MORPHOLOGICAL STUDY OF THE ILEAL PEYER'S PATCHES OF MAMMALS

BY

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ABSTRACT

Ileal Peyer's patches of some mammals such as sheep and goats have similar morphological characteristics to the avian bursa of Fabricius. The objective of this study was therefore to obtain more data on the morphological properties and life history of ileal Peyer's patches in a wider range of mammalian species. Thus, gross and histologic methods were used to evaluate the morphological changes in the ileal Peyer's patches during fetal, youth and adult periods in wildebeests, reedbucks, cattle, donkeys, pigs, dogs, cats, wild rats, giant rats, baboons and vervet monkeys. In each age group three animals were used. Grossly, no Peyer's patches were visible in duodenum, jejunum and ileum of fetuses except donkey fetuses, which had 29 ileal Peyer's patches and 40 jejunal Peyer's patches. However, microscopic evaluation of fetal ileal samples from fetuses of all mentioned species except rats at about mid gestation, showed accumulation of lymphoid cells in the sub-mucosa. During late fetal period, cattle and donkey fetuses had conical follicles while dogs had round follicles. In addition, bovine fetus ileal Peyer's patch follicles showed distinct cortico-medulla differentiation. Young wildebeests, reedbucks, cattle, pigs, dogs and cats had long continuous band of ileal Peyer's patch that covered the ileum and extended into the jejunum. Donkeys, baboons and vervet monkeys possessed 32, 1, 3 oval shaped individual ileal Peyer's patches respectively, while rats had no patches. Young wildebeests, reedbucks, cattle, cats, baboons and vervet monkeys possessed oval shaped jejunal Peyer's patches numbering 32, 10, 26, 3, 12 and 18, respectively. Foals and puppies had round jejunal Peyer's patches that numbered 48 and 8, respectively. No jejunal Peyer's patches were seen in piglets and rats. Duodenal Peyer's patches were only seen in puppies. Microscopically, ileal Peyer's

patch showed follicles that were sac-like, tightly packed, with clear cortico-medulla differentiation and small domes, corona and interfollicular areas. The adults of wildebeest, reedbuck, cattle, pig, donkey, dog and cat had no grossly visible ileal Peyer's patches. However, microscopically, sub-mucosa was filled with connective tissue. Nevertheless, wild rats had 2 and giant rats had 8 small round ileal Peyer's patches that contained pear-shaped follicles, wider domes, conspicuous corona and large interfollicular areas. All animals under study possessed jejunal Peyer's patches, which were bigger than those of respective youths and their numbers averaged 55 in wildebeest, 15 in reedbuck, 37 in cattle, 8 in pig, 57 in donkey, 9 in dog, 6 in cat, 3 in wild rat and 17 in giant rat. Again, 3 duodenal Peyer's patches were seen in dogs. Adults of non-human primates were not obtained. The study has therefore demonstrated presence of a continuous long ileal Peyer's patches in young animals in orders; Artiodactyla and Carnivora while young animals of the order Perissodactyla had discrete round patches. At least, the study has also demonstrated a prenatal maturation of the ileal Peyer's patches in the mammalian orders Artiodactyla, Perissodactyla and Carnivora which is followed by full maturation at young age and lastly by involution at adulthood except in rats. This morphological pattern resembles that of avian bursa of Fabricius.

DECLARATION

I, HANDO HUSSEIN ANTALLO do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has not been submitted for a degree award in any other University.

Signature.....

Date.....26 October 2001

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This work is dedicated to my mother Mrs. Ulla Antallo, my father the late Mr. Antallo Saki, my wife Mrs. Neema Antallo and our beloved son Mr. Antallo H. Antallo.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

µm	Micrometre
Bov	Bovine
cm	Centimetre
DNA	Deoxyribose Nucleic Acid
DPX	Distrene Plasticizer Xylene
Fig.	Figure
GALT	Gut – Associated Lymphoid Tissues
Ig	Immunoglobulin
IgA	Immunoglobulin A
IgG	Immunoglobulin G
IgM	Immunoglobulin M
M-Cell	Membraneous cell
mm	Millimetre
Mon	Month/Months
RB	Reedbuck
SE	Standard Error of Mean
WB	Wildebeest
Yr	Year/Years

CHAPTER ONE

1.0 INTRODUCTION

1.1 LYMPHOID ORGANS

The lymphoid organs of vertebrates are divided into three groups namely primary, secondary and tertiary lymphoid organs (Faulk *et al.*, 1971; Agostini *et al.*, 1993; Tizard, 1996). This division is based on a number of non-immunological criteria amongst which are morphology, stage of development and pattern of cellular repopulation (Faulk *et al.*, 1971; Tizard, 1996).

The primary lymphoid organs include the thymus, which is found in both mammals and birds, bursa of Fabricius in birds and bursa equivalent in mammals. The bone marrow being responsible with the differentiation and maturation of the immunocompetent lymphoid cells destined to make humoral antibodies is thus also regarded as a primary lymphoid organ (Burnet, 1968; Osmond, 1980; Agostini *et al.*, 1993; Tizard, 1996).

The secondary lymphoid organs include spleen, lymph nodes, tonsils and certain Peyer's patches. They originate from the embryonic mesoderm. These secondary lymphoid organs persist in the adulthood and upon their removal there is no or very little development of antibody deficiency. Generally, these secondary lymphoid organs are responsible with the trapping, accumulation and processing of antigens (Agostini *et al.*, 1993; Tizard, 1996).

Tertiary lymphoid tissues are considered to be all other body tissues that have few lymphoid elements, but that upon local antigenic stimulation, represents sites of accumulation of memory lymphocytes that have previously been generated in the secondary lymphoid organs. Such tissues include udder, lungs, and urogenital tracts (Agostini *et al.*, 1993; Tizard, 1996)

1.2 LYMPHOCYTES DIFFERENTIATION

Lymphocytes are differentiated into three types, namely T-lymphocytes, B-lymphocytes and Natural killer (NK) lymphocytes (Tizard, 1996). The differentiation of T-lymphocytes takes place in the thymus in both mammals and birds and they are primarily responsible for immunologic reaction called cellular immunities that include allergies and graft-versus-host reactions (Cooper *et al.*, 1967; Tizard, 1996). The differentiation of B-Lymphocytes on the other hand, takes place in the bursa of Fabricius in the birds whereas in the mammals, they are said to differentiate in an organ homologous to the bursa of Fabricius of bird. B-lymphocytes are responsible with the humoral mediated immunity (Cooper *et al.*, 1967; Tizard, 1996). Natural Killer (NK) lymphocytes are neither T-lymphocytes nor B-lymphocytes. Despite the fact that they are said to originate from the same haemopoietic stem cell as other T-lymphocytes, they do not undergo thymic processing. They are found in small numbers in the blood and are widely distributed throughout the secondary lymphoid organs (Weiss, 1983; Tizard, 1996).

Although, differentiation and maturation of B-Lymphocytes in mammals is widely accepted to occur in the bone marrow (Osmond, 1980; Tizard, 1996), mammals are believed to posses a bursa equivalent, which is related to the gut as an avian bursa.

The bursa of Fabricius and the bursa-equivalent are lymphoepithelial tissues which originated from the ecto-endo-dermal junction, develops early in embryonic life, involutes at puberty, unresponsive to antigenic stimulation and upon their removal there is development of antibody deficiency conditions. Peyer's patches have thus been identified as possible bursa equivalent (Tizard, 1996).

However, other studies have shown that there are some behaviours of the Peyer's patches, which makes them not to qualify as a bursa equivalent. These behaviours includes the finding that in mice, upon intraluminal antigen sensitization, the Peyer's patches produce precursors of IgA secreting plasma cells which eventually home to the intestinal mucosa (Craig and Cebra, 1971). It has also been reported that in the mice, the pattern of cellular repopulation of the Peyer's patches is similar to that of lymph node but not of thymus (Evans *et al.*, 1967). Lymphopoiesis in the Peyer's patches has also been shown to be at least antigen-dependent in the rabbits, following surgical *in situ* isolation of the Peyer's patches from normal gut-lumen flow without disrupting the vascular integrity (Perey and Good, 1968; Stramignoni and Mollo, 1968). The behaviour of antigen dependency has also been shown when studying the development of bovine immune system (Schultz *et al.*, 1973). Other behaviours include the presence of T-lymphocytes in the developing Peyer's patches (Evans *et al.*, 1967). The demonstrations that cultures of the Peyer's patch cells from non-primed rabbit responded *in vitro* to sheep red blood cells is similar to the spleen cultures (Henry *et al.*, 1970) and a finding of B-lymphocytes in a fetal liver and bone marrow instead of the Peyer's patches (Owen *et al.*, 1974; Osmond, 1980) are also in support of a suggestion that the Peyer's patches are not a bursa equivalent.

Nevertheless, Peyer's patches display heterogeneity in their location in the intestines, life history and morphology. There are thus four types of Peyer's patches, namely, discrete patches located in the duodenum termed duodenal Peyer's patches. Many discrete Peyer's patches are located in the jejunum and termed jejunal Peyer's patches. Single continuous ileal Peyer's patch is located in the ileum while other discrete patches occur in the colon and are termed colic Peyer's patches (Reynolds and Morris, 1983; Larsen and Landsverk, 1985; Reynolds *et al.*, 1985; Assey, 1988; Liebler *et al.*, 1988; Parson *et al.*, 1989; HogenEsch and Felsburg, 1990; Barman *et al.*, 1997).

The empirical evidences on the prenatal maturation, persistence and lymphopoiesis relate the ileal Peyer's patches to the avian bursa. This has been reported in sheep (Reynolds and Morris, 1983; Miyasaka *et al.*, 1984; Larsen and Landsverk, 1985; Reynolds *et al.*, 1985) and in goats (Assey, 1988).

1.3 OBJECTIVE OF THE STUDY

Immune system of mammals is composed of a cellular component controlled by thymus in the mammals and birds and humoral component controlled by bursa of Fabricius in birds and bursa equivalent in mammals (Tizard, 1996). Ileal Peyers patches have been related to the bursa of Fabrius of birds both functionally and morphologically in sheep and goats (Reynolds and Morris, 1983; Assey, 1988). However, no such studies have been done in other mammalian species. Therefore, the objective of the present study was to obtain more data on the morphological

characteristics of the ileal Peyer's patches in a wider range of mammalian species. The species involved in this study belonged to five mammalian orders, which were Carnivora including dogs (*Canis familiaris*) and cats (*Felis domestica*); Rodentia including giant rats (*Cricestomys gambianus*) and wild rats (*Mastomys natalensis*); Primates including baboons (*Papio cynocephalus*) and vervet monkey (*Cercopithecus aethiops*); Perissodactyla including donkeys (*Asinus africanus*); Artiodactyla including cattle (*Bos indicus*), blue Wildebeest (*Connochaetes taurinus*), common reedbuck (*Redunca arundinum*) and pigs (*Sus scrofa*). The study involved gross and microscopic evaluation of the prenatal and postnatal development of the ileal Peyer's patches.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 GUT-ASSOCIATED LYMPHOID TISSUE (GALT)

The gut-associated lymphoid tissue is the major sub-division of the immune system along the gut. The gut-associated lymphoid tissue is composed of lymphoid cells and reticuloendothelial cells in the epithelium and lamina propria, aggregated lymphoid follicles (*folliculi lymphatic aggregati*) called the Peyer's patches (Owen and Jones, 1974; Fahel and Morris, 1978; Reynolds and Morris, 1983; Kanamori *et al.*, 1996), isolated lymphoid follicles (*Folliculi lymphatic solitari*) in the esophagus, stomach and colon (Assey, 1988; Liebler *et al.*, 1988; Parson *et al.*, 1989; Parson *et al.*, 1991; Kanamori *et al.*, 1996), appendix and *Sacculus Rotundus* (Faulk *et al.*, 1971; Waksman *et al.*, 1973) and the avian bursa (Glick *et al.*, 1956).

Therefore, gut-associated lymphoid tissue contains several lymphoid tissue populations that can be distinguished on morphological consideration. In fact the sub-epithelial lymphatic tissue of the digestive tract contains three times as many lymphocytes as there found in the circulating blood and when taken as a whole, the sub-epithelial lymphatic tissue of the digestive tract amounts to 6.5 times that of all the other lymphatic tissues combined (Grau, 1979). The gastro-intestinal tract contains mixtures of antigens such as microorganisms or potentially harmful foreign substances. It is therefore, not surprising to find that the mucosa is heavily populated with the cells of the immune system (Fahel and Morris, 1978; Neutra, 1983).

2.2.PEYER'S PATCHES.

Grossly, Peyer's patches are circumscribed lymphoid tissues in the gut mucosa involved in the uptake of antigen and induction of gut-associated immune responses. They are seen as patches on the side of intestinal luminal wall opposite the mesenteric border (Doughri, *et al.*, 1972; Reynolds and Morris, 1983; Binns and Licence, 1985; Assey , 1988; Parson *et al.*,1989).

Microscopically Peyer's patches contains follicles that are located primarily in the sub-mucosa. However, they may reach the lamina propria (Doughri *et al.*, 1972). Each follicle (Fig.1) has a cap called the corona covered by the dome. The dome is covered by dome epithelium or follicle associated epithelium (FAE). The FAE is composed of a non-columnar absorptive cell (enterocytes), intraepithelial lymphocytes, specialized micropinocytotic cells (M-Cells) and lacks mucous cells (Faulk *et al.*, 1971; Waksman *et al.*, 1973; Bockman and Cooper, 1973; Landsverk, 1984; Owen and Jones, 1974; Burns, 1982; Reynolds and Morris, 1983; Ermak and Owen, 1986; HogenEsch and Felsburg, 1990; Gerbert, 1997). However, there are a few mucous cells in the pigs (Chu et al., 1979). Between the apices of adjacent follicle there is an area called the interfollicular area (IFA) or thymus dependent area (TDA) containing post-capillary high-endothelial-venules (Waksman *et al.*, 1973; Reynolds and Morris, 1983; Reynolds et al., 1985; HogenEsch and Felsburg, 1990). Above the IFA there is an area called the mushroom which is covered by normal gut epithelium (Sobhon, 1971; Waksman *et al.*, 1973; Chu *et al.*, 1979; Landsverk, 1984; Sharpnack *et al.*, 1984). Reynolds and Morris (1983) reported that despite the

fact that the dome and follicles were joined, both regions are quite distinct histologically.

