejunum and ileum were also measured and compared.

Materials and Methods

Animals: Three calves of three-month old local breed were used in this study. The calves were supplied by the University Research Park, Universiti Putra Malaysia. The calves were lefted free grazing with their mother in the field. The calves were apparently healthy and clinical examination carried out showed no abnormalities. The protocol of the study has been approved by the Faculty's Ethics Committee.

Light Microscopic Examination: Upon slaughtering, the small intestine was taken out and washed with phosphate buffer saline solution (pH 7.3) to remove the content. The small intestine was ligated at five different sites: proximal end, middle and distal end of jejunum, middle of the ileum and at the ileocaecal junction. The intestinal lumen was then inserted with 10% buffered formalin. After 30 min, the ligations were opened and the intestine was cut and opened along the mesenteric attachment site. The mucosal surface was rinsed gently with cold water for 2 hr and then transferred into the freshly prepared fixative solutions.
For microscopic examination, three sites (proximal, middle and distal parts) of each jejunum and ileum containing lymphoid follicles were sampled and fixed in 10% neutral buffered formalin solution (pH 7.3), embedded in paraffin, sectioned at 4µm and stained with Haematoxylin and Eosin. The slides were examined under light microscope with 40x objective and field diameter of 1 mm was used to count Intraepithelial Lymphocytes (IEL). The IEL were counted in the portion of randomly selected villi and crypts, which filled the field. A total of four field from six villi and crypts were examined and classified as subnuclear, nuclear and supranuclear according to their plane with relationship to the nucleus of adjacent absorptive cells. The IEL count was based on morphological appearance. The results were presented as mean±standard deviation per 50 enterocytes.

Scanning Electron Microscopy: Sample of the intestinal tissue approximately 1.0x1.0x1.0 cm were taken and fixed in 4% glutaradehyde buffer (pH 7.4) using 0.1 M sodium cacodylate buffers at room temperature for 24 hr. After primary fixation, the samples were washed and then post-fixed in 1% osmium tetroxide buffer (pH 7.4) for 2 h at room temperature. The samples were then dehydrated in increasing series of ethanol, infiltrated with amylacetate and subjected to critical point drying by CO_2 substitution. These dried samples were mounted with aluminium stub and coated with gold. The samples were examined under SEM at 15 KV and photographed.

Transmission Electron Microscopy: All preparations for Transmission Electron Microscope (TEM) involving handling of the specimens were carefully and gently performed on a flat surface. The samples were of equal length. They were fixed with 4% glutaraldehyde for 24 hr and washed with 0.1 M sodium cacodylate buffer (pH 7.4) for 3 times. Samples were washed three times in buffer solutions for 10 min and postfixed for 2 hr with 1% osminum tetroxide. The specimens were rinsed and dehydrated in a series of acetone solutions. Dehydrated specimens were infiltrated with resin-acetone mixture. The fixing and rinsing stages were carried out at 4°C. Specimens were embedded in capsule with resin mixture and polymerised in oven at 60°C for 24-48 hr. Thick (1µm) and thin (80-90 nm) sections were stained with toludine and double stain with uranyl acetate and lead citrate respectively. The stained sections were examined using transmission electron microscope.

Results and Discussion

Histological Examination: The distribution of IEL of villi and IEL of crypts are shown in Tables 1 and 2 respectively. The mean numbers of intraepithelial lymphocytes (IELs) were varied at different sites of small intestine. Generally, the proximal part of jejunum contained more IEL of villi and crypts and the number was gradually decreased toward the distal end of small intestine in all calves. The mean number of IELs of villi was significantly ($p<0.05$) higher than those of the crypts at different parts of small intestine in each individual calf (Table 3). Statistical analyses of IELs between villi and crypts showed significant difference ($p<0.05$) between jejunum and ileum of small intestine of calves of the same age (Table 4). The nuclear level of IELs was classified as subnuclear, nuclear and supranuclear

Table 1: Mean number of intraepithelial lymphocytes (IELs) of villi at different parts of small intestine of three-month old calves. Data were expressed as number of IEL/50 absorptive cells, mean±SE

Calf	Proximal part of jejunum	Middle part of jejunum	Distal part of jejunum	Proximal part of ileum	Middle part of ileum	Distal part of ileum
1	10.3±0.6 ^{a}	8.8±0.6^{ab}	7.3±0.6^{b}	6.6±0.7^{c}	5.2±0.5^{cd}	5.1±0.4^{d}
2	13.6±0.8 ^{a}	12.0±0.9^{a}	11.3±1.2^{b}	11.0±1.0^{b}	10.5±1^{c}	9.5±0.6^{d}
3	13.6±1.3 ^{a}	12.7±2.0^{ab}	12.0±0.9^{b}	9.8±1.6^{c}	6.9±1.0^{cd}	5.5±1.2^{d}

^{a,b,c,d}Mean with different superscript within a column were significantly different at p< 0.05 using ANOVA

Table 2: Mean number of intraepithelial lymphocytes (IELs) of crypts at different parts of small intestine of three-month old calves. Data were expressed as No. of IEL/50 absorptive cells, mean±SE

