



UNIVERSITI PUTRA MALAYSIA

**MORPHOLOGY OF GUT-ASSOCIATED AND BRONCHUS-ASSOCIATED LYMPHOID
TISSUES OF CALVES IN RELATION TO AGE**

SAW PO PO

FPV 2006 5



**MORPHOLOGY OF GUT-ASSOCIATED AND BRONCHUS-ASSOCIATED
LYMPHOID TISSUES OF CALVES IN RELATION TO AGE**

By

SAW PO PO

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,
in Fulfilment of the Requirements for the Degree of Doctor of Philosophy**

June 2006



**MORPHOLOGY OF GUT-ASSOCIATED AND BRONCHUS-ASSOCIATED
LYMPHOID TISSUES OF CALVES IN RELATION TO AGE**

SAW PO PO

**DOCTOR OF PHILOSOPHY
UNIVERSITI PUTRA MALAYSIA**

2006



DEDICATION

**This thesis is dedicated to my parents and my grandmothers for their
encouragement and gratitude
And
To my sisters and my teachers for their kindness and love**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for degree of Doctor of Philosophy

MORPHOLOGY OF GUT-ASSOCIATED AND BRONCHUS-ASSOCIATED LYMPHOID TISSUES OF CALVES IN RELATION TO AGE

By

SAW PO PO

June 2006

Chairman: Md Zuki Abu Bakar @ Zakaria, PhD

Faculty: Veterinary Medicine

The macroscopic, light and scanning and transmission electron microscopic structure of gut-associated lymphoid tissue (GALT) of small and large intestines and bronchus-associated lymphoid tissue (BALT) were studied in the 3, 6 and 8 month-old calves. Results showed that two different types of Peyer's patches (discrete and continuous) were present in the small intestine. Discrete Peyer's patches (dPP) were located in the duodenum and jejunum and continuous Peyer's patches (cPP) were located in the ileum. The number, size, shape and distribution of Peyer's patches (PP) in small intestine varied between individuals of the same group and between age groups. There were significantly more ($p < 0.05$) dPP found in the jejunum part compared to that of duodenum. The number of dPP increased significantly ($p < 0.05$) from 3 to 6 month but this number of dPP significantly ($p < 0.05$) declined after 6 month of age. However, the size and distance between dPP were not significantly ($p > 0.05$) differences between 6 and 8 month-old calves. The cPP of the ileum was found to be raised above the level of mucosa in the 6 month-old calves. Its size decreased significantly ($p < 0.05$) after 6 month.

Under light microscope revealed the presence of elongated follicles with very small interfollicular areas (IFAs) in ileal PP (cPP) and small pear shape follicles with large IFAs were found in the duodenal and jejunal PP (dPP). Based on the morphometric analysis, the number of jejunal lymphoid follicles and size of ileal lymphoid follicles of the 6 month-old calves were significantly higher ($p < 0.05$) than those of 3 and 8 month-old calves. The width of IFAs of PP of small intestine increased with age. The number and size of dome of PP in small intestine of 6 month-old calves were significantly higher ($p < 0.05$) than those of 3 and 8 month-old calves. Two types of follicle-associated epithelium (FAE), FAE of villi and FAE of crypts were found in this study. The intrafollicular invaginations of dome epithelium of duodenal PP (dPP) were found in all ages.

Three morphological changes in the involuted Peyer`s patches were found in the ileum of the 8 month-old calves. The changes were: 1) the dome epithelium invaded into the follicle and formed intrafollicular surface epithelium, 2) depletion of follicle with replacement by the connective tissue and formation of acellular cavity in the follicles and 3) the domes became atrophied and all villi were thickened and shrunken. M cells in FAE of dPP were randomly distributed and the morphological structures of M cells in FAE of cPP were found to be same characteristic.

SEM findings revealed that the apical surface of M cells of dPP had irregular, sparse microvilli and some M cells were completely encroached up by adjacent absorptive cells. In cPP, the luminal surface of some M cells had few or lack microvilli and various sizes of vacuoles containing particles or lymphocytes on their surface.

Under the transmission electron microscope, mature and immature M cells were observed to be present in FAE of dPP and cPP of all age of studied. Mature M cells of dPP were tall columnar shape cells with less electron dense cytoplasm containing mitochondria with closely packed cristae and lymphocytes include into the cytoplasmic pocket. An irregular shaped nucleus was located toward the base of the cell and vesicles were presence at the base of microvilli. Multivesicular electron-dense bodies were found in the cytoplasm of mature M cell of cPP. Immature M cells containing many mitochondria in the cytoplasm were found in lower dome epithelium. There are cytoplasmic protrusions on the luminal surface of M cells with interdigitating cell membrane at the periphery. In cPP, luminal surface of immature M cells had small microvilli and vacuole, mitochondria; rounded nucleus located at the apical part of the cell.