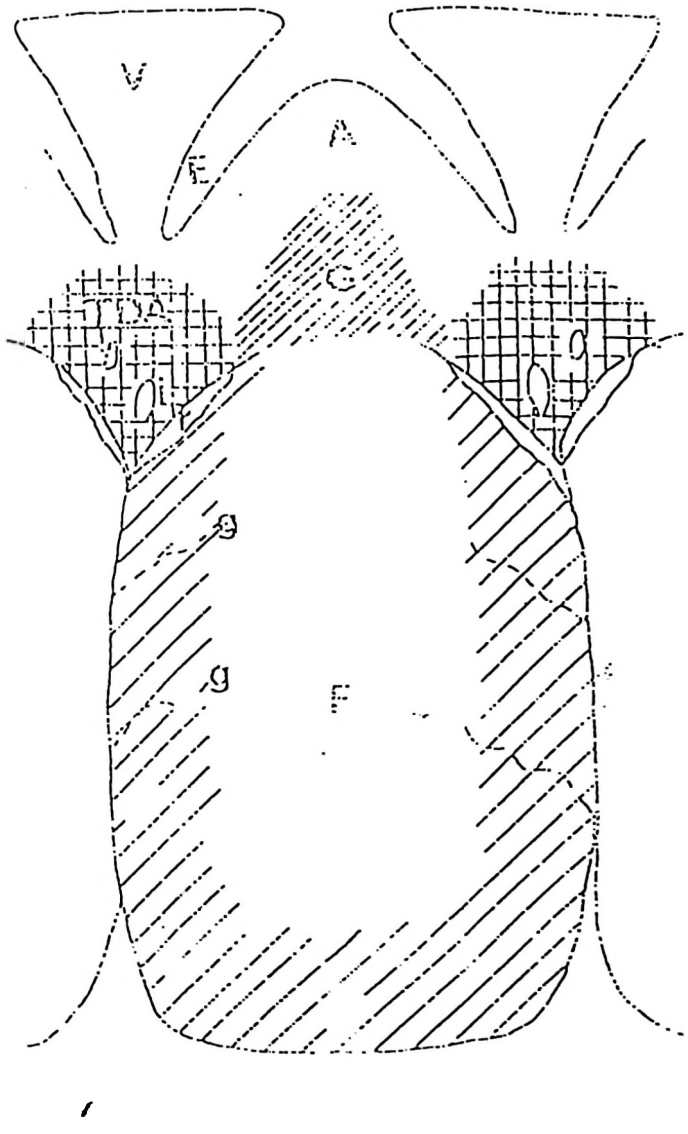

Figure 1: Diagram to show the morphologic elements of a Peyer's patch follicle. Dome (A), crypts extending from lumen (E), follicle germinal center (F), Corona (C), mushroom of adjacent villi (V), thymus dependent area (TDA), capillaries (g), post capillary venules (i) and lymphatics (r) (With minor modification from Waksman *et al.*, 1973)

2.3 HETEROGENEITY OF THE PEYER'S PATCHES

Peyer's patches are classified according to location, life history, morphological features and functional characteristics. There are therefore four types of Peyer's patches i.e. duodenal, jejunal, ileal and colic (Reynolds and Morris, 1983; Larsen and Landsverk, 1985; Reynolds *et al.*, 1985; Assey, 1988; Parson *et al.*, 1989; HogenEsch and Felsburg, 1990; Barman *et al.*, 1997).

2.3.1 Duodenal Peyer's patches.

Grossly, duodenal Peyer's patches appear as small raised and roundish patches located in the duodenum of dog (HogenEsch and Felsburg, 1990) and occasionally reported in goat (Assey, 1988). Histologically, they have small dome, pear-shaped follicles and are also characterized by the presence of small invagination from dome epithelium. In terms of their life history and functional characteristics they resemble the jejunal Peyer's patches (Assey, 1988; HogenEsch and Felsburg, 1990).

2.3.2 Jejunal Peyer's Patches

Grossly, jejunal Peyer's patches are seen as discrete patches located in the jejunum. They are raised, oval or elongated with round ends. They persist during entire lifetime of all mammals and their size and number increase with age (Gerber, 1979; Reynolds *et al.*, 1981; Reynolds and Morris, 1983; Larsen and Landsverk, 1985; Reynolds *et al.*, 1985; Assey, 1988; HogenEsch and Felsburg, 1990; Parson *et al.*, 1991).

length (Parson *et al.*, 1989; Parson *et al.*, 1991). Prenatal studies on the bovine gut indicated that they appear grossly as recognizable structure in the eighteenth week of the fetal life and are well developed by the end of twentieth week (Titkemeyer and Calhoun, 1955).

In sheep, it is reported that there are 25-40 patches scattered throughout the jejunum with individual patch size of up to 10cm length and 1.5 cm width (Gerber, 1979; Reynolds, 1980; Reynolds *et al.*, 1981; Reynolds and Morris, 1983; Larsen and Landsverk, 1985; Reynolds *et al.*, 1985).

Kids are born with an average of 35 Peyer's patches in the jejunum with a length ranging from five follicles to 4.5-cm long patch. At the age of three months, goats are reported to possess about 38 Peyer's patches in the jejunum with size ranging from five follicles to about 5.4 cm long patch. At 8 to 12 months there are about 33 jejunal Peyer's patches with individual length ranging from five follicles to 8.1 cm long patch (Assey, 1988).

Horses are reported to have 25 patches that are intermittently arranged in the jejunum with their individual size varying from 1.5 to 2.0 cm length (Titkemeyer and Calhoun, 1955).

In dogs, the patches appear as circumscribed elevations with the size of up to 2.0-cm length and 1.5 cm width. Their numbers range from 17 to 26 with an average of 22 patches and are more numerous in the cephalic portion of the jejunum. Unlike the

other species; they are visible even from the serosal surface (Titkemeyer and Calhoun, 1955). In the cat, the Peyer's patches are so small such that their visualization is only possible microscopically (Titkemeyer and Calhoun, 1955).

In the human beings, Peyer's patches are reported to be ovoid in shape. Their number reaches up to 239 at puberty. However, Peyer's patches having more than 25 follicles were reported to be 40 – 100 in number at puberty. Therefore, there is general tendency for the Peyer's patches to increase in size and number with increasing age until puberty (Cornes, 1965)

Microscopically, these patches are composed of pear shaped follicles containing large population of T-lymphocytes. They also have broad domes, conspicuous corona, large interfollicular areas containing numerous post-capillary high-endothelial venules. These venules contain recirculating lymphocytes as reported in sheep (Gerber, 1979; Reynolds, 1980; Reynolds *et al.*, 1981; Reynolds and Morris, 1983; Larsen and Landsverk, 1985; Reynolds *et al.*, 1985), pigs (Barman *et al.*, 1997) and dogs (HogenEsch and Felsburg, 1990). Veil cells which resemble the antigen presenting cells of the skin has been reported to occur in the interfollicular areas of rats, guinea pigs and pigs (Wilders *et al.*, 1983).

2.3.3 Ileal Peyer's patches.

These appear as a single continuous band covering an area from ileocecal junction and extend into the jejunum. Thus, it is also termed ileo-jejunal Peyer's patch. Involution starts from its proximal end (located in the jejunum). In cattle there is a

presence of one long patch in the ileum (Parson *et al.*, 1989; Parson *et al.*, 1991). In the sheep it is reported that the patch measures 2.5-meter long covering the entire ileum length (Gerber, 1979; Reynolds *et al.*, 1981; Reynolds and Morris, 1983; Larsen and Landsverk, 1985; Reynolds *et al.*, 1985). In newborn kids, the patch measures about 0.8 meters but at the age of three months the patch increases to about 1.4 m in length. However, between 8 to 12 months of age, the jejuno-ileal Peyer's patches appears to undergo involution (Assey, 1988). In horses there are no continuous ileal Peyer's patch. Instead, there are discrete Peyer's patches, which begin at about 1.2 meters from the pylorus and are located intermittently in both the jejunum and ileum. Their total number in the jejunum and ileum was found to be 25 with their size ranging from 1.5 to 20 cm in length (Titkemeyer and Calhoun, 1955). In pigs, the ileal Peyer's patch also form a long continuous band covering the ileum and extends into the jejunum (Binns and Licence, 1985).

Microscopically the ileal Peyer's patch is characterized by having sac-like tightly packed follicles containing mostly B-cells expressing IgM surface receptors whereas T-Cells accounts for less than 1% of the total cells. Their dome is slender villus-like, have an inconspicuous corona and very small interfollicular area (Gerber, 1979; Reynolds and Morris, 1983; Larsen and Landsverk, 1985; Reynolds *et al.*, 1985; Assey, 1988; Parson *et al.*, 1989; HogenEsch and Felsburg, 1990; Parson *et al.*, 1991; Barman *et al.*, 1997). In the horse, ileal Peyer's patch comprises of proprial follicles, lymphoglandular complexes that are found in the submucosa and follicles that extend from the lamina propria to the submucosa (Lowden and Heath, 1996).

Follicles are surrounded by dense connective tissue capsule isolating each follicle from its neighbours. Each follicle is divided into two distinct parts namely follicular medulla and follicular cortex. The follicular medulla is centrally situated containing primarily small dark cells (have IgA surface receptors) with low labeling index. The follicular cortex is peripherily situated containing primarily large cells (have IgM surface receptors) with high labeling index showing that they are in great rate of DNA synthesis. Thus, these cells are highly proliferating. A few macrophages containing bacteria and dendritic cells are also present in the cortex of the follicle (Fitchelius, 1968; Meuwissen *et al.*, 1969; Sobhon, 1971; Waksman *et al.*, 1973; Reynolds and Morris, 1983; Spalding *et al.*, 1983; Larsen and Landsverk, 1985; Reynolds *et al.*, 1985; Ermak and Owen, 1986; Lebman and Coffman, 1988; Bjerk *et al.*, 1988; Parson *et al.*, 1989; Ermak *et al.*, 1990; Sharma *et al.*, 1998).

The dome is a slender villus-like structure covered by dome epithelium or follicle associated epithelium (FAE). The epithelium covering the dome is composed of non-columnar absorptive cells (enterocytes), intraepithelial lymphocytes and specialized micropinocytotic cells (M-Cells) (Faulk *et al.*, 1971; Waksman *et al.*, 1973; Owen and Jones, 1974; Burns, 1982; Reynolds and Morris, 1983; Landsverk, 1984; Ermak and Owen, 1986; HogenEsch and Felsburg, 1990; Gerbert, 1997). Mucous cells are reported to be lacking in most of the mammalian gut-associated lymphoid tissue epithelium. However, mucous cells have been observed in the pig (Chu *et al.*, 1979) and in the goat (Assey, 1988).

The intraepithelial lymphocytes seem to reside within the epithelial cells but in actual fact they are found between the epithelial cells (Chu *et al.*, 1979). Several observations suggest that the intraepithelial lymphocytes migrate from the intestinal lamina propria and it is believed that, partially if not completely is of T-cell origin following antigenic stimulation (Guy-Grand *et al.*, 1974). However it is also reported that they are a mixed population of T- and B-lymphocytes similar in composition to those of Peyer's patches, spleen and lymph nodes (Ferguson and Parot, 1972). A study with electron microscope has shown that the intraepithelial lymphocytes are larger than peripheral or circulating lymphocytes (Guy-Grand *et al.*, 1974). Furthermore, it is reported that the number of intraepithelial lymphocytes increase with age and cranial portion of the small intestine contain much more than the caudal portion (Chu *et al.*, 1979; Reynolds, 1980; Assey, 1988)

The M-Cells are specialized for the trans-epithelial transport of lymphocytes and antigens (Giannasca and Neutra, 1994). Their apical membranes have lectin binding sites (Gerbert and Hach, 1993). In mice it has been reported that these lectin binding sites have fucosylated glycoconjugate unique to the apical surface and cytoplasmic content (Clara *et al.*, 1993; Falk *et al.*, 1994; Sharma *et al.*, 1996). In pigs, the M-cells differs from the enterocytes in the composition of their cytoskeleton which is called Cytokeratin 18 (Gebert *et al.*, 1994). In cattle, the M-Cells are hidden between enterocytes such that they are only detected by their cytoplasmic protrusion into the lumen (Parson *et al* 1991). M- cells are more numerous in the ileal Peyer's patches than in the jejunal Peyer's patches in lambs, calves and kids (Tores-Medina, 1981) while in pigs there is no difference (Chu and Liu, 1984).

The follicle associated epithelium of the ileal Peyer's patches have also a specific characteristic behaviour of shading membrane bound carbonic anhydrase particles (cap) of 50ηm size (Reynolds and Morris, 1983; Landsverk, 1987). These particles are said to be rich in carbonic anhydrase due to their derivation from the luminal plasma membrane (Landsverk, 1990). It is therefore, suggested that these particles are taken up by follicle lymphocytes by attaching and indenting to their plasma membranes and their function is not yet known (Landsverk *at el.*, 1990).

The dome also contains a network of reticular fibers interspersed with large and medium lymphocytes mixed with blast cells, few small lymphocytes and few macrophages containing bacteria (Waksman *et al.*, 1973; Reynolds and Morris, 1983; Sharma *et al.*, 1998). The corona is inconspicuous and composed of a network of few reticular fibers with small lymphocytes and a few macrophages containing bacteria (Waksman *et al* 1973; Butcher *et al.*, 1982; Bjerk *et al.*, 1988).

The interfollicular area (IFA) or thymus dependent area (TDA) is a very small triangular area situated between the apices of adjacent follicles (see Fig.1). It is composed of small lymphocytes and post-capillary high-endothelia venules (Sobhon, 1971; Waksman *et al.*, 1973; Bockman and Cooper, 1973; Reynolds and Morris, 1983; Reynolds *et al.*, 1985; Larsen and Landsverk, 1985). This area is said to be responsible with the recirculation of lymphocytes. Thus, it is an analogue to the cortico-medullary region of the lymph nodes (Parot and Ferguson, 1974). Rodents contain well-developed high endothelial venules while in sheep, they are

not as developed as in rodents (Reynolds, 1980). In mice the post-capillary high-endothelial venule are small and are located near the base of a follicle (Sobhon, 1971).

The mushroom of the ileal Peyer's patches surround the dome to a height of about 250-300 μm above the level of dome apex and do not prevent the visualization of the dome from the luminal surface. The epithelium of these villi is just like that of a normal mucosa containing enterocytes, numerous mucous cells, plasma cells, eosinophils and very few lymphocytes (Waksman *et al.*, 1973; Sharpnack *et al.*, 1984).