Calf	Proximal part of jejunum	Middle part of jejunum	Distal part of jejunum	Proximal part of ileum	Middle part of ileum	Distal part of ileum
1	4.2±0.6^{a}	4.0±0.4^{a}	3.7±0.6^{b}	3.0±0.6^{c}	2.7±0.4^{cd}	2.2±0.8^{d}
2	7.3±1.3^{a}	6.8±0.6^{b}	6.0±0.6^{b}	5.8±1.2^{bc}	5.0±1.0^{c}	4.2±0.8^{d}
3	6.5^{a}±1.3	6.3±0.5^{a}	5.5±1.3^{b}	4.8±0.6^{c}	4.0±1.3^{cd}	3.8±1.2^{d}

^{a,b,c,d}Mean with different superscript within a column were significantly different at P< 0.05 using ANOVA

Table 3: Mean number of intraepithelial lymphocytes of villi and crypts of small intestine of three-month old calves

Calf	Intraepithelial lymphocytes of villi mean±SE	Intraepithelial lymphocytes of crypts mean±SE
1	13.3±0.5^{ya}	6.0±0.2^{xyb}
2	16.3±0.6^{xa}	7.3±0.4^{xb}
3	9.6±0.9^{za}	4.3±0.4^{yb}

^{x,y,z}Mean with different superscript within column were significantly different at (p< 0.05) using ANOVA
^{a,b}Mean with different superscript within column were significantly different at (p< 0.05) using Mann-Whitney u test

Table 4: Mean number of intraepithelial lymphocytes of villi and crypts of the jejunum and ileum in three-month old calves

Part of small intestine	Number of intraepithelial Lymphocytes of villi Mean±SE	Number of intraepithelial Lymphocytes of crypts Mean±SE
Jejunum	14.5±0.6^{a}	7.9±0.4^{b}
Ileum	12.3±0.6^{a}	7.1±0.3^{b}

^{a,b}Mean with different superscript within column were significantly different at (p< 0.05) using Mann-Whitney u test

according to their places with relation to the nuclei of adjacent absorptive cells. More IEL had the subnuclear level in all parts of small intestine. Normal lymphocytes as well as degenerated cells were found on the surface of mucosal epithelium.

Jejunal Peyer`s patches which composed of DPPs were mostly characterized by pear-shaped lymphoid follicles with large domes and intrefollicular areas. There were three to four rows of lymphoid follicles of jejunal Peyer`s patches observed in all calves and some follicles had germinal centre and distinct corona (Fig. 1). Germinal centre could be subdivided into the central light zone and the basal dark zone. The central light zone composed of lymphoblast, reticular cells and macrophages, while the basal dark zone consisted of small lymphocytes and lymphoblasts. An indefinite layer or mantle zone was seen in the periphery of germinal centre of lymphoid follicles. Dome villi were conical upper end, cylindrical and rounded in shape (Fig. 2). Dome crypts were oval or kidney shaped in cross section (Fig. 3). The dome of lymphoid follicles of jejunal Peyer`s patches were also well developed. Lymphocytes within the dome were tightly packed, relatively larger and stained lighter than the lymphoid follicles. These lymphocytes have large nuclei. Some distinct corona had lightly stained lymphocytes and some tightly arranged corona were made up of compact small lymphocytes, which were more deeply stained than those in the germinal centre. A few plasma cells were seen under the corona. Many empty vacuoles were observed in the cytoplasm of enterocytes. Neutrophils were seen in the lamina propria and epithelium close to the crypt regions.

The ileal CPP composed of long sac-like follicles tightly packed with other slender vilus-like dome (Fig. 4). There were poorly developed interfollicular areas and an inconspicuous corona. The poorly developed interfollicular area comprised only a very small triangular area and some interfollicular areas were situated below the muscularis mucosae at the apex of the follicles (Fig. 5). The dark zone of the follicles extended up along the lateral side of the germinal centre to the muscularis mucosae. The villi of ileum were shorter than that of jejunum. Some of the follicles had prominent dome regions where there were no villi and only a thin layer of epithelial tissues present (Fig. 6). Other

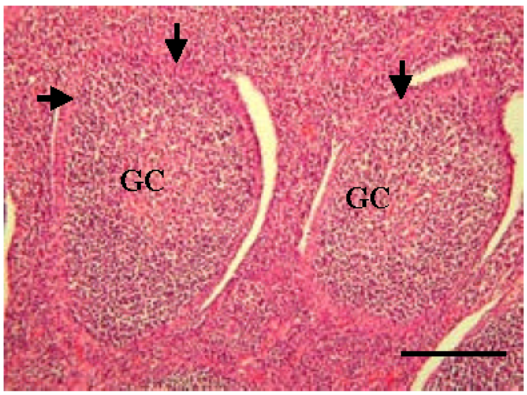


Fig. 1: Histological section of the jejunal Peyer's patches shows the lymphoid follicles with distinct corona (arrows) and germinal centre (GC). H&E, Bar = 20µm