The number and width of Peyer`s patches of large intestine varied with regions and ages. But it was not found that significantly difference ($p < 0.05$) between different ages. Under light microscope, the number and size of the lymphoid follicles of LGC and lymphoid nodules varied with location and age. The age related increase changes in number and size of lymphoid follicles were found in the colonic PP. The width of IFAs I the rectal and colonic PP gradually increased with increasing age. The number and size of dome of rectal PP were significantly less ($p < 0.05$) than those of other parts of the large intestine in all age groups. The biggest domes were found in the 6 month-old calves. Under the scanning electron microscope, the star-like structures of intestinal mucosa correspond with the lamina propria nodule and the LGCs related to the pit openings of intestinal mucosa. Under transmission electron microscope, the pseudopodia-like cytoplasmic protrusion were observed in the luminal surface of M

cell of FAE in lamina propria nodule. Intermediate cells containing electron lucent cytoplasm were found in FAE of LGC. The high cellular densities of diffuse lymphoid tissues of GALT of small intestine were found in the 6 month-old calves. The diffuse lymphoid tissues of GALT of large intestine were most prominent in the 8 month-old calves.

Five different types of BALT found in this study have dissimilar distribution in different lobes of the individual animal and between different animals of different age groups. The greatest number of BALT was found in the cranial lobes of the lung in 8 month-old calves. The number of plasma cells and IEL of BALT increased with age. Non-ciliated cuboidal and flattened shape of epithelial cells in the lymphoepithelium of the 3 month-old calves changed to non-ciliated columnar shape in the 6 and 8 month-old calves. Under the scanning electron microscope, the non-ciliated columnar cells area of epithelium were mostly found in the 8 month-old calves. Ultrastructurally, two different types of non-ciliated epithelial cells were found in LPE of lymphoid nodule. Type 1 cells had cylindrical shaped nuclei with clumped chromatin materials; the electron-dense cytoplasm had numerous mitochondria. In Type 2 cell, the nucleus was cuboidal shaped and its cytoplasm contained numerous vesicles of various size electron dense granules and abundant mitochondria. The luminal surface of type 2 cell had short microvilli. So it can be concluded that the morphological and immunological development of BALT reaches the maximal level at 8 month-old calves compared to that of 3 and 6 month-old calves.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

MORFOLOGI TISU LIMFOID BERKAIT USUS DAN BRONKUS ANAK LEMBU BERHUBUNG DENGAN UMUR

Oleh

SAW PO PO

Jun 2006

Pengerusi: Md Zuki bin Abu Bakar @ Zakaria, PhD

Fakulti: Perubatan Veterinar

Kajian keatas morfologi tisu limfa berkait usus (GALT) usus besar, usus kecil, dan limfa berkait bronkus (BALT) anak lembu berumur 3, 6 dan 8 bulan telah dijalankan secara makroskopi dan menggunakan mikroskop cahaya, mikroskop elektron pengimbas dan transmisi. Hasil kajian menunjukkan terdapat dua jenis tompok Peyer's (diskrit dan difusa) dalam usus kecil. Tompok Peyer's diskrit (dPP) terletak di duodenum dan jejunum dan tompok Peyer's difusa (dfPP) terletak di dalam ileum. Bilangan, saiz, bentuk dan taburan tompok Peyer's di usus kecil berbeza di antara individu dalam kumpulan yang sama dan individu di antara kumpulan umur. Terdapat lebih banyak ($p < 0.05$) dPP di dalam bahagian jejunum usus berbanding dengan bahagian duodenum. Bilangan dPP meningkat secara signifikan ($p < 0.05$) dari anak lembu berumur 3 hingga 6 bulan tetapi menurun secara signifikan ($p < 0.05$) selepas 6 bulan. Walau bagaimanapun saiz dan jarak dPP didapati tidak berbeza secara signifikan ($p > 0.05$) di antara anak lembu berusia 6 dan 8 bulan. Tompok Peyer difusa dalam ileum didapati melebihi paras mukosa pada anak lembu

berumur 6 bulan. Saiz tompok berkurangan secara signifikan ($p < 0.05$) dalam anak lembu berusia selepas 6 bulan.

Pemeriksaan histologi di bawah mikroskop cahaya menunjukkan kehadiran folikel berbentuk memanjang dan kawasan intrafolikel yang sangat kecil (IFAs) pada PP ileum (dfPP) dan folikel berbentuk pear yang kecil IFA yang besar di dapati pada duodenum dan PP jejunum (dPP). Analisis morfometri menunjukkan bilangan folikel limfa jejunum dan saiz folikel limfa ileum anak lembu berumur 6 bulan adalah tinggi secara signifikan ($p < 0.05$) berbanding dengan folikel limfa anak lembu berusia 3 dan 8 bulan. Pusat germina ditemui di kebanyakan folikel limfa PP jejunum dalam anak lembu berumur 6 dan 8 bulan. Ukuran garis pusat IFAs di dalam PP usus kecil meningkat dengan umur. Bilangan umur dan saiz kubah PP adalah tinggi secara signifikan ($p < 0.05$) dalam usus kecil anak lembu berusia 6 bulan berbanding dengan anak lembu berumur 3 dan 8 bulan. Dua jenis folikel berkait epithelium (FAE) ditemui dalam kajian ini: FAE villi dan FAE kripta. Invaginasi intrafolikular kubah pada epithelium duodenum ditemui dalam semua peringkat umur.