Comparing the gut-associated lymphoid tissue of rabbit and sheep it has been shown that the appendix of rabbit is similar to the ileal Peyer's patches of the sheep while the ileal Peyer's patches of rabbit are similar to the jejunal Peyer's patches of the sheep. Furthermore, Assey (1988) reported morphological similarity between the ileal Peyer's patches of goats and the appendix of rabbit. In addition, jejunal Peyer's patches of goats are equivalent to the to ileal Peyer's patches of rabbit and colic Peyer's patches were equivalent to the jejunal Peyer's patches of the rabbits, goats and cattle (Waksman *et al.*, 1973; Reynolds *et al.*, 1985; Assey, 1988; Liebler *et al.*, 1988; Parson *et al.*, 1989).

2.3.4 Colic Peyer's patches.

These are small, raised and roundish patches located in the colon. In goats there are two patches (Assey, 1988). While in cattle there is a single roundish patch (Liebler

et al., 1988; Parson *et al.*, 1989; Parson *et al.*, 1991). Histologically, they are composed of short, roundish or oval-shaped follicles. In terms of life history and functional characteristics, the colic Peyer's patches are similar to the jejunal Peyer's patches in goats (Assey, 1988) and cattle (Liebler *et al.*, 1988; Parson *et al.*, 1989).

2.4 ILEAL PEYER'S PATCHES AS BURSA EQUIVALENT.

The history of the bursa of Fabricius started when Glick *et al* (1956) described its role in the development of the humoral immunity in birds. These authors quite incidentally observed that female chickens, which were bursectomized twelve days after hatching subsequently, failed to produce detectable antibodies following intravenous injection of heat inactivated *Salmonella typhimurium* organisms. Since then, there has been search for an organ, which could be responsible for the development of humoral immunity in the mammals. Thus, in the process of searching for a mammalian equivalent organ Cooper *et al* (1965) presented a clear morphological and functional definition of the dichotomy of the immune system of chicken and showed the possibility that a similar system may exist in mammals.

The finding of the existence of heterogeneity of Peyer's patches is noteworthy. Among four types of Peyer's patches, the ileal Peyer's patches is the one considered to be the possible bursa equivalent (Reynolds and Morris 1983; Miyasaka *et al.*, 1984; Reynolds *et al.*, 1985; Larsen and Landsverk, 1985; Assey, 1988). Similarities between ileal Peyer's patches and bursa of Fabricius have been established to be morphological and functional. The morphological criteria includes changes that take place during prenatal and postnatal development (Faulk *et al.*,

1971; Reynolds *et al.*, 1981; Tizard, 1996). These morphological criteria are as follows:

2.4.1 ORIGIN

Experimental studies using chromosomal markers and histological techniques, in combination with other techniques like parabiosis and transplantation procedures have demonstrated the inflow of blood borne stem cells into chick embryo thymic region and bursal primodium during embryogenesis. Therefore, these blood borne stem cells are shown to be responsible for the development of the thymus (Moore and Owen, 1967) and bursa of Fabricius (Moore and Owen, 1966).

When these blood-borne stem cells have reached their respective areas, they proliferate and differentiate into thymus-dependent lymphocytes and bursa-dependent lymphocytes, respectively. Their epithelial cell component is responsible for furnishing an inductive environment for the said proliferation and differentiation of the stem cells (Moore and Owen, 1966. 1967). Furthermore, supporting the above finding, it was proposed that the lymphoid stem cells perhaps of vascular or marrow origin migrate to the lymphoepithelial sites where local environmental influence or directs their proliferation and differentiation along cell lines that are expressed in the periphery lymphoid tissues. The thymus-influenced cells are directed towards the specialized function of cellular immunity and the cells influenced by the bursa of Fabricius in birds or bursa equivalent in mammals are directed towards the immunoglobulin production and humoral immunity (Good *et al.*, 1967; Cooper *et al.*, 1968).

2.4.2 MORPHOLOGICAL SIMILARITIES

Archer *et al* (1963) compared the development of the bursa of Fabricius and the appendix of the rabbit taking into account their relationship with the epithelium and reported that the two are morphologically similar, since both are gut-associated lymphoepithelial tissues with follicular organization. Furthermore, the proximity to the contents of the intestinal lumen was postulated to be essential for the special lymphopoietic activity of the bursa of Fabricus (Thompson and Cooper, 1971) and the gut-associated lymphoid tissue (Perey and Good, 1968). Therefore, morphological similarities between the bursa of Fabricius and Peyer's patches can be further seen as follows:

2.4.2.1 Ontogeny

The development of these primary lymphoid organs is said to start early in the embryonic life with unknown stimuli causing cell proliferation in the bursa of Fabricius. In the mammalian embryo too, proliferation of lymhocyets in the Peyer's patches is reported to be not due to extrinsic antigens (Kincade and Cooper, 1971; Tizard, 1996).

The proliferation (lymphopoiesis) continues in these organs even after their isolation from digestive tract as in sheep (Reynolds, 1980) and rabbits (Perey and Good 1968). It has been reported that somatic hypermutation occurs in the ileal Peyer's patches independent of the antigen influence. Thus, this increases the number of genetically determined B-cell repertoire (Reynaud *et al.*, 1991). It is

believed that majority of mammals show antigenic independent lymphopoesis because type of placenta they possess does not allow placental antigenic exchange (Reynolds and Morris, 1984). When studying the development of the bursa of Fabricius by using histochemical and morphological procedures, it was noted that the formation of lymphoid follicles starts when the hemopoietic stem cells invade connective tissue in the bursa which, takes place by the tenth day of incubation appearing as an accumulation of lymphoid cells, clusters of pale staining reticuloendothelial cells and large cells with abundant cytoplasm and their proliferation continues until the mature follicles are formed at around hatching (Shiojiri and Takahashi, 1991).

The primordial Peyer's patches appear as an accumulation of lymphoid cells, clustered together with pale staining reticuloendothelial cells and large cells with abundant cytoplasm. This develops at day 60 of pregnancy in sheep (Reynolds and Morris, 1983), from day 50 in pigs (Chapman *et al.*, 1971) and from day 120 in cattle (Ishino *et al.*, 1991).

In about mid-gestation the growing Peyer's patches are seen as accumulation of lymphoblast cells mixed with some darkly stained and lightly stained epithelial cells clustered together to form a characteristic follicular distribution (Doughri *et al.*, 1972; Schutz *et al.*, 1973; Chapman *et al.*, 1974; Reynolds and Morris, 1983; Ishino *et al.*, 1991).

During late pregnancy or late incubation period and early postnatal life, these lymphoepithelial tissues are reported to be histologically mature as they are seen to have villous-like domes, sac-like follicles with connective tissue capsule, small triangular interfollicular area and very small or sometimes inconspicuous corona. The follicles are divided into a periphery follicular cortex containing primarily large cells with high labeling index and central follicular medulla containing primarily small cells (Reynolds and Morris, 1983; Assey, 1988; Ishino *et al.*, 1991; Shiojiri and Takahashi, 1991; Barman *et al* 1997).

Investigations in man (Cornes, 1965) and dog (Bryant and Shifrine, 1972) also revealed that the accumulation of lymphoid tissue in the gut occur before birth. In man these accumulations are seen at 98 days of gestation (Kyriazj and Esterly, 1970) while in dogs, they are seen at day 62 of pregnancy (Bryant and Shifrine, 1972).

2.4.2.2 The young stage:

The rate of cell division in the follicles is higher in the last stage of pregnancy as reported in sheep fetus (Reynolds *et al.*, 1981). Thus, these organs reach maturity at around birth when cell division is high. This type of cell division continues postnatally until the organs attain their greatest sizes. In the pigeons, it is reported to be 10 days post hatching (Ciriaco *et al.*, 1989), In the sheep it is at three to four months post-birth (Gerber, 1979; Reynolds, 1980; Reynolds and Morris, 1983). In goats greatest size of Peyer's patch follicles is reached at three months of age (Assey, 1988) while in cattle it occurs just after birth (Parson *et al.*, 1989).

2.4.2.3 The adult stage.

These lympho-epitheial tissues undergo involution once an animal has reached sexual maturity (Tizard, 1996). In the pigeon involution of the bursa of Fabricius commence between 90 to 180 days post hatching (Ciriaco *et al.*, 1989). In sheep, it has been reported that after attaining the age of three to four months, involution starts and is completed by the age of 15 months (Gerber, 1979; Reynolds, 1980; Reynolds and Morris, 1983; Reynolds *et al.*, 1985; Larsen and Landsverk, 1985). In goats, involution of the ileal Peyer's patches occurs between the age group of 8 to 12 months (Assey, 1988) and in the cattle involution commences soon after birth (Parson *et al.*, 1989).

In mammals where the ileal Peyer's patches are grossly visible, the involution is seen as a diminution of the patch starting from its distal end and progresses to the proximal. The process of involution is seen as a distal tapering of the patch. Finally, in the adulthood the patch completely disappears as reported in sheep, cattle and goats (Gerber, 1979; Reynolds, 1980; Reynolds and Morris, 1983; Reynolds *et al.*, 1985; Larsen and Landsverk, 1985; Assey, 1988; Parson *et al.*, 1991).

Microscopic studies revealed that the involuting patch initially shows that the follicles are regressing toward the center and there is a decrease in cellularity. Areas devoid of cells are filled with connective tissue and macrophages. However, in the periphery of the follicles some lymphocytes are still proliferating. In progressing involution sometimes only shriveled follicles remain. When the involution is about to be complete, the remaining dome has a heterogeneous mixture of cells including

plasma cells. At the end, the lymphoid tissue is completely replaced by fibrous tissue (Reynolds and Morris, 1983; Reynolds et al., 1985; Larsen and Landsverk, 1985; Assey, 1988; Ciriaco *et al.*, 1989; Parson *et al.*, 1989).

2.4.2.4 Relationship to a specialized epithelium

The epithelium in the bursa of Fabricius and ileal Peyer's patches has a specific characteristic behaviour of shading membrane bound carbonic anhydrase particles (cap) of 50ηm size (Reynolds and Morris, 1983; Landsverk, 1987). However, in the bovine, the colic Peyer's patches do also shade these carbonic anhydrase particles (Parson *et al.*, 1991: Landsverk *et al.*, 1991). Another characteristic feature of these epithelia in the bursa of Fabricius of chicken, appendix of rabbit and Peyer's patches of mouse is their capability to transport ferritin or Indian ink traces from the gut lumen (Bockman and Cooper, 1973).

2.4.2.5 Lymphopoiesis

Fitchelius (1968) when searching for an organ which is the precursor of B-lymphocytes he concurred with the hypothesis that gut Peyer's patches are avian bursa equivalent by using phylogenetic data and *in vivo* observation that lymphocytes in the follicles of gut epithelium lymphoid tissue are in a stage of DNA synthesis. Thus, this proofs that these cells are in active stage of cell proliferation. Labelling technique showed that appendix, *Sacculus Rotundus* and Peyer's patches of rabbit appeared to be the primary location for cell replication because in their follicles more cells were in DNA synthesis especially in the

follicular cortex. This is a similar characteristic to that of bursa of Fabricius (Meuwissen *et al.*, 1969).

By using immunofluorescent markers, it has been demonstrated that there is presence of surface immunoglobulins in developing fetal Peyer's patches lymphocytes at a time when the Peyer's patches contain the dome and interfollicular area only (Chapman *et al.*, 1974). However, before these findings, it was reported that maturation of the dome cells gave rise to corona and follicles (Stramignomi and Mollo, 1968). Using endotoxin-stimulated cultures, it was also shown that B-Cell proliferation started from the dome, which then accumulate in the corona. Thus, the dome is said to be the supplier of the precursor of the immunocompetent B-Cells (Andersson *et al.*, 1972; Gery *et al.*, 1972).

The cells from the corona then enter the upper side of the follicle. These cells again move towards the center of the follicle then to the bottom of the follicle where they are initiated to undergo blast transformation. Thus, there is high rate of cell division (mitosis). As mitosis is completed, the proportion of the small cells increase and these cells (immature B-cells) enter the septal lymphatic sinuses in the follicle where they are ready for emigration. This process is said to take three to four days (Waksman *et al.*, 1973). In sheep the germinal centers in the ileal Peyer's patches seems to be undergoing centripetal migration of lymphocytes and there is increasing amount of surface immunoglobulin M (IgM) as cells migrating inward which is suggestive of progressive maturation (Larsen and Landsverk, 1985).

The abundance of pyknotic figures in the follicles and stathmokinetic study results has established the fact that there are signs of newly formed B-cell deaths in the follicles. Therefore, among the B-cells produced in the follicles many of them die within the follicles (Miyasaka *et al.*, 1984).

It is further reported that despite the deaths of many newly formed B-cells within the follicles some are emigrating. The extent of these emigrating newly formed B-cells from the follicles is sufficient to replace the B-Cells pool in the periphery blood within two to five days (Reynolds and Pabst, 1984; Miyasaka *et al.*, 1984). Eventually, the emigrated B-cells home back to the gut mucosal surface. Here these B-cells further proliferate within the ileal Peyer's patches in mammals and bursa of Fabricius in birds where they differentiate to a sedentary, local antibody producing plasma cells (Neutra, 1983).

2.4.3. FUNCTIONAL SIMILARITIES

2.4.3.1 Response to antigens

These lymphoepithelial tissues are unresponsive to antigens (Tizard, 1996). For example, antigens such as ferritin or killed *Brucella abortus* organisms when injected in the intestines of the fetal lamb one-month before birth causes dramatic reaction in the lymph nodes and spleen. The same causes no reaction or influence in the morphology of the Peyer's patches (Müller-Schoop and Good, 1975; Reynolds, 1980; Gerber, 1979; Reynolds and Morris, 1984). Experiments in the chickens involving the injection of antigens in the Bursa of Fabricius produced same results as those of lambs (Kincade and Cooper, 1971).

2.4.3.2 Effect after their removal.

Early removal of the gut-associated lymphoid tissues, like early removal of bursa (Cooper *et al.*, 1966), should result in a selective deficiency of that line of cells having the capacity for immunoglobulin production (Cooper and Lawton, 1972). Unfortunately, in most mammalian species, gut-associated lymphoid tissues are extensively distributed along the small intestine and are richly endowed with lymphopoietic activity long before birth. Nevertheless, appendectomized rabbit developed a suppression of antibody-producing capacity (Sutherland *et al.*, 1964; Perey *et al.*, 1970).

Removal of the bursa of Fabricius followed with nearly lethal irradiation of the whole body of a chicken produced early elimination of germinal centers, plasma cells and circulating immunoglobulins. When same chickens were injected with unirradiated lymphoid cells from their extirpated bursa the defects were restored (Cooper *et al.*, 1966). It was also reported that removal of Peyer's patches, *Sacculus Rotundus* and appendix from a young adult rabbit coupled with high doses of total body irradiation produced an effect of immunological suppression similar to those observed in chicken (Cooper *et al.*, 1966). Surgical removal of the appendix, *Sacculus Rotundus* and Peyer's patches from the neonatal rabbit resulted in a series of abnormality in immunoglobulin synthesis, but the cell mediated immunity was not affected (Cooper *et al.*, 1968). Surgical removal of ileal Peyer's patch before birth or during first week of post natal life by excising the terminal 1.0 – 1.5 meter of an ileum resulted in a severe reduction in number of circulating B-cells in lambs (Gerber, 1979; Reynolds *et al.*, 1981).