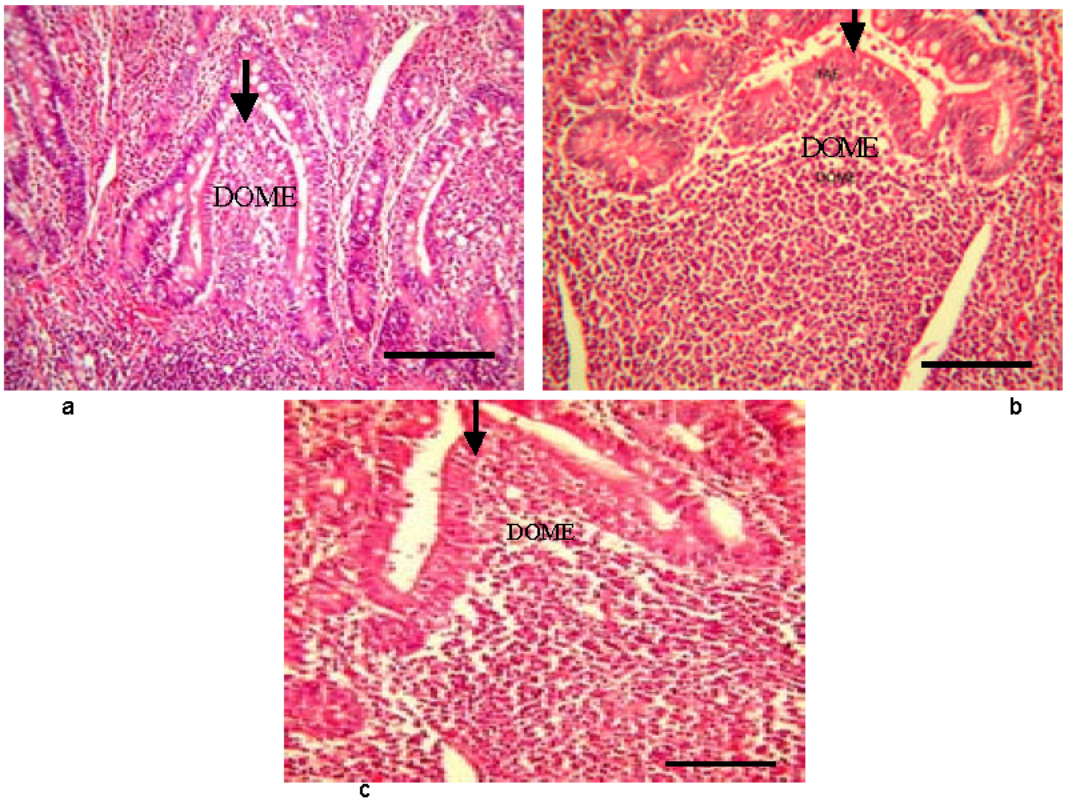


Fig. 2: Histological sections of the discrete Peyer`s patches of jejunum show the different shape of dome villi (arrows) a) cylindrical, b) rounded and c) conical. H&E, Bar = 20µm

follicles had two villi above them and no defined domes. More lymphoid aggregations were seen covering the mucosa of the ileum than on the jejunum. These aggregations could not completely be covered by dome epithelium. The follicle associated epithelium of villi and crypts was lined by single layer of cells. The surface characteristics of the epithelial cells of FAE were particularly distinct from those of the epithelial cells covering the normal intestinal villi or crypts. This epithelium was composed of enterocytes, columar shaped M cells, intraepithelial lymphocytes, plasma cells and lack of goblet cells. M cells were not identified because the criteria for identification depended on ultrastructural features requiring electron microscopy. The FAE of villi and crypts was usually a little thinner and shorter but contained more intraepithelial lymphocytes (IELs). The IELs were apparently more numerous in FAE of villi. IELs were situated close to the basement membrane. Size and morphological appearance of IELs varied but

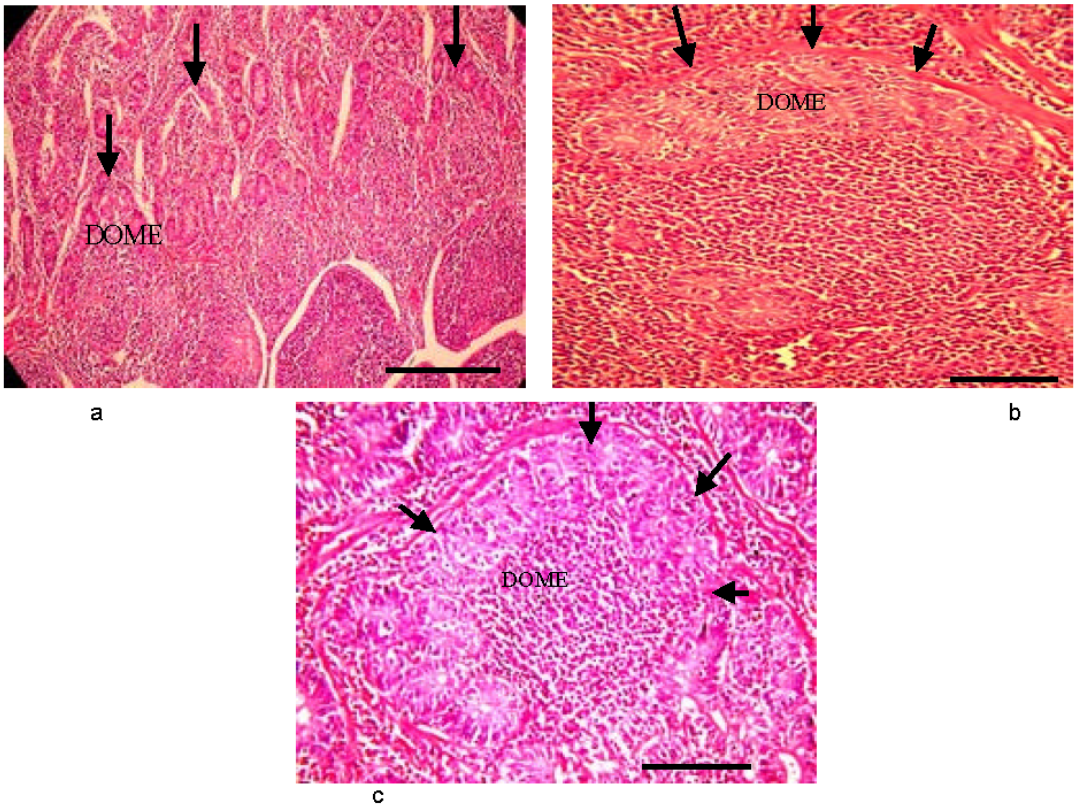


Fig. 3: Histological sections of the discrete Peyer`s patches of jejunum show the different shapes of dome crypts (arrows) a) conical, b) kidney and c) oval. H&E, Bar = 20µm