Tiga perubahan morfologi di dalam tompok peyer's berinvolusi didapati pada ileum anak lembu berusia 8 bulan. Perubahan tersebut adalah: 1) kubah epithelium menyusup masuk folikel dan membentuk permukaan epithelium intrafolikular; 2) pengurangan folikel dan diganti dengan tisu penyambung dan pembentukan kaviti tidak bersel di dalam folikel dan 3) kubah tersebut mengecil dan semua vilus menebal dan memendek. FAE terdiri daripada sel bermembran (sel M) berselerak secara rawak di antara sel penyerap dan struktur morfologi sel M pada FAE cPP digambarkan mempunyai ciri-ciri yang sama.

Dari pemeriksaan dengan SEM menunjukkan permukaan apikal sel M dPP mempunyai mikrovilus yang tidak rata dan longgar dan beberapa sel M menunjukkan ditutupi secara keseluruhan dengan sel penyerap di sekelilingnya. Pada cPP, permukaan berongga pada sel M mempunyai sedikit mikrovilus, dan beberapa jenis vakuol mengandungi partikel atau limfosit yang kelihatan di permukaan sel.

Dari segi struktur, sel M matang dan tidak matang ditemui pada FAE dPP dan cPP di dalam anak lembu pada semua umur yang dikaji. Sel M yang matang mempunyai bentuk kolumnar tinggi dan mempunyai sedikit sitoplasma tumpat elektron yang mengandungi mitokondria dengan kristae yang padat dan limfosit dalam kantungnya. Nukleus yang tidak sekata bentuknya terletak pada bes sel dan vesikel didapati pada bes mikrovilus. Jasad tumpat multivesikular elektron ditemui pada cPP sel M matang. Elektron Sel M yang tidak matang ditemui pada bahagian bawah kubah epithelium dan vesikelnya mempunyai banyak mitokondria. Permukaan berongga mempunyai unjuran sitoplasma dan membran sel menginterdigitat. Dalam cPP, permukaan berongga sel M tidak matang mempunyai mikrovilus yang kecil dan vakuol, mitokondria dan nukleus berbentuk bulat ditemui pada bahagian apeks sel tersebut.

Bilangan dan saiz folikel limfa tompok Peyer usus besar berbeza mengikut lokasi dan perbezaan umur, tetapi perbezaan umur tidak berbeza secara signifikan ($p < 0.05$). Pemeriksaan mikroskopi cahaya mendapati bilangan dan saiz folikel limfa pada LGC dan nodul limfa berbeza mengikut lokasi dan umur. Perbezaan signifikan berhubung dengan umur pada bilangan dan saiz folikel limfa hanya didapati pada PP kolonik.

Kebanyakan IFA ditemui pada LGC dan saiznya pada rektum dan PP kolon meningkat secara berjadual mengikut umur. Bilangan dan saiz kubah pada PP rektum adalah berkurangan secara signifikan ($p < 0.05$) berbanding dengan lain-lain bahagian usus besar dalam kesemua peringkat umur. Kubah terbesar dijumpai pada anak lembu berusia 6 bulan. Pemeriksaan mikroskopi elektron imbas mendapati, struktur berbentuk seperti bintang pada mukosa usus adalah bersangkutan dengan nodul lamina propria dan LGC berkaitan dengan pembukaan pada mukosa usus. Dengan TEM, unjuran sitoplasmik berbentuk pseudopodia ditemui pada permukaan rongga sel M FAE pada nodul lamina propria. Sel intermedia mempunyai sitoplasma lutsinar elektron juga ditemui pada FAE LGC. Ketumpatan tinggi tisu limfa berkait usus (GALT) usus kecil ditemui pada anak lembu berumur 6 bulan. Tisu limfa GALT difusa usus besar adalah paling ketara pada anak lembu berusia 8 bulan.

Lima jenis BALT ditemui dalam kajian ini tetapi taburannya berbeza pada lobus pada haiwan yang sama di antara individu dan kumpulan umur. Bilangan BALT tertinggi ditemui pada lobus krania paru-paru anak lembu berumur 8 bulan. Bilangan sel plasma dan IEL pada BALT meningkat mengikut umur. Pada anak lembu berumur 3 bulan, sel epithelium jenis limfoepitelium (LPE) nodul limfa adalah kuboid tetapi bentuk sel epithelium berubah kepada kolumnar dalam anak lembu berumur 6 dan 8 bulan. Bentuk sel LPE mungkin berbeza