2.5 PEYER'S PATCHES NOT BEING BURSA EQUIVALENT.

Despite the above suggestions that the Peyer's patches are mammalian bursa equivalents, there are some contrary views. However, these contradicting views were present before the opinion that mammalian bursa equivalent was the ileal Peyer's patches. Thus, the said contrary views include:

2.5.1 Antigen – dependent lymphopoiesis

Lymphopoiesis is arrested in the gut-associated lymphoid tissue of rabbit following *in situ* surgical isolation by using methods of cecal ligation, isolation of appendix, *Sacculus Rotundus* and distal portion of the Peyer's patches without disrupting the vascular integrity (Perey and Good, 1968; Stramignoni and Mollo, 1968). The development of the lymphoid tissue in the rabbit appendix in the first two weeks of life has been studied with both light and electron microscope. The results indicated that the area where appendix developed was originally represented by connective tissue with abundant ground substance, few collagen fibrils, dark and light cells. Afterwards, small lymphocytes appeared in the area and formed solid nodules. Later, possibly as a consequence of reactive changes, numerous large pyroninophilic cells with ultrastructural features of the so-called immunoblasts were seen; under these circumstances a great number of mitoses were observed. These changes are obvious in the lymphoid nodules. In the same, a large number of macrophages appear in the central part of the nodule. Thus this is a typical case of antigen-dependent reaction (Stramignoni and Mollo, 1968).

Subsequent, experiments in the rabbit showed that there are extensive lymphopoiesis in the gut after birth, thus, proving antigens-dependent lymphopoiesis (Perey and Good, 1968). These findings were further supported by similar observations seen in the mice (Ferguson and Parot, 1972). An additional study on the development of bovine immune system in support of the effect of natural antigenic stimulation theory was done. Both embryos and fetuses were evaluated for the lymphoid tissue development, immunoglobulin producing cells and serum immnoglobulins. The methods used for the assay include immunofluorence, radial diffusion, electrophoresis and special staining technique. Possible sources of antigenic stimuli on the fetuses were determined using standard bacteriologic, virologic and serologic methods. Results concluded that the proliferation in the Peyer's patches was antigen-dependent (Schultz *et al.*, 1973).

Later, studies using young adult goats produced results on the relative proliferative activity of periphery lymphoid organs tested by triated thymidine showed antigen-dependent proliferation in the Peyer's patches and these antigens has been said to be absorbed from the intestinal lumen. Thus, they supported the hypothesis that under normal circumstances, the magnitude of the proliferation activity in the periphery lymphoid organs including the Peyer's patches may be determined by the degree of antigenic stimulation (Gulliani *et al.*, 1974).

Studies in the rats and mice have shown that the Peyer's patches on responding to the intraluminal antigen produced the precursors of the immunoglobulin A (IgA) – secreting plasma cells, which eventually home to the intestinal mucosa (Craig and

Cebra, 1971; Befus *et al.*, 1978; Butcher *et al.*, 1982). In rabbits it has been reported that follicles in the appendix from a functional sense are similar to the germinal centers of lymph nodes and spleen because they develop after birth (Waksman *et al.*, 1973).

2.5.2 Pattern of cellular repopulation

Studies in mice showed that when lethally irradiated mice were injected with 10^5 bone marrow and 10^7 lymphoid cells carrying a distinct chromosome marker, the pattern of cellular repopulation of the Peyer's patches resembles that of lymph nodes, not of a thymus (Evans *et al.*, 1967).

2.5.3 Presence of T-Lymphocytes

Presence of T-lymphocytes in the developing Peyer's patches is also an indication that lymphocytes differentiation in these organs is in part, thymic dependent (Evans *et al.*, 1967). Sutherland *et al* (1974) also reported that some of the thymus-derived cells migrate to the mammalian gut-associated lymphoid tissues, but they occur in an area outside the organized lymphoid follicles.

Studies using Peyer's patches cell cultures from nonprime rabbit has shown that these cells respond experimentally to sheep erythrocytes. These responses take place in the absence of the epithelial cells covering the lymphoid patches. The response is similar to that of spleen culture from normal rabbit. In contrast, thymic cultures can not mount primary immune response *in vitro*. However, these findings did not consider the possibility that some of the reacting cells of the Peyer's patches could

be those which may have homed to the patches through post-capillary high-endothelial venules (Henry *et al.*, 1970). Furthermore, using immunofluorescent markers, it has been demonstrated that prominent accumulations of T-lymphocytes occurring in Peyer's patches of mice are contrary to the characteristics of primary lymphoid organs (Friedberg and Weissman, 1974). Similar observations were reported in rodents when studying the appendix, *sacculus rotundus* and Peyer's patches with the electron microscope (Bockman and Cooper, 1973).

2.5.4 Other possible sources of B-lymphocytes

Studies in mice using fetal livers showed that there is development of IgM, IgG and probably IgA-bearing lymphocytes in organ culture of fetal liver removed on the 14^{th} day of gestation, a time well before immunoglobulin-bearing cells or cells of lymphoid morphology could be detected in an intact animal. This could be possibly associated with the differentiation of B-lymphocytes in the liver. Thus, they came up with a conclusion that liver is a possible source of B-lymphocytes instead of Peyer's patches (Owen *et al.*, 1974).

Studies on the ontogeny of B-lymphocytes in neonatal mice showed that although some B-lymphocytes are generated in the Peyer's patches, the rate is too low to account for the neonatal increase in the number of these lymphocytes in the spleen. Thus in mice and other rodents the bone marrow is the site where B-lymphocytes are produced (Fiedberg and Weisman, 1974). This finding was supported by the report that in the postnatal life there is continuous generation and dissemination of B-lymphocytes from the bone marrow. Report further narrates that although these

lymphocytes lack their distinctive properties, they undergo marked changes in their surface properties and functional responsiveness during maturation that takes place in the spleen and lymph nodes (Osmond, 1980).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 EXPERIMENTAL SETUP

In this work morphological investigation of the Peyer's patches was carried out in five mammalian orders: i.e.

Carnivora:	Dogs (*Canis familiaris*) and Cats (*Felis domestica*).
Rodentia:	Giant rats (*Cricestomys gambianus*) and Wild rats (*Mastomys natalensis*)
Primates:	Baboons (*Papio cynocephalus*) and Vervet monkey (*Cercopithecus aethiops*)
Perissodactyla:	Donkeys (*Asinus africanus*).
Artiodactyla:	Cattle (*Bos indicus*), Blue Wildebeest (*Connochaetes taurinus*), Common reedbuck (*Redunca arundinum*) and Pigs (*Sus scrofa*).

In each of the species, three age groups i.e. prenatal, young and adult were used and for each age group, 3 animals were studied.

3.2 AGE ESTIMATION

In the domestic animals, age data was obtained from owners. In wild ruminants, age estimation was done by using dentition technique and horn ring technique (Morris, 1972). In the case of the primates body size and dentition methods was used (Halternoth and Diller, 1980). In the case of bovine fetuses the crown-rump-length

technique was used (Evans and Sack, 1973; Richardson *et al.*, 1976) while for other domestic animal fetuses, age data from owners was used. The age of fetuses of wild ruminants was estimated by using the knowledge of an experienced game officer (Mr. Kauzeni - personal communication) Wild rats and giant rats were aged by using age data from SUA pest Management Centre (Table 1).

3.3 KILLING OF ANIMALS

Cattle, pigs, donkeys, dogs, cats and rats were killed by exsanguination through the jugular vein after sedation with xylazine while wildebeests, reedbucks, baboons and green monkeys were shot by game officer.

3.4 SAMPLE COLLECTION AND EXAMINATION

Within five minutes after death, data on dentition and horn rings were taken for age estimation. Midline incision was made to expose the small intestines and markings were put for the duodeno-jejunal and jejuno-ileal boundaries. Approximately one centimeter long of the terminal part of the ileum was cut and immediately fixed in 10% Neutral Buffered Formalin (NBF). The reminder of small intestine was subsequently opened through the mesenteric border and washed with clean water until there was no ingesta, blood or mucus (Cornes, 1965).

3.5 MACROSCOPIC EVALUATION

The length of small intestine was measured using tape measure and the length recorded. The intestines were then placed in 3% solution of acetic acid for 24 hours for the purpose of fixing the nuclei. Knowing that the Peyer's patches consist almost entirely of nuclei material the fixed patches would stand out clearly as white superficial plaques against semi-translucent grayish background (Cornes, 1965). To make the follicular contents of the patches more visible the intestines were stained with 0.5% polychrome methylene blue for one minute. The number, size, distribution and gross appearance of the Peyer's Patches were recorded.

Some macrographs of the patches were taken.

3.6 HISTOLOGICAL EVALUATION

The intestinal samples were fixed for 7 – 10 days at 4°C then dehydrated by using ascending concentrations of ethanol, cleared by xylene, embedded in paraffin wax or Historesin. These were later sectioned at 4μm and 3μm thickness for paraffin and historesin embedded sections respectively. Sections were stained with Hematoxylin and Eosin using standard procedures and mounted with DPX (Kiernan, 1990). From the prepared histological sections the following parameters were assessed and evaluated:

a) Presence or absence of Peyer's patches follicles.

b) The longest 5 follicles were used for the various measurements i.e. the height of the follicles which was obtained by measuring the highest point of the height of the follicle, and breadth of the follicle which was obtained by measuring the middle part of the follicle i.e. about equidistant between apex and the base. Other measurements

taken were depths of the dome, corona, inter-follicular area and cortex medulla ratio by dividing width of cortex over that of medulla and convert into parcentage and sizes of 10 randomly selected small and large lymphocytes using calibrated graticule.

c) Indication of lymphoproliferation by presence of germinal centres.

d) Micrographs from representative samples were prepared.

3.7 DATA ANALYSIS

Means and standard error of means of both gross and microscopic measurements were obtained using the SAS programme (SAS, 1988) and data analysis was done by parametric data analysis methods.

Table 1: Estimated and actual ages of animals used in this study and their age at sexual maturity and gestation periods

Species	Age of fetuses	Age of youths	Age of adults	Sexual Maturity age	Gestation period
Wildebeest	5 months each	< 5m,5-12m,1.5yr	4,6,6 years	1.5 years	9 months
Reedbuck	2 months each	6-12 months each	3,4,4 years	1.5 years	7.75 months
Cattle	130,140,182 days	1,2,3 month	3,5,5 years	8 months	9 months
Pigs	6 weeks each	2,4,4 weeks	3,4,4 years	7 months	114 days
Donkeys	4 ,7,8 months	2 ,3,3 months	3, 4,4 years	12 months	11 months
Dogs	3,3,2 weeks	2 ,2,5 weeks	2,2,2.5 years	6 months	68 days
Cats	3 weeks each	2 ,3,3 weeks	2 years each	6 months	2 months
Wild rats	10, 10, 10 days	1,1,1 weeks	6,6,6 months	2.5 months	23 days
Giant rats	7,7,7 days	7,7,7 days	2,12,12 months	6 months	32 days
Baboons	Not obtained	12,13,15 months	Not obtained	3.5-4 years	187 days
G/Monkeys	Not obtained	1,1,1 year	Not obtained	2-2.5 years	175 days

Table was prepared using aging technique adopted from Evans and Sack (1973), Kingdon (1974), Richardson *et al.*, (1976), Merck's manual (1979), Morris (1972), Data from farmers (2001) and personal communication with a game officer Mr. Kauzeni (2001).

CHAPTER FOUR

4.0 RESULTS.

4.1 MORPHOLOGICAL CHANGES OF THE ILEAL PEYERS PATCHES WITH AGE

4.1.1 Gross morphological changes

4.1.1.1 During prenatal period

Fetuses that were obtained from wildebeests and whose age was estimated to be five months (or second trimester period) had no grossly visible Peyer's patches. Similarly, fetuses that were collected from cattle and whose age was estimated to be 130 days, 140 days and 182 days had no grossly visible Peyer's patches. The same was true for the fetuses that were obtained from pigs (6 weeks old), dogs (3 weeks and 2 months old), donkeys (4 months old), cats (3 weeks old), wild rats (10 days old), giant rats (7 days old) and reedbucks (about 2 months old). However, in two donkey fetuses that were estimated to be 7 and 8 months old, there were 29±4 grossly visible Peyer's patches in the ileum. These patches were round in shape and intermittently arranged on the antimesenteric border of the ileum, with an average size of 0.5 cm × 0.3 cm for each individual patch. The patches in the jejunum of these fetuses were 40±9 in number and were round in shape with individual patches measuring up to 0.7 × 0.5 cm (Figs. 2 and 3). Unfortunately fetal specimen could not be obtained from the primates i.e. baboons and vervet monkeys.

Fig. 2: A section of an ileum of 8 month old donkey fetus showing ileal Peyer's patches (arrow).

Fig. 3: A section of jejunum of 8 month old donkey fetus showing jejunal Peyer's patches (arrow).

4.1.1.2 During youth period

Gross examination of the ileum of young wildebeests (less 5 months-1.5 years old), reedbucks (6-12 months old), calves (1-3 months old), piglets (2-4 weeks old), puppies (2-5 weeks old) and kittens (2-3 weeks old) revealed presence of long continuous patch that extended from the ileocecal junction through the entire ileum and extending into the jejunum (Fig. 4-6). The distal part of ileum (in the jejunum) was narrower and tapering towards the jejunum while the proximal part covered almost the whole circumference of the ileum. In dogs and cats, the proximal part extended for about one centimeter into the caecum. The mean length of the ileal Peyer's patches in the young wildebeests was 1.5±0.07 m, 0.9±0.04 m in young reedbucks, 0.85±0.05 m in a calves, 0.38±0.01 m in a.piglets, 0.19±0.02 m in a puppies and 0.1±0.002 m a kittens.

In young donkeys (2-3 months old), young baboons (1.5 years) and young vervet monkeys (12 months), the ileal Peyer's patches were in a form of discrete patches. In the donkeys, there were 32±3 patches and individual patches were round in shape (Fig.7) with an individual average size of 0.7 cm in length x 0.6 cm width, thus the total length of ileal Peyer's patches been 0.203±0.003 m. The patches in the donkeys were intermittently arranged along the anti-mesenteric surface of the ileum. In young baboons and vervet monkeys the patches were oval. In the baboons, there was a single patch in the ileum that was 0.4 cm × 1.2 cm in size. However, in the vervet monkeys there was three patches in the ileum with sizes of 0.2 cm × 0.4 cm, 0.2 cm × 0.4 cm, and 0.5 cm × 1.4 cm.

patches in the ileum with sizes of 0.2 cm x 0.4 cm, 0.2 cm x 0.4 cm, and 0.5 cm x 1.4 cm.