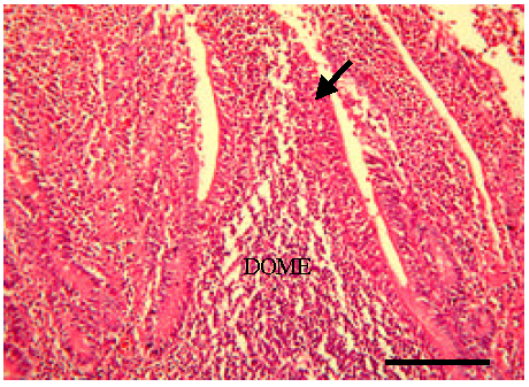


Fig. 4: Histological section of the continuous Peyer`s patches of Ileum shows the cylindrical like small dome villi (arrow). H&E, Bar = 20µm

they were distinguished from epithelial cells mainly by smaller size of the nuclei. The dome epithelium contained more intraepithelial lymphocytes (IELs) than the ordinary villous epithelium. More IELs were usually occurred singly rather than groups and found in the villus epithelium in comparison with the crypts (Fig. 7).

Electron Microscopic Findings: In scanning electron microscopy, there were leaf-like and finger-like absorptive villi in the small intestine of all calves (Fig. 8). Dome villi of DPPs completely covered by the leaf-like absorptive villi could not be seen. In contrast, dome villi of CPPs were covered by finger like absorptive villi and found in between the absorptive villi. CPPs were composed of long sac like-follicles, small interfollicular area and dome region. The T lymphocyte was seen in interfollicular area (Fig. 9).

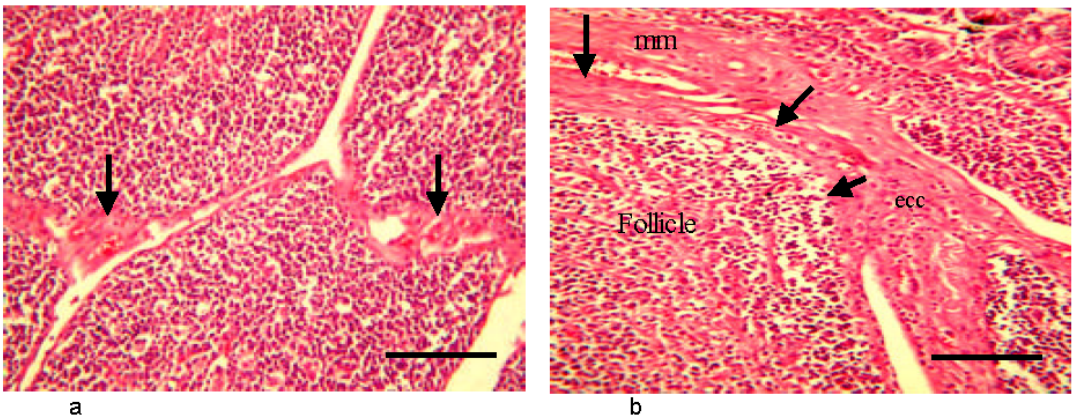


Fig. 5: Histological sections of the continuous Peyer`s patches of ileum show a) the poorly developed interfollicular area (IFA) (arrows) and b) some IFA (arrows) were located below the muscularis mucosae (mm). ecc, encapsulated connective tissue. H&E, Bar = 20µm

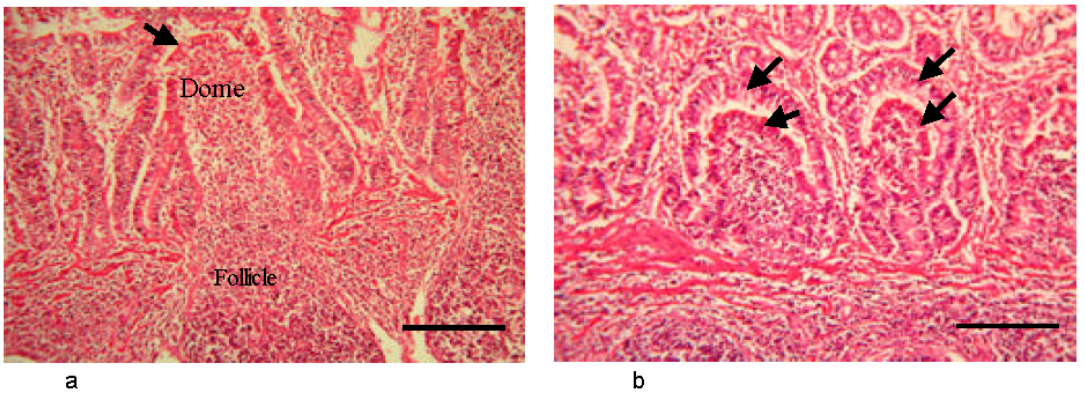


Fig. 6: Histological sections of the continuous Peyer`s patches of Ileum show a) the developed dome area (arrow) consists of thin layer of epithelium and indistinct corona area are found in some follicles, b) the double villi (arrows) cover the undifferentiated dome area of some lymphoid follicles. H&E, Bar = 20µm