No ileal Peyer's patches were seen in the ileum of young wild rats (1 week old) and giant rats (1 week old).

Fig. 4a.

Fig. 4b.

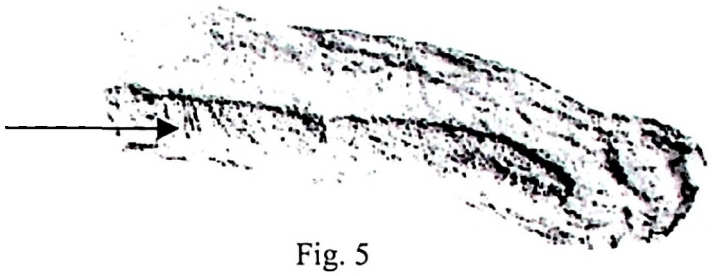

Fig. 5

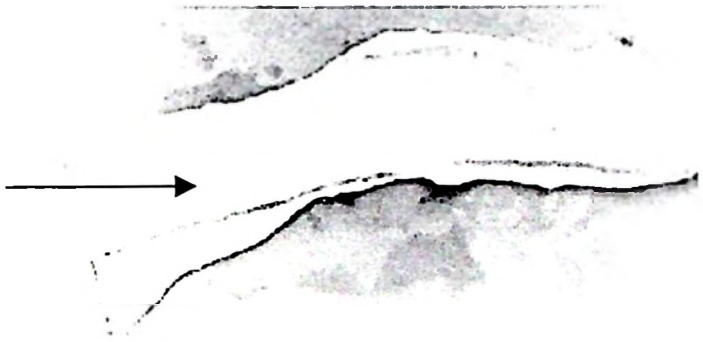

Fig. 6

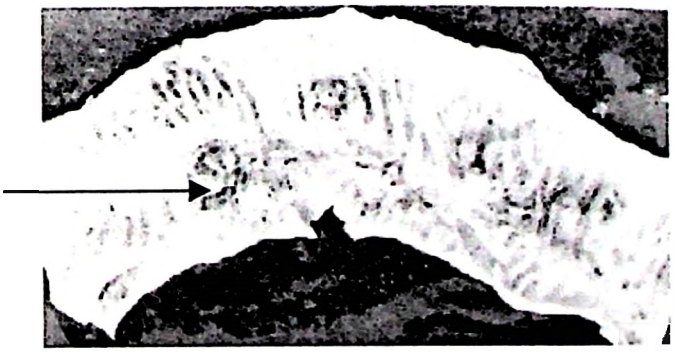

Fig. 7

Legend for figures 4, 5, 6 and 7.

Figure 4a: Gross morphology of proximal part of ileal Peyer's patch from a young wildebeest (arrow). Note that almost the whole circumference of ileum is covered by band of ileal Peyer's patch. Magnification x 0.5

Figure 4b: Gross morphology of distal part of ileal Peyer's patch from a young wildebeest (found in the jejunum). Note its breadth as compared to that of the proximal part (arrow) (Fig. 4a). Magnification x 0.5

Figure 5: Gross morphology of the distal part of ileal Peyer's patch (arrow) from a young reedbuck. Magnification x 0.5

Figure 6: Gross morphology of the mid part of ileal Peyer's patch of a calf. Note the broader proximal part (arrow) of this band as compared to distal part (D) that is narrower. Magnification x 0.5

Figure 7: Ileal Peyer's patches of a young donkey appearing as discrete patch intermittently arranged along the ileum (arrow). Magnification x 0.5

Jejunal Peyer's patches of young wildebeests, young reedbucks, calves, kittens, baboons and vervet monkeys were oval with round ends (Fig. 8-12). In young wildebeests, the mean number of Peyer's patches was 32±2 and their sizes ranged from 0.5 cm × 0.7 cm to 1.2 cm × 11.6 cm. Young reedbucks had a mean of 10±2 patches and their sizes ranged from 0.9 cm × 0.1.1 cm to 0.9 cm × 4.1 cm. Calves had a mean of 26±2 patches and their sizes ranged from 0.3 cm × 0.8 cm to 1.3 cm × 9.8 cm. Kittens had a mean of 3±1 patches with sizes ranging from 0.2 cm × 0.3 cm to 0.4 cm × 0.5 cm. In vervet monkeys and baboons, there were 12±1 and 18±1 patches respectively. The sizes of individual patches for vervet monkeys ranged from 0.5 cm × 0.8 cm to 0.8 cm × 2.4 cm and in the baboons sizes of the individual patches ranged from 0.6 cm × 0.9 cm to 1.1 cm × 2.9 cm.

Young donkeys and puppies had round patches (Fig. 11-12). Young donkeys had a mean of 48±3 patches and their individual average sizes was 0.8 cm × 0.6 cm. Puppies had a mean of 8±1 patches with their sizes ranging from 0.3 cm × 0.3 cm to 1.3 cm × 1.4 cm.

No jejunal Peyer's patches were seen in the jejunum of piglets, wild rats and giant rats.

The mean total length of the jejunal Peyer's patches was 1.4±0.08 m in the young wildebeests, 0.3±0.04 m in young reedbucks, 1.2±0.02 m in calves, 0.38±0.006 m in young donkeys, 0.38±0.001 m in puppies and 0.09±0.002 m in kittens.

Dogs showed the presence of 3 round duodenal Peyer's patches. Their sizes were 0.8 cm x 0.6 cm, 0.9 cm x 0.8 cm and 1.0 cm x 0.9 cm. They covered 2.96 ± 0.004% of the total length of the small intestine.

The numbers, sizes and distribution of the patches on the small intestine of the young animals are summarized in table 2.

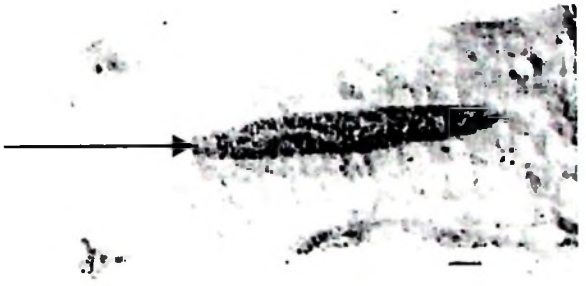

Fig. 8

Fig. 9

Fig. 10

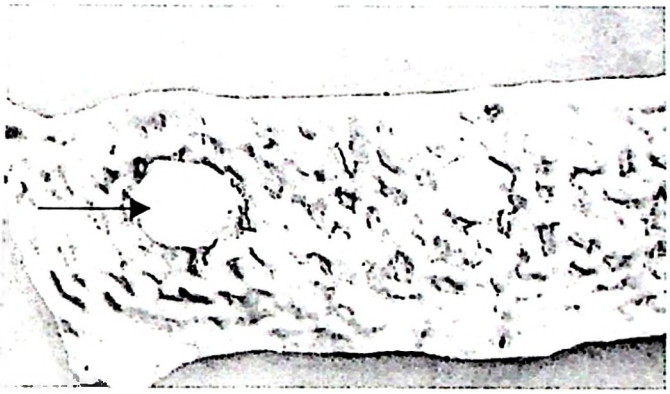

Fig. 11

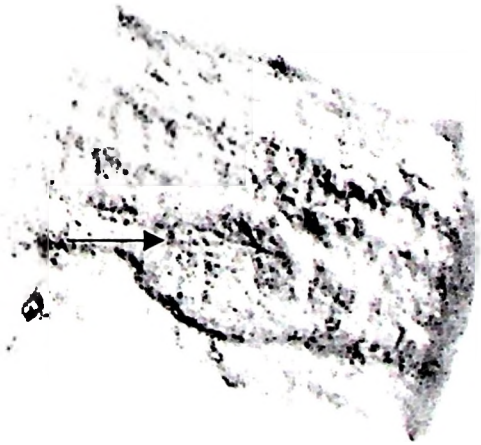

Fig. 12

Legend for figures 8, 9, 10, 11 and 12.

Fig. 8: Jejunal Peyer's patch of a young wildebeest (arrow). Magnification x 0.5

Fig. 9: Jejunal Peyer's patch of a young reedbuck (arrow) Magnification x 0.5

Fig. 10: Jejunal Peyer's patch of a calf (arrow) Note its elongated oval shape. Magnification x 0.5

Fig. 11: Jejunal Peyer's patch (arrow) of a young donkey. Note its round shape. Magnification x 0.5

Fig. 12: Jejunal Peyer's patch of a puppy (arrow) Note its round shape. Magnification x 0.5

Table 2: Means and standard error of Means (SE) of Peyer's patch sizes in young animals in meters.

Species		LI	LJ	LD	NIP	NJP	NDP	LIPP	LJP	LDP	PICI	PJCJ	PDD	TLSI	PSP
Wildebeest	Mean	1.4	16.8	2.3	1	32	0	1.5	1.4	0	7.3	6.8	0	20.5	14.1
	SE	0.03	3.6	1.5	1	2.0	0	0.7	0.8	0	0.5	0.4	0	5	3.2
Reedbuck	Mean	0.8	3.6	0.7	1	10	0	0.9	0.3	0	17.6	5.9	0	5.1	23.5
	SE	0.5	0.1	0.04	1	2.0	0	0.04	0.04	0	5.9	2.02	0	0.2	0.6
Cattle	Mean	0.7	11.5	0.7	1	26	0	0.85	1.2	0	6.6	9.3	0	12.9	15.9
	SE	0.05	0.4	0.02	1	2.0	0	0.5	0.2	0	1.7	0.13	0	0.4	1.01
Pigs	Mean	0.6	2.9	0.7	1	0	0	0.8	0	0	19.2	0	0	4.2	19.2
	SE	0.9	0.4	0.04	1	0	0	0.01	0	0	3.9	0	0	0.4	0.3
Donkeys	Mean	0.6	3.1	0.5	29	48	0	0.203	0.38	0	4.8	9.05	0	4.2	13.9
	SE	0.1	0.8	0.06	7	3.0	0	0.003	0.006	0	0.4	0.2	0	0.9	1.9
Dogs	Mean	0.14	0.61	0.16	1	8	4	0.15	0.06	0.07	16.5	6.6	1.9	0.91	24.0
	SE	0.03	0.04	0.01	1	1.0	4	0.002	0. 001	0.04	1.8	1.3	1.2	0.007	0.8
Cats	Mean	0.091	0.32	0.09	1	3	0	0.1	0.009	0	21.0	1.8	0	0.501	22.8
	SE	0.004	0.02	0.006	1	1.0	0	0.002	0.002	0	2.4	0.4	0	0.2	0.65
Baboon	Mean	0.12	0.53	.009	1	12	0	.004	.029	0	3.0	5.4	0	.659	5.0
	SE	.04	.25	.003	0	1.0	0	.001	.003	0	.05	.7	0	.002	.8
Green monkey	Mean	.10	.32	.008	3	18	0	.009	.022	0	9.0	7.0	0	.328	13.0
	SE	.003	.004	.002	1.0	1.0	0	.002	.006	0	2.0	1.6	0	.003	2.4
Wild rats	Mean	.007	.06	.005	0	0	0								
	SE	.002	.003	.001	0	0	0								
Giant rats	Mean	.009	.24	.007	0	0	0								
	SE	.001	.004	.002	0	0	0								

Key for table 2

LI	Length of ileum
LJ	Length of jejunum
LD	Length of duodenum
NIP	Number of Ileal Peyer's patches
NJP	Number of Jejunal Peyer's patches
NDP	Number of Duodenal Peyer's patches
LIP	Length of ileal Peyer's patches
LJP	Length of jejunal Peyer's patches
LDP	Length of duodenal Peyer's patches
TLSI	Total length of small intestine
PICI	Percentage of ileum covered by ileal Peyer's patches
PJCJ	Percentage of jejunum covered by jejunal Peyer's patches
PDCD	Percentage of duodenum covered by duodenal Peyer's
PSP	Percentage of small intestine covered by Peyer's patches

4.1.1.3 During adulthood period

Examination of the ileum showed that the ileal Peyer's patches were not grossly visible in the adults of wildebeest (4-6years old), reedbuck (3-4 years old), cattle (3-5 years old), pig (3-4 years old), donkey (3-4 years old), dog (2-2.5 years old) and cat (2 years old). However, wild rats (3 months old) had 3±1 individual ileal Peyer's patches and giant rats (10 months old) had 8±1 individual ileal Peyer's patches. These ileal Peyer's patches were round in shape. The total mean length of ileal Peyer's patches was 0.006±0.003 m in wild rats and 0.03±0.003 m in giant rats (Fig. 13 and 14). Adult baboons and vervet monkeys were not obtained

Examination of the jejunum revealed presence of different sizes and numbers of jejunal Peyer's patches in the different species (Fig.15-18). Wildebeests had 55±4 patches which were oval in shape and their sizes ranged from 0.5 cm × 0.8 cm to 3.9 cm × 14.1 cm. Reedbucks had 15±2 oval shaped patches and their size ranged from 0.5 cm × 0.7 cm to 1.5 cm × 8.7 cm. Cattle had 37±3 oval shaped patches with sizes range from 1.0 cm × 2.5 cm to 3.9 cm × 16.0 cm. Pigs had 8±1 oval shaped patches and their sizes ranged from 0.5 cm × 3.7 cm to 1.5 cm × 8.0 cm. Donkeys had 57±10 round patches and their average sizes were 1.1 cm × 1.2 cm. Dogs had a mean of 9±1 round patches and their sizes ranged from 0.5 cm × 0.7 cm to 2.1 cm × 2.2 cm. In cats the mean was 6±1 oval patches with sizes ranging from 0.4 cm × 0.5 cm to the largest being 1.0 cm × 1.3 cm. In wild rats, there were 3±1 round patches and the sizes ranged from 0.2 cm × 0.2 cm to 0.4 cm × 0.5 cm. Giant rats had a mean of 17±3 round patches with the smallest having a size of 0.2 cm × 0.3 cm while the size of the largest patch was 0.5 cm × 0.6 cm.

The mean total length of jejunal Peyer's patches was 2.9±0.08 m in the wildebeests, 0.8±0.04 m in reedbucks, 97±0.18 m in cattle, 0.5±0.004 m in pigs, 0.63±0.003 m in donkeys, 0.24±0.004 m in dogs, 0.197±0.002 m in cats, 0.005±0.002 m in wild rats and 0.5±0.002 m in giant rats.

Dogs had 3 duodenal Peyer's patches of sizes 1.2 cm × 1.1 cm, 1.3 cm × 1.2cm and 1.6 cm × 1.5 cm.

Percentage of small intestine coverage by the Peyer's patches in the young animals was 14.1±3.2% in wildebeests, 23.5±0.6% in reedbucks, 15.9±1.01% in a calves, 19.2±0.2% in piglets, 13.9±1.9% in a foals, 24.0±0.8% in a puppies and 22.8±0.065% in a kittens. However, in the adults it was 10.6±3.2% in wildebeest, 12.6±0.6% in reedbuck, 13.3±1.01% in cattle, 2.8±0.2% in pig, 5.9±0.09% in donkey, 19.0±0.8% in dog, 6.6±0.04% in cat, 18.3±0.3% in wild rat and 14.3±0.3% in giant rat.

The numbers, sizes and distribution of the patches on the small intestine of the adults is summarized in table 3.

Fig. 13

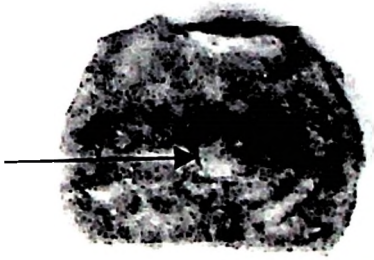

Fig. 14

Fig. 15

Fig. 16

Fig. 17

Fig. 18

Legend for figures 13 - 18.

Fig. 13: Ileal Peyer's patches of an adult wild rat. The upper strip is a view from lumen and lower strip is a view from the serosal surface (arrow). Magnification x 2

Fig. 14: Ileal Peyer's patch of an adult giant rat (arrow). Magnification x 2

Fig. 15: Jejunal Peyer's patch of an adult wildebeest (arrow) Magnification x 0.14

Fig. 16: Jejunal Peyer's patch of an adult reedbuck (arrow) Magnification x 0.16

Fig 17: Jejunal Peyer's patch of an adult cattle (arrow) Magnification x 0.15

Fig. 18: Jejunal Peyer's patch of an adult donkey (arrow) Magnification x 0.67

Table 3: Means and standard error of Means (SE) of Peyer's patch sizes in adults animals in meters

Species		LI	LJ	LD	NIP	NJP	NDP	LIP	LJP	LDP	TLSI	PICI	PJCJ	PDC	PSP
Wildebeest	Mean	1.9	23.4	2.06	0	55.0	0	0	2.9	0	27.36	0	10.6	0	10.6
	SE	0.03	3.6	0.15	0	4.0	0	0	0.8	0	0.5	0	0.3	0	0.3
Reedbuck	Mean	1.07	4.4	0.9	0	15.0	0	0	0.8	0	6.37	0	12.6	0	12.6
	SE	0.05	0.1	0.04	0	2.0	0	0	0.04	0	0.02	0	2.02	0	0.6
Cattle	Mean	1.17	20.3	0.9	0	37.0	0	0	2.97	0	22.37	0	13.3	0	13.3
	SE	0.05	0.4	0.02	0	3.0	0	0	0.18	0	1.3	0	1.3	0	1.01
Pigs	Mean	2.1	13.9	1.8	0	8	0	0	0.5	0	17.8	0	2.8	0	2.8
	SE	0.9	0.9	0.04	0	1.0	0	0	0.04	0	0.4	0	0.4	0	0.3
Donkey	Mean	0.93	9.0	0.8	0	57.0	0	0	0.63	0	10.73	0	5.13	0	5.9
	SE	0.13	0.8	0.06	0	10.0	0	0	0.003	0	0.9	0	0.2	0	.09
Dogs	Mean	.23	1.05	0.2	0	9.0	3	0	0.24	0.041	1.48	0	16.2	2.8	19
	SE	0.03	0.04	0.01	0	1.0	1.0	0	0.04	0.003	0.007	0	1.8	1.2	0.8
Cats	Mean	0.97	1.1	0.9	0	6.0	0	0	0.197	0	2.97	0	6.6	0	6.6
	SE	0.004	0. 02	0.06	0	1.0	0	0	0. 002	0	0.02	0	0.4	0	0.65
Wild rats	Mean	0.04	0.06	0.05	2.0	3.0	0	0.006	0.005	0	0.15	4.0	3.3	0	18.3
	SE	0.002	0.002	0.002	0.2	1.0	0	0.003	0.002	0	0.004	0.08	0.03	0	0.3
Giant rats	Mean	0.1	0.37	0.09	8.0	17.0	0	0.03	0.05		0.56	5.4	8.9	0	14.3
	SE	0.004	0.005	0.002	1.0	3.0		0.003	0.002		0.003	1.1	1.2	0	0.03

Key for tables 3 in Meters.

LI - Length of ileum
LJ – Length of jejunum
LD – Length of duodenum
NIP – Number if Ileal Peyer's patches
NJP – Number if Jejunal Peyer's patches
NDP – Number if Duodenal Peyer's patches
LIP – Length of ileal Peyer's patches
LJP -- Length of jejunal Peyer's patches
LDP – Length of duodenal Peyer's patches
TLSI – Total of small intestine
PICI – Percent of ileum covered by ileal Peyer's patches
PJCJ – Percent of jejunum covered by jejunal Peyer's patches
PDCD – Percent of duodenum covered by duodenal Peyer's
PSP – Pencent of small intestine covered by Peyer's patches

4.1.2 Microscopic Morphology

4.1.2.1 During prenatal period

Microscopic examination of the ileum of the fetuses of wildebeest (5 months - second trimester), reedbuck (2 month old), cattle (130 and 140 days), donkey (4 months old), pig (6 weeks old), dog (3 weeks old) and cat (3 weeks old) revealed an accumulation of lymphoid cells in the sub-mucosa (Fig. 19).

In the ileum of 8 weeks old fetuses of dog, there were tightly packed round shaped follicles that were covered with a capsule, which was not well defined. Follicles had no clear cortex and medulla differentiation. Domes had an overlying epithelium, which was composed of low columnar epithelial cells, mononuclear cells and lacked mucous cells (Fig. 20).

However, in the cattle fetus of 182 days old there were mature follicles that were conical in shape (Fig. 21). These follicles were surrounded by connective tissue capsule. The follicles had distinct cortex and medulla. The domes were elongated and their epithelium was composed of columnar epithelial cells, mononuclear cells occurred within the epithelium and mucous cells were lacking. Interfollicular areas were not well defined.

The ileum of the donkey fetuses of 7 and 8 months old revealed presence of well-defined follicles that were sac-like in shape tightly packed in the sub-mucosa and had small, medium and large lymphocytes, which were almost evenly distributed. Follicles had distinct cortex and medulla. The domes were conical in shape and the

evenly distributed. Follicles had distinct cortex and medulla. The domes were conical in shape and the epithelium was composed of columnar epithelial cells, mononuclear cells and lacked mucous cells. However, there were no follicles in the fetuses of wild rats (10 days old) and giant rats (7 days old).

Data for follicle height, follicle breadth, dome depth, corona depth, interfollicular area depth, cortical-medulla ratio and lymphocyte sizes for the species studied are summarized in table 4.

Fig. 19

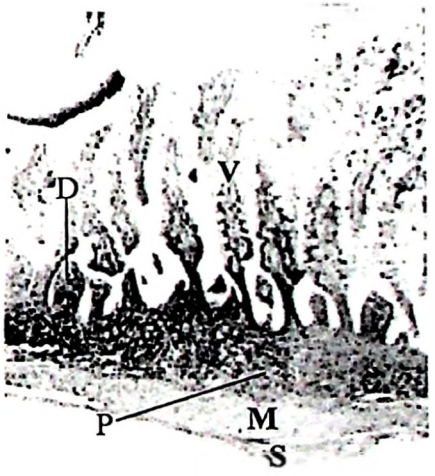

Fig. 20

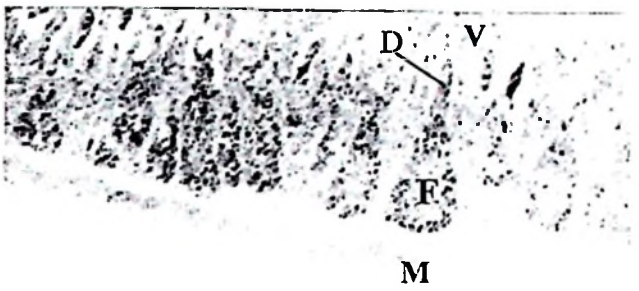

Fig. 21

Legend for figures 19 - 21.

Figure 19: Light micrograph of a primordial ileal Peyer's patch follicle of a dog fetus of 3 weeks old. Note the clustering of lymphoid cell (P), villi (V) and the muscular layer (M). Magnification x 120.

Figure 20: Light micrograph of ileal Peyer's patch follicles of a dog fetus of 2 months old. Note the follicle (P), villi (V), the muscular layer (M), the dome (D) and serosa (S). Magnification x 50.

Figure 21: Light micrograph of ileal Peyer's patch follicle of cattle fetus of 182 days old. Note the follicle (P) with an inner medulla and an outer cortex, villi (V), muscular layer (M) and dome (D). Magnification x 50.

Table 4: Means and standard error of Means (SE) of size of follicles, domes, corona, IFA and lymphocytes in fetuses.

Species		FH	FB	DD	CD	ID	CMR	10SML	10LGL
Cattle	Mean	156.57	38.72	51.26	0	40.7	31.07	1.12	4.46
	SE	115.77	27.47	14.27	1.09	11.5	6.98	0.38	1.14
Donkeys	Mean	1108.08	527.56	272.71	32.43	52.91	50.02	5.3	7.25
	SE	161.52	91.75	52.69	3.96	7	5.88	0.63	0.89
Dogs	Mean	58	43	23.63	0	0	0	0.93	2.46
	SE	40.08	28.98	15.94	0	0	0	0.21	0.54

Legend for table 4 (measurements in µm).

Follicle height - FH

Follicle breadth - FB

Dome depth- DD

Corona depth - CD

Interfollicular area depth - ID

Cortico - Medullary ratio - CMR

10 small lymphocytes mean sizes - 10SML

10 large lymphocytes mean sizes - 10LGL

4.1.2.2 During young period

Microscopic studies of the ileal Peyer's patches revealed presence of tightly packed sac-like shaped follicles in the sub-mucosa. These follicles were surrounded by a layer of connective tissue capsule and were well differentiated into an outer dark cortex and an inner lighter medulla. The cortex contained mainly small sized lymphocytes (about 3-8 μm) and medium lymphocytes (about 9-12 μm), which had condensed chromatin that looked as black spots while medulla contains mostly large sized lymphocytes (about 13-24 μm). The domes were small, conical in shape and were covered by epithelium that was composed of columnar epithelial cells and mononuclear cells. Mucous cells were lacking. The interfollicular areas were small in size and contained post - capillary high endothelial venules. The coronae were very small such that their visibility was sometimes difficult. However, there were quite a number of species differences noted from this study.

The follicles of young wildebeest had clear and well differentiated cortex and medulla. The domes were conical in shape and epithelium was composed of columnar epithelial cells and mononuclear cells. Mucous cells were not evident. Interfollicullar area was clearly marked, but the corona was inconspicuous (Fig. 22).

The follicles of young reedbuck were long and cortex and medulla differentiation was visible. Domes were conical in shape covered with an epithelium, which was composed of columnar epithelial cells and mononuclear cell. Once again mucous cells were absent. Interfollicullar area and corona were not visible (Fig. 23).

Calves follicles had clear cortex and medulla differentiation. Domes were conical in shape and the epithelium was composed of columnar epithelial cells, mononuclear cells and lacked mucous cells. The interfollicullar area was small and triangular while coronae were inconspicuous (Fig. 24).

Piglets showed presence of sac-like follicles that had poorly distinct capsules. Domes were conical in shape and were covered by an epithelium that contained columnar epithelial cells, mononuclear cells and few mucous cells. The cortex and medulla were not well differentiated. Interfollicullar areas and corona were not well defined (Fig. 25).

Follicles in the donkeys had very clearly differentiated cortex and medulla. Domes were conical in shape and epithelium was composed of columnar epithelial cells, mononuclear cells and lack mucous cells. Interfollicullar areas were visible between the apices of the adjacent follicles and the corona was inconspicuous (Fig. 26).

In dogs, the follicles had clear cortex and medulla differentiation. Domes were conical in shape covered by an epithelium, which was composed of columnar epithelial cells, mononuclear cells and lacked mucous cells. Interfollicullar area and depth of corona were not clearly marked.

In the cats, the follicles were also sac-like in shape. However, there was an indication that at a young age, follicles were cylindrical whereas in slightly older cats the follicles became more saccular in shape. The domes had long and slender

apices with varying shapes of either pyramidal or triangular. Epithelium was composed of epithelial cells of varying shapes of either columnar or cuboidal epithelial cells and mononuclear cells. However mucous cells were lacking. Interfollicullar areas were visible between the apices of the adjacent follicles while the corona was inconspicuous (Fig. 27).

Data for follicle height, follicle breadth, dome depth, corona depth, interfollicular area depth, cortico-medulla ratio and lymphocyte sizes for the species studied are summarized in table 5.

Fig. 22

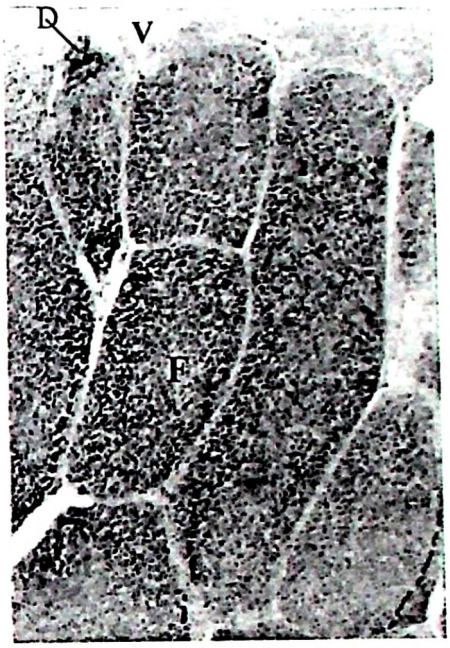

Fig. 23

Fig. 24

Fig. 25

Fig. 26

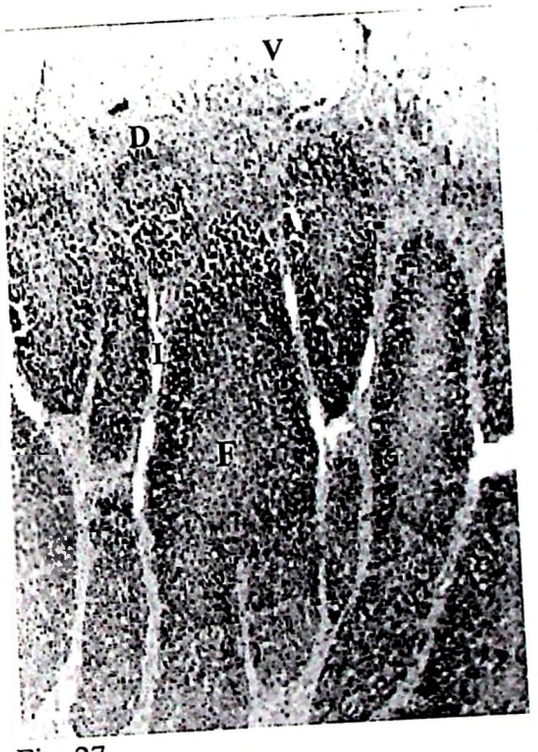

Fig. 27

Legend for figures 22 - 27.