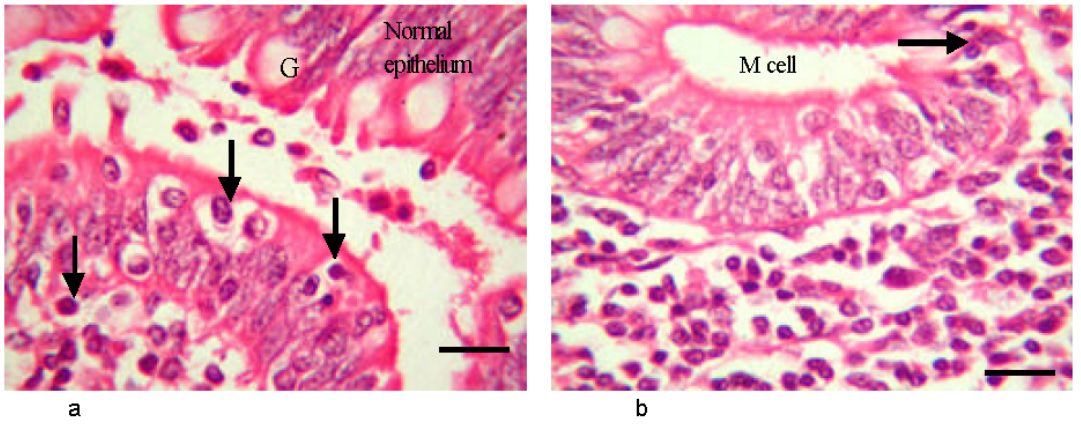
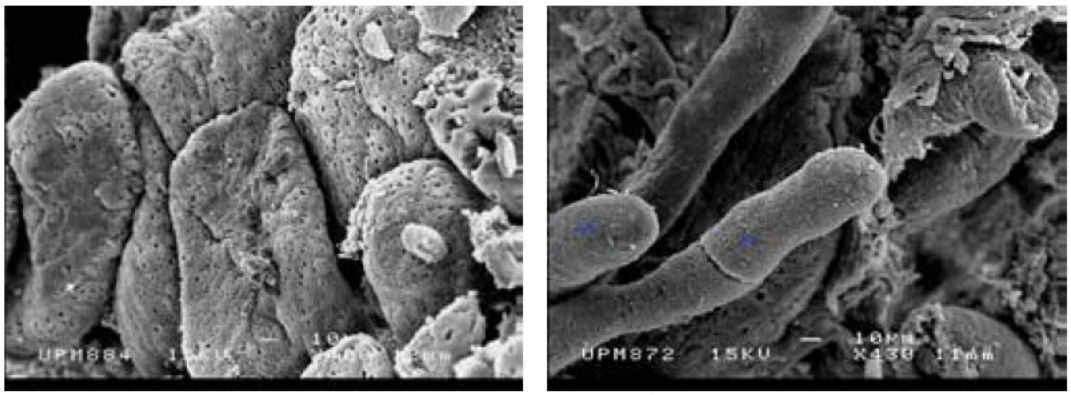
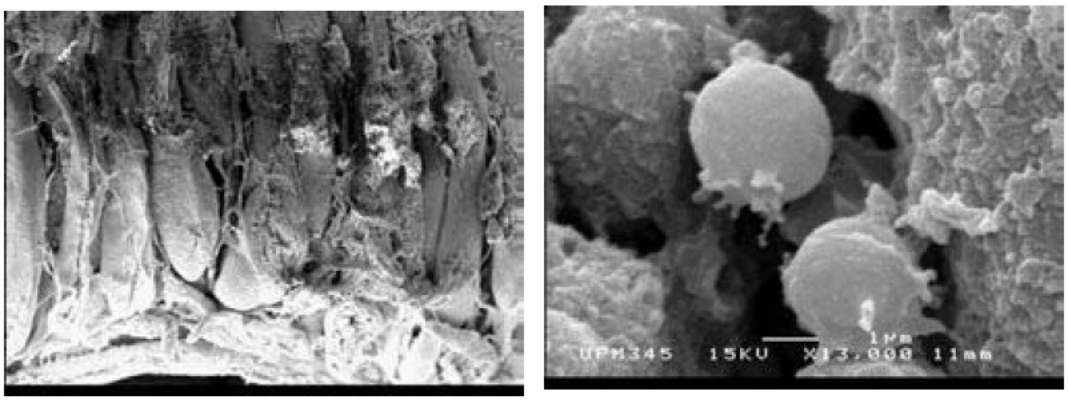


Fig. 7: Histological sections of the Peyer`s patches of small intestine show follicle associated epithelium of villi (a) and crypt (b) containing with intraepithelial lymphocyte (arrows). Note that the FAE of villi contains more IEL than in FAE of crypts. G, Goblet cells. H&E, Bar = 10µm



a b

Fig. 8: Scanning electron micrographs of two different types of intestinal absorptive villi in small intestine of calves, a) leaf-like and b) finger-like



a b

Fig. 9: Scanning electron micrographs of a) CPPs in small intestine and b) interfollicular area. Note that the CPPs are composed of long sac like lymphoid follicles, FAE, dome region and interfollicular area, while in the interfollicular area the T lymphocytes (arrow) are present

In transmission electron microscopy, the FAE of the DPPs were lacked of goblet cells and consisted of M cells. M cells were interspersed between the more numerous enterocytes. They were tall columnar in shape and lack of cytoplasm vacuole (Fig. 10). Their cytoplasm was often less electron-dense than adjacent epithelial cells. The cytoplasm contained mitochondria with closely packed cristae. An irregular nucleus was situated toward the base of the cell. Vesicles were found at the base of microvilli. M cells had irregular, short microvilli and microfolds on their luminal surface and were rounded in all calves (Fig. 11). Some M cells were rested beside the surrounding enterocytes, but were visible. The epithelial cells were usually arranged irregularly and mixed with both small and large lymphocyes. The FAE of CPPs contained lack of goblet cells and absorptive cells. M cells of CPPs had round nuclei located at the base of the cell. On the apical surfaces of those cells there were numerous microfolds, which were much shorter, thicker and fewer than the microvilli of the enterocytes. In the apical cytoplasm, four to five mitochondria, numerous free ribosome, undeveloped granular endoplasmic reticulum (ER) and the Golgi apparatus were recognized. Membrane bound particles were found in close association with large dense bodies. Some were found in intercellular vesicles and intercellular space in the DPPs and CPPs (Fig. 12). Intraepithelial lymphocytes had small nucleus and pale cytoplasm. They had few mitochondria, rough endoplasmic reticulum and well-developed Golgi apparatus. Furthermore, mononuclear cells were seen towards the base of the epithelium not associated with lymphoid follicles. Within lymphoid follicles, lymphocytes were the main cell type and seen beneath the epithelium, germinal centre and diffuse lymphoid tissues. The small lymphocytes had a thin rim of relative electron-dense cytoplasm (Fig. 13). Few cytoplasmic organelles were seen in the small lymphocytes. Large lymphocytes had less dense cytoplasm than small lymphocytes. Plasma cells had the typical clock-face arrangement of nuclear chromatin (Fig. 14). They were larger than other lymphoid cells. Mitochondria and small round dense granules were present.

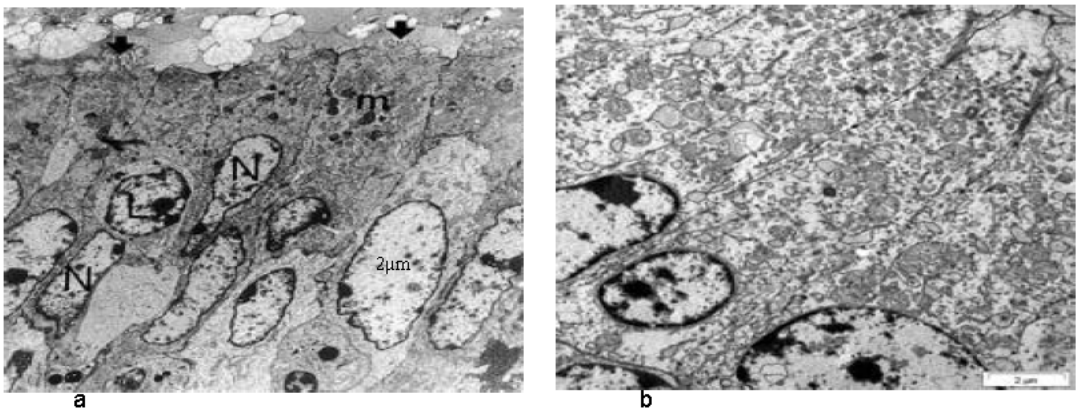


Fig. 10: Transmission electron micrographs of jejunum in calf show a) the mitochondria (m) nucleus (N), lymphocyte (L) and microvilli of M cell of dome of DPP, b) the characteristics of M cell (columnar shape). Note that the lack of cytoplasmic vacuole and interdigitating cell membranes of adjacent cells in b

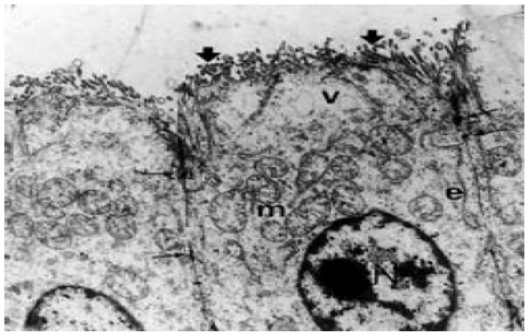


Fig. 11: Transmission electron micrograph of the ileum demonstrates the M cell with small microfolds (arrows). v, vacuole, m, mitochondria, N, nucleus. Bar= 2µm