Fig. 22: Light micrograph of ileal Peyer's patch follicles of young wildebeest. Note the villi (V), follicle (F) with an outer darker cortex and a pale inner medulla, dome (D) and intefollicular area (I) Magnification x 50

Fig. 23: Light micrograph of ileal Peyer's patch follicles of young reedbuck. Note the villi (V), follicle (F) with an outer darker cortex and a pale inner medulla and dome (D). Magnification x 50

Fig. 24: Light micrograph of ileal Peyer's patch follicles of a calf. Note the villi (V), follicle (F) with an outer darker cortex and a pale inner medulla, dome (D) and intefollicular area (I). Magnification x 50

Fig. 25: Light micrograph of ileal Peyer's patch follicles of a piglet. Note the villi (V), follicle (F) with ill-defined medulla and cortex being not well marked, dome (D) and mucous cell (M). Magnification x 120

Figure 26: Light micrograph of ileal Peyer's patch follicles of young donkey. Note the villi (V), follicle (F) with an outer darker cortex and a pale inner medulla. dome (D) Magnification x 50

Figure 27: Light micrograph of ileal Peyer's patch follicles of a kitten. Note the villi (V), long narrow follicles (F) with well-defined medulla and cortex, intefollicular area (I).and dome (D). Magnification x 50

Table 5: Means and standard error of Means (SE) of size of follicles, domes, corona, IFA and lymphocytes in youths in µm.

Species		FH	FB	DD	CD	ID	CMR	MSSL	MSLL
Wildebeest	Mean	2779.99	980.81	202.6	98.63	155.23	88.51	11.34	24.31
	SE	129.97	61.42	12.13	8.7	11.8	1.8	0.07	0.1
Reedbuck	Mean	2462.3	1193.57	218.84	54.61	131.03	70.93	3.3	
	SE	105.46	51.88	18.03	2.78	11.32	2.56	0.02	
Cattle	Mean	2389.0	545.4	113.0	20.26	105.59	76.27	3.8	12.3
	SE	115.77	27.47	14.27	1.09	11.5	6.98	0.38	1.14
Pigs	Mean	1012.4	391.43	218.58	88.21	0	0	3.2	9.36
	SE	35.34	21.08	7.31	4.48	0	0	0.03	0.11
Donkeys	Mean	2726.0.	1452.82	802.81	51.0	70.1	90.4	8.04	12.02
	SE	161.52	91.75	52.69	3.96	7.0	5.88	0.63	0.89
Dogs	Mean	960.1	398.3	230.15	99.26	74.04	79.54	4.23	7.94
	SE	40.08	28.98	15.94	3.25	8.59	3.0	0.21	0.54
Cats	Mean	1201.0	553.06	232.0	83.87	134.43	69.24	3.64	9.63
	SE	44.87	40.04	16.18	6.27	7.57	2.75	0.19	0.51

Legend for table 5 (measurements in µm).

Follicle height - FH
Follicle breadth - FB
Dome depth - DD
Corona depth - CD
Interfollicular area depth - ID
Cortico - Medullary ratio - CMR
Mean sizes small lymphocytes - MSSL
Mean sizes large lymphocytes- MSLL

4.1.2.3 During adulthood stage

Ileal Peyer's patches were not grossly visible in wildebeests, reedbucks, cattle, donkeys, pigs, dogs and cats. However, microscopic examination of the ileal Peyer's patches in the adults of some species of wildebeest, reedbuck, cattle, pig, donkey, dog and cat revealed the presence of connective tissue in places which were initially occupied by follicles. The lamina propria of the ileum of the mentioned animals were also infiltrated with numerous mononuclear cells. However, there was also histological evidence of the presence of shrinked ileal Peyer's patch follicles in cows aged 3 years old which had only one follicle (Fig. 28). Donkey with same age had single follicle (Fig. 29) and cats at early adulthood had at least some remaining follicles (Fig. 30)

Data for follicle height, follicle breadth, dome depth, corona depth, interfollicular area depth, cortico-medulla ratio and lymphocyte sizes for cattle, donkeys and cats are summarized in table 6.

In wild and giant rats, the ileal Peyer's patches (Figs. 31 and 32) had pear-shaped follicles with cortical area containing densely packed small lymphocytes intermingled with other large mononuclear cells. The corona was distinct. Domes were large and covered by epithelia containing cuboidal epithelial cells, mononuclear cells and no mucous cells. Interfollicular areas were large and extended to the bases of the follicles. Post-capillary high endothelial venules were evident in the interfollicular areas.

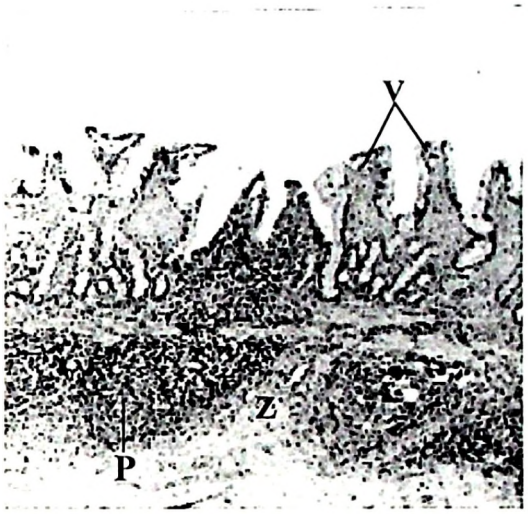

Fig. 28

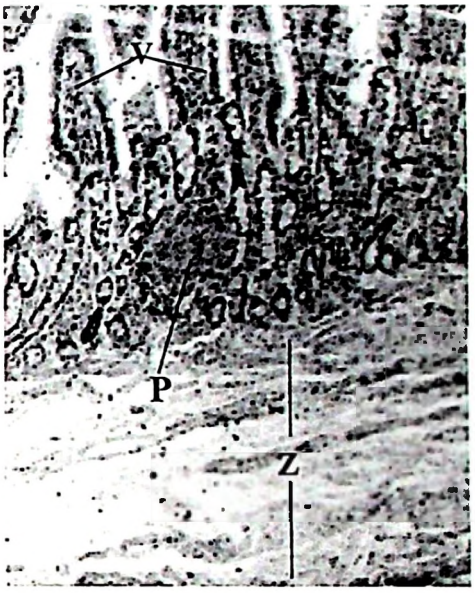

Fig. 29

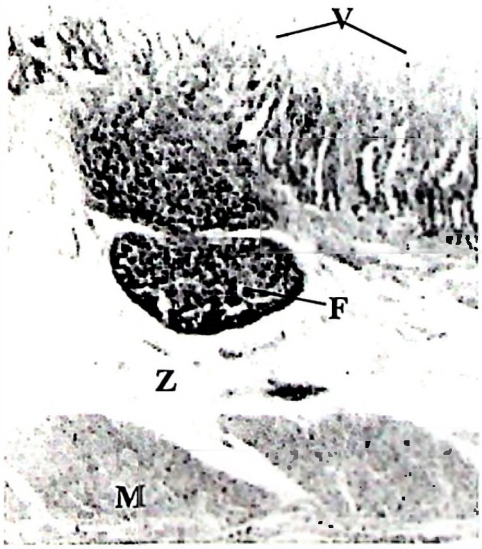

Fig. 30

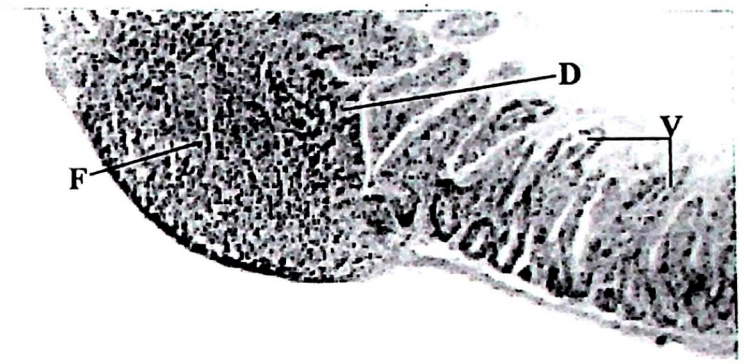

Fig. 31

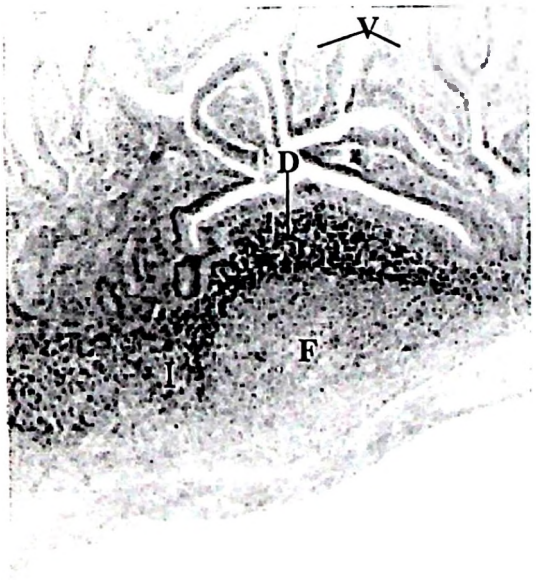

Fig. 32

Legend for figures 28 - 32.

Fig. 28: Light micrograph of an ileum of an adult cattle showing atrophied ileal Peyer's patch follicles. Note the shrinked follicle (P), wider connective tissue zone (Z), and villi (V). Magnification x 50

Fig. 29: Light micrograph of an ileum of an adult donkey showing atrophied ileal Peyer's patch follicles. Note the shrinked follicle (P), connective tissue zone (Z) and villi (V). Magnification x 50

Fig. 30: Light micrograph of an ileum of an adult cat showing atrophied ileal Peyer's patch follicles. Note the shrinked follicle (F), wider connective tissue zone (Z), villi (V) and tunica muscularis (M). Magnification x 50

Fig. 31: Light micrograph of the ileum of wild rat showing ileal Peyer's patch follicle. Note the follicle (F), dome (D) and villi (V). Magnification x 50

Fig. 32: Light micrograph of the ileum of giant rat showing ileal Peyer's patch follicle. Note the follicle (F), dome (D), wider interfollicular area (I), and villi (V). Magnification x 50

Table 6: Means and standard error of Means (SE) of size of follicles, domes, corona , IFA and lymphocytes in adults

Species		FH	FB	DD	CD	ID	CMR	MSSL	MSLL
Cattle	Mean	165.7	96.93	19.21	0	0	0	1.19	1.97
	SE	1.15	2.7	1.4	0	0	0	0.38	0.14
Donkeys	Mean	193.3	21.7	0	0	0	0	0.308	0.71
	SE	1.6	0.9	0	0	0	0	0.006	0.009
Cats	Mean	111.1	84.9	19.17	0	0	0	0.77	2.29
	SE	4.4	4.0	1.6	0	0	0	0.01	0.1

Legend for table 6 (measurements in µm).

Follicle height - FH

Follicle breadth - FB

Dome depth- DD

Corona depth - CD

Interfollicular area depth - ID

Cortico - Medullary ratio - CMR

Mean sizes small lymphocytes - MSSL

Mean sizes large lymphocytes - MSLL

CHAPTER FIVE

5.0 DISCUSSION

5.1 Ileal Peyer's patch changes during the prenatal period.

Gross morphological results of the species studied did not reveal gross presence of ileal Peyer's patches in mid gestation in the majority of the animals studied. However, at late prenatal period fetuses of donkeys had 29 patches whose individual length measured up to 0.5 cm. Earlier studies in cattle fetuses has indicated that Peyer's patches were not grossly visible during prenatal period (Lubis *et al.*, 1982). On the contrary, sheep fetus Peyer's patches are grossly vissible at late gestation period (Reynolds, 1980; Reynolds and Morris, 1983). It seems therefore, that gross appearance of Peyer's patches during prenatal period varies between species and fetal age. However, in the present study only a few fetuses were obtained, and therefore there is a need of collecting more data from fetuses of all age groups in various mammals before any concrete conclusion is made.

Although, ileal Peyer's patches were not grossly visible in the majority of the fetuses in the species under study, microscopic examination of the ileum of cattle and wildebeest during the second trimester, 2 months in reedbuck, 4 months in donkey, 6 weeks in pigs, 3 weeks in dogs and 3 weeks in cats showed the existence of ileal Peyer's patch follicles in form of accumulations of lymphoid cells in the sub-mucosa. Whether the lymphoid cells are residents of the gut wall or have migrated from another locus/tissue remains to be determined.

During the third trimester, cattle fetuses had well-defined sac-like mature follicles that had an outer cortex and inner medulla. The later was composed of mainly lymphocytes that measured up to 2.0µm. However, separation of a cortex and medulla in the donkey and dog fetuses was not as distinct as in the cattle fetuses.

Furthermore, unlike the above species, ileal sections from fetuses of wild rat of 10 days old and giant rat of seven days old did not reveal the presence of ileal Peyer's patch follicles.

Observation of ileal Peyer's patch follicles in cattle, wildbeest, reedbuck, donkey, pig, dog, and cat concurs with previous observations in sheep, pig, cattle, dog, and man (Cornes, 1965; Bryant and Shifrine, 1972; Chapman *et al.*, 1974; Reynolds, 1976; Gerber, 1979; Reynolds and Morris 1983; Ishino *et al.*, 1991).

The development of mature follicles in later stage of prenatal life accompanied with high rate of cell proliferation shown by clear differentiation of cortex and medulla concurs well with previous reports in sheep and cattle (Doughri *et al.*, 1972; Reynolds, 1980). In the goat it was argued that since mature follicles were present at birth then their maturation started prenatally (Assey, 1988) and in the pigs mature follicles in the ileal peyer's patches were also reported to be seen at birth (Chapman *et al.*, 1974).