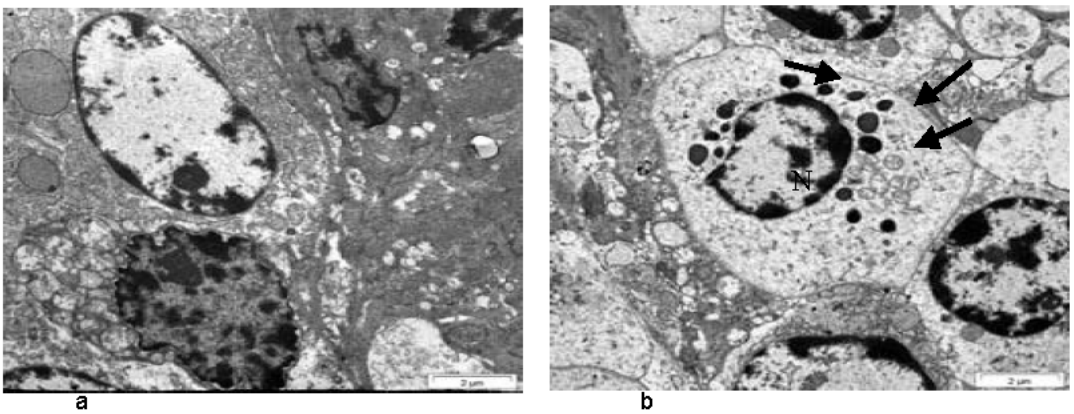


Fig. 12: Transmission electron micrographs of the jejunal Peyer's patches of three-month old calf show a) membrane bound particles (arrows) with either an opaque or dense are present in the intercellular space of folliclcular epithelial cells at dome villus of a discrete Peyer`s patches, b) membrane bound particles (arrows) present in a perinuclear vacuole in the follicle associated epithelium of continuous Peyer's patches. N, nucleus

The cytoplasm was mostly filled by the endoplasmic reticulum. Macrophages were present in moderate numbers throughout the lymphoid tissue. They often contained phagocytosed material; dead and dying

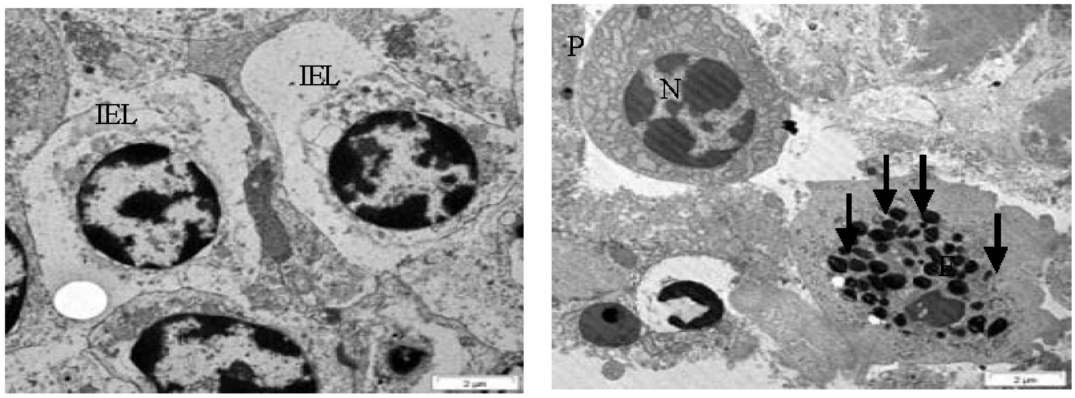


Fig. 13: Transmission electron micrographs of a) intraepithelial lymphocytes of FAE and b) mononuclear cells [plasma cell (P) and eosinophil (E) with electron- dense internum (arrows)] in lymphoid follicles. N, nucleus

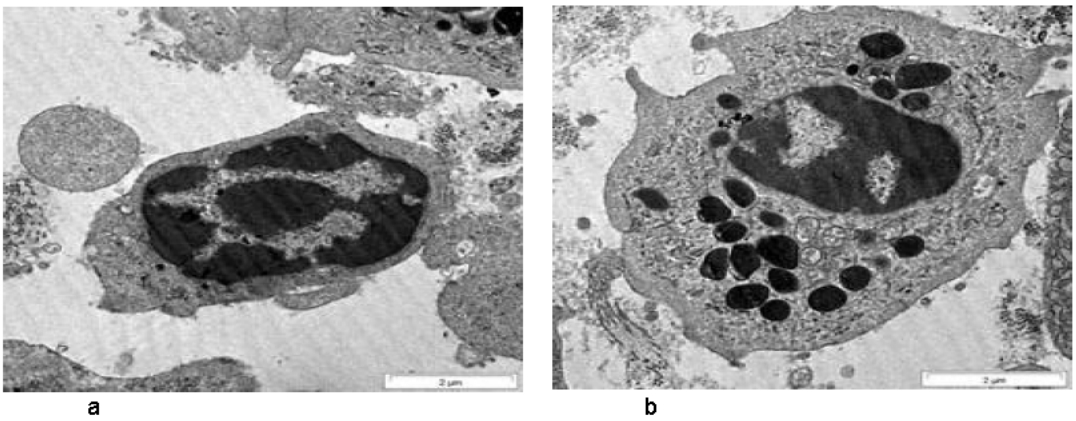


Fig. 14: Transmission electron micrographs of a) the plasma cell containing nuclear chromatin and b) the macrophage with the dead or dying cells (arrows) within vacuoles in cytoplasm. N, nucleus

The cytoplasm was mostly filled by the endoplasmic reticulum. Macrophages were present in moderate numbers throughout the lymphoid tissue. They often contained phagocytosed material; dead and dying cells could be seen within vacuoles in the cytoplasm. The nuclear membrane was often indented by phagocytosed material.