However, unlike mammals of the orders of Artiodactyla, Perissodactyla and Carnivora, mammals of the order Rodentia did not exhibit ileal Peyers patch

follicles. In the mammals studied therefore, ileal Peyer's patch follicles developed at least by mid-gestation except in rodents. Thus, the ileal Peyer's patch follicles did show prenatal maturation in a majority of the mammals. This morphological pattern resembles that of avian bursa of Fabricius (Cooper and Lawton, 1972; Ciriaco *et al.*, 1989; Shiojiri and Takashari, 1991).

Nevertheless, the differentiation of follicles into cortex and medulla during prenatal period is of interest, especially in the domesticated ruminants, which have syndepitheliochorial type of placenta (Dantzer, 2001-Personal communication). Grossly, the placentas of wildebeest and reedbucks were of the cotyledonary type as in other ruminant species (personal observation). Thus, their placentas are very likely also of syndepitheliochorial type. The placentas of donkeys and pig are of epitheliochorial type. Dogs and cats have on the other hand an endotheliochorial type of placenta (Dyce *et al.*, 1996). These types of placentas create an environment where the fetuses are not exposed to external antigens (Simpson – Morgan and Smearton, 1972). Thus, the observed lymphoproliferation at least in the mammals studied is most likely taking place without extrinsic antigenic stimulation.

Antigenic-independent histological development of the ileal Peyer's patches before birth has also been confirmed by results obtained from a study where antigens such as *Brucella arbotus* or ferritin were injected into the intestine of the lamb fetuses of one month old. Results from these studies revealed a dramatic reaction in the lymph nodes and spleen but not in the ileal Peyer's patch follicles (Reynolds, 1980).

Antigenic stimulation of chick embryos produced comparable results (Kincade and Coopers, 1971).

Nevertheless, current view also suggest that somatic hypermutation occurs in the ileal Peyer's patch follicles independent of the influence of antigens, thus increasing the genetically determined B-cells repertoire (Reynaud *et al.*, 1991, 1995). Therefore, this could also apply to the animals that showed antigen-independent lymphopoiesis in this study.

Furthermore, interfollicular areas with post-capillary high endothelial venules were observed in some fetuses of cattle, donkey and dog. This finding is in agreement with those in sheep and cattle (Doughri *et al*, 1972). These areas represent the site for lymphocyte recirculation. Therefore, this implies that at least the avenues for lymphocyte recirculation are established before birth.

5.2 Ileal Peyer's patches changes during young stage

Gross examination of the ileum of young wildebeests, reedbucks, cattle, pigs, dogs and cats revealed the presence of a long continuous patch that extended from the ileocecal junction covering the entire ileum and extending into the jejunum. The distal part (in the jejunum) was narrower and tapering while the proximal part covered almost the whole circumference of the ileum. In dogs and cats, the proximal part extended for about one centimeter into the caecum. Previous studies in calves, lambs, kids and piglets also showed that the ileal Peyer's patches extended from the ileum to the jejunum (Reynolds and Morris, 1983; Assey, 1988; Parson *et al.*, 1989;

Barman *et al.*, 1997). The current finding of the ileal Peyer's patch morphology in wild ruminants seems to suggest therefore that the ileal Peyer's patches of the mammalian orders of Artiodactyla, Perrisodactyla and Carnivora are similar during early postnatal life.

In the donkeys, baboons and vervet monkeys the ileal Peyer's patches were distributed in a form of discrete patches. However, the number of patches in the donkey is higher than reported in the horse (Titkemeyer and Calhoun, 1955). In baboons and vervet monkeys the shape and distribution of Peyer's patches in the ileum and jejunum resembled those of man but their numbers were 13 for baboon and 21 for vervet monkey which were much lower than those of man where about 40-100 patches with more than 25 follicles have been reported (Cornes, 1965). Nevertheless, species difference could be the reason for the differences in their numbers.

The number of jejunal Peyer's patches in the calves was within the range of 18-40 that was reported by Parson *et al* (1989). Interestingly, the mean number of jejunal Peyer's patches in the wildebeest (32±10) was also within the same range as in cattle (Parson *et al.*, 1989). Despite the fact that reedbuck, goats and sheep are small ruminants the number of jejunal peyer's patches in reedbuck is lower than that of sheep and goat (Reynolds and Morris, 1983; Assey, 1988). The number of jejunal Peyer's patches in donkey was higher than that reported in horse (Titkemeyer and Calhoun, 1955). The number of jejunal and ileal Peyer's patches in baboon was about 13 and vervet monkey was 21 which were lower than that reported for man

having 40-100 patches with more than 25 follicles (Cornes, 1965). The current study has shown that dogs have about 8 jejunal Peyer's patches compared to a range of 17-26 reported by Titkemeycr and Calhoun (1955). On the other hand, previous studies did report absence of jejunal Peyer's patches in cats (Titkemeyer and Calhoun, 1955). In this study an average of three patches were seen. The current methodology of using acetic acid and 0.5% of polychrome methylene blue, which was not done in the referred work, could have enhanced the visualization of the Peyer's patches. The number of jejunal Peyer's patches do therefore show a wide variation between species (Reynods and Morris, 1983; Assey, 1988; Parson *et al.*, 1989; Barman *et al*,.1997). It is very likely that the number of jejunal Peyer's patches may also affect the percentage of intestine covered by jejunal Peyer's patches. Thus, this could dictate animal's ability to fight enterogenic diseases.

The length of small intestine covered by Peyer's patches in this study was 14.1% in young wildebeest, 23.5% in young reedbuck, 15.9% in a calf, 19.2 % in a piglet, 13.1 % in a foal, 24.0 % in a puppy and 22.8% in a kitten. A similar study has reported 17% of the total length of the small intestine to be covered by Peyer's patches in young sheep and kids (Reynolds and Morris, 1983; Assey, 1988). The gastro-intestinal tract (GIT) presents a vulnerable surface challenged to a variety of lumen antigens (Fahel and Morris, 1978; Neutra, 1983). Thus, the Peyer's patches forms the first line of defense for guarding the intestine against such a variety of antigens. Hence the percentage of the coverage by Peyer's patches is an indication of reliable protection for the young. Moreover, carnivores seem to have a relatively more protected gut, which could be related to their eating habits.

The ileal Peyer's patches of young wildebeests and reedbucks and as well as those of foals calves, piglets, puppies and kittens had tightly packed sac-like follicles in the sub-mucosa and their domes buldging into the lumen. These follicles were surrounded by a layer of connective tissue capsule and were well differentiated into an outer dark cortex and an inner lighter medulla. The cortex contained mainly small and medium lymphocytes while medulla contained mostly large lymphocytes. These observations are in agreement with those reported for young sheep, kids, calves, and piglets (Reynods and Morris, 1983; Assey, 1988; Parson *et al.*, 1989; Barman *et al,.*1997). Furthermore, these observations imply that there is high lymphopoietic activity in the ileal Peyer's patches at young age and therefore, it is also presumably true for the young wildebeests and reedbbuck, also for calves, piglets, foals, puppies and kittens as many lymphocytes were seen in the follicles. The implications of the observations is that the body of an animal is now exposed to a great variety of intestinal antigens such that it has to produce more lymphocytes to combat the situation.

The domes of young wildebeests and reedbucks as well as calves, foals, puppies and kitten were covered by epithelium that was composed of columnar epithelial cells and mononuclear cells but lacked mucous cells. However the dome of the ileal Peyer's patch follicles of piglets contained few mucous cells. Previous studies in young sheep, kids, calves and piglets (Reynolds and Morris, 1983; Chu and Liu, 1984; Assey, 1988, Parson *et al*, 1989) have also reported a similar finding. The role of the mucous cells is suggested to be trapping of bacteria (Shrank and Verwey,

1976) and nematodes (Millers *et al.*, 1981), thus, facilitating their removal. However, the significance of presence or absence of mucous cells in dome epithelium of the Peyer's patches requires further investigation.

Lymphocyte sizes in ileal Peyer's patch follicles in the young wildebeests and reedbucks and as well as in calves, piglets, foal, puppies and kittens were in the ranges of 11-24 µm, 3-19 µm, 4 -12 µm, 3 -9 µm, 8 -12 µm, 4 -9 µm and 4 -10 µm respectively. The size of the lymphocytes seems therefore to vary with the animal's body size. In rabbits, the small lymphocytes had a size less than 8 µm, medium lymphocytes ranged from 8-12 µm and large lymphocytes ranged from 13-15 µm (Sobhon, 1971). Medium and large lymphocytes are known to undergo proliferation as demonstrated by presence of a large number of mitotic figures (Faulk *et al.*, 1971; Waksman *et al.*, 1973). On the other hand small lymphocytes are for recirculation (Reynolds and Pabst, 1984; Miyasaka *et al.*, 1984).

Young wild and giant rats showed the absence of Peyer's patches in the ileum and jejunum both grossly and microscopically. This is similar to the results obtained from rabbits where no patches could be identified at birth. However, in the rabbits it was further reported that by day 15 of birth the patches were evident and their number ranged from two to ten patches (Faulk *et al.*, 1971). It is also possible that the ileal Peyer's patch follicles are formed after birth and that by this age (i.e. day 7) when ileal samples were collected they had not developed. This is substantiated by their occurrence in the adult stage in these species. Nevertheless, a study involving

younger rats of different ages could possibly come up with the exact age at which ileal Peyer's patch follicles appear.

5.3 Ileal Peyer's patches changes during adult stage

Examination of the ileum showed that the ileal Peyer's patches were not grossly visible in the adults of wildebeest, reedbuck, cattle, pig, donkey, dog and cat. Nevertheless, microscopic examination of the ileal Peyer's patches in the adult cattle of three years old, donkey of the same age and cats of 2 years old showed the presence of shrinked ileal Peyer's patch follicles. Furthermore, the older animals among the adults had an increased presence of fibrous tissue in the sub-mucosa, in the same place, which was occupied by lymphoid follicles in their young age. In addition, lamina propria of the ileum was infiltrated with many lymphoid cells. Previous reports have also indicated that ileal Peyer's patches atrophy at sexual maturity in sheep, goats, cattle and pigs (Reynolds and Morris, 1983; Binns and Licence, 1985; Assey, 1988; Parson *et al.*, 1989). Evidence from the present study showed that microscopically ileal Peyer's patch follicles are still evident beyond sexual maturity. Therefore, complete atrophy of the ileal Peyer's patches occurs sometimes later after sexual maturity. Presence of lymphoid cells along the gut is vital even in adult stage and very likely the infiltration of lamina propria by many lymphoid cells is an effort to balance the effect of atrophied ileal Peyer's patches. This pattern of atrophy is similar to those obtained in the bursa of Fabricius of adult birds (Ciriaco *et al.*, 1989).

Present data on the pattern of jejunal Peyer's patches in wildebeests, reedbucks, cattle, pigs, donkeys, dogs and cats from the young ones to adults showed an

indication that the sizes of the patches increase with age and their numbers also differ with species. This finding concurs to those reported in sheep, cattle, goats and pigs (Reynolds and Morris, 1983; Binns and Licence, 1985; Assey, 1988, Parson *et al.*, 1989). The jejunal Peyer's patches are reported to posses more of T- cells and therefore are concerned with cellular immunities (Larsen and Landsverk, 1985; Tizard, 1996). Thus, their increase in numbers with age indicates that as gut is increasing in size it is very likely that more antigens are coming in.

Generally the proportion of the total length of the small intestine of the adult covered by Peyer's patches was lower than at the young age stage. In goats the proportion of intestine that is covered by Peyer's patches has also been shown to decrease with age (Assey, 1988). This implies that the rate of atrophy of ileal Peyer's patches with age is greater than the rate of increase of total length of jejunal Peyer's patches. Nevertheless, it is very likely that the infiltrated lymphoid cell in the lamina propria of the ileum now takes the role of ileal Peyer's patches. Subsquently, this suggests that the gut of young animals is more well protected.

The present study showed histological similarity between jejunal Peyer's patches and ileal Peyer's patches follicles of wild rats and giant rats which is similar to the rabbits as reported by Faulk *et al* (1971). Nevertheless, reports have also shown that the ileal Peyer's patch follicles of the rabbits are morphologically and functionally similar to the jejunal Peyer's patches in sheep and goats (Reynolds, 1980; Assey, 1988). The jejunal Peyer's patches are reported to be present during the whole life time of an animal and they increase in size with age as seen in sheep, cattle, pigs,

goats and dogs (Reynolds and Morris, 1983; Binns and Licence 1985; Assey, 1988; Parson *et al.*, 1989; HogenEsch and Felsberg, 1991). Thus, the ileal Peyer's patches and jejunal Peyer's patches of wild rats and giant rats are very likely playing similar functional roles.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 CONCLUSIONS

From the results of the present study, it can be concluded that:

(a) There is prenatal maturation of ileal Peyer's patch follicles in the mammalian orders Artiodactyla, Perissodactyla and Carnivora. In addition, bovine fetus ileal Peyer's patch follicles showed clear cortex and medulla differentiation. Prenatal maturation is one of the criteria for a primary lymphoid organ.

(b) Young animals of mammalian orders Artiodactyla and Carnivora had long band-like ileal Peyer's patch covering the whole ileum and extending into the jejunum while mammalian order Perissodactyla had ileal Peyer's patches that are in a form of discrete patches.

(c) Mammalian orders Artiodactyla, Perissodactyla and Carnivora showed an involution of ileal Peyer's patches during the period of their adulthood. Involution of a lymphoid organ at adult hood is also a feature for a primary lymphoid organ.

(d) Mammalian order Rodentia have discrete ileal Peyer's patches, which do not undergo involution at adulthood period.

(e) The size of the jejunal Peyer's patches increases with age in the mammalian orders Artiodactyla, Perissodactyla and Carnivora.

(f) With advancing age, mammals of orders Artiodactyla, Perissodactyla and Carnivora had a decrease in the total coverage of small intestine by Peyer's patches.

(g) Generally, the study has demonstrated a prenatal maturation of the ileal Peyer's patches in the mammalian orders Artiodactyla, Perissodactyla and Carnivora; which is followed by involution at adulthood except in rats. These morphological behaviour and their proximity to the gut epithelium places the ileal Peyer's patches to the equivalence of avian bursa of Fabricius

6.2 RECOMMENDATION

Further studies are suggested in rats and primate to obtain more data on the role of gut-associated lymphoid tissues. The studies should involve more areas of the gut-associated lymphoid tissue rather than concentrating on the Peyer's patches of small intestine only.

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