Discussion

Peyer`s patches are an important composition of gut associated lymphatic tissues. These are the sites where antigens are recognized and are transported to the mucosa by M cells. Peyer`s patches induce an immune response. Peyer`s patches of small intestine have a facilitative effect on the healing of intestinal wound by promoting both epithelial cell migration, epithelial cell proliferation in the crypts adjacent to the wound and by decreasing the rate of wound contraction (Saxena *et al.*, 1997). Based on the histological findings in this study, follicles of Peyer`s patches in the ileum were densely packed together with scant interfollicular area. While, in the jejunum, follicles were more widely dispersed and separated by prominent interfollicular regions. Thus, the results are an accord with Wolf *et al.*, 1981. The current study also found that the shape of lymphoid follicle of ileum is long sac-like shape. Numerous IELs were found in the villus epithelium in comparison to the crypts. This is an accord to Barman *et al.* (1997). Glaister, (1973) reported that when germ free mice were exposed to normal environment, the number of IELs increased markedly. Ferguson and Parrott (1972) and Ferguson (1977) reported that the intestine of normal mouse contains no IELs before the age of 2 to 3 weeks. Thus, they concluded that the age, together with some factors within the intestine and intraluminal antigenic stimulation influenced the production of IELs. However, IEL numbers in cats were not affected by age (Sturgess *et al.*, 2001). Chu and Rober (1979) reported that the numbers of IELs in the small intestine of pig were much higher than those found in the small intestine of other

animals. Their study demonstrated that the pig had 3.3±0.6 IEL per 100 enterocytes at birth and 38.9± 3.5 IEL per 100 enterocytes after 31 days. According to our results, the calf had 12.3±1.2 IEL of vilius per 50 enterocytes and 4.7±1.3 IEL of crypt per 50 enterocytes in 3month-old calves. Thus, our results are agreed with the findings by Chu and Rober, (1979). Results of this study also showed that IELs were found in the subnuclear level of adjacent epithelial cells and supported the results of previous studies (Chu and Rober, 1979; Meander and Lander. 1967; Glaister, 1973; Guy–Grand *et al.,* 1974 and Marsh, 1975).

The follicle associated epithelium (FAE) of Peyer`s patches in three-months old calves is described in current study. Scanning electron microscopy and transmission electron microscopy demonstrated the absence of goblet cells in the FAE and the presence of specialized cells similar in appearance to M cells as described previously in small intestine of calves (Liebler *et al.,* 1988). FAE of the jejunal resembled that of reported in other mammalian species. M cell ultrastructure, certain characteristics were uniformly found in all mammalian species. The luminal surface of these cells was variable and short, sparse microvilli or microfolds were seen. This FAE had poorly developed microvilli, with numerous cytoplasmic tubules, vesicles and vacuoles similar to those in FAE described in chicken bursa of Fabricius, man, pig and mouse Peyer`s patches (Owen and Jones, 1974). The differences between the jejunal and ileal were readily seen. Scattered cells lacking a distinct brush border were found in the FAE of jejunal PPs and FAE of ileal PPs that had homogeneous population of cells. Landsverk (1987) mentioned that a structural and functional maturation of PP developed before birth and their qualification as primary lymphoid organs. Torres-Medina (1981) stated that the luminal surface of bovine M cells of FAE had densely packed blunt microvilli that were irregular, short and thick. In contrast, the luminal surface of M cells of FAE was covered with loosely blunt microvilli in this study. In this study, the ileal M cells had fewer and shorter microvilli. This is similar to the finding by Hogenesch and Felsburg (1990). In contrast, M cells of FAE of the ileal Peyer`s patches in the Rhesus Macaque was covered with irregular membrane productions (Kuhn and Kaup, 1996). Torres- Medina (1981) described that FAE contained only one population of M cells in calf. However, the FAE of DPPs and CPPs of small intestine of three-month old calves in this study had two cell types (columnar and rounded shaped) of M cell. The luminal surface of M cells had irregular microvilli and others had variable cytoplasmic protrusion. This surface structure may be related to the degree of maturation of M cells (Bye *et al.,* 1984). The M cells, enterocytes and goblet cells were rapidly renewing cell population. Thus, the immature M cells were abundant and better-defined microvilli than mature M cells (Bye *et al.,* 1984). The M cell of FAE of small intestine demonstrated in this study may be immature form. Thus, the structures were slight different. In addition, Smith *et al.* (1980) found that the FAE of Peyer`s patches of mouse and horses contained goblet cells. However, in this study, the FAE of Peyer`s patches of calves had no goblet cells.

The M cells form to be basic for the establishment of a local secretory immune response through the phagocytosis of intestinal antigens and their transport to their intracytoplasmic lymphoid cells (Wolf *et al.*, 1981). In addition to their physiological role, the M cells are referred to as an intestinal entry for a variety of systemic infections. Two different routes have to be postulated for the initiation of an immune response to gut luminal antigens: luminally processed antigen is taken up by FAE absorptive enterocytes and presented to intra and subepithelial T cells. M cells take up unprocessed luminal antigens and transport them to the underlying antigen-presenting cells (Kuhn and Kaup 1996). The results presented here demonstrated the normal ultrastructural of jejunal and ileal Peyer`s patches of small intestine. The ultrastructural of M cells is important to study of immune response to the antigens and detection of pathologic lesion. The present study described the characteristic of M cells and intraepithelial lymphocytes of calves. In addition, the study also emphasized the important uses of SEM for the proper identification of epithelial covering the dome of lymphoid follicles.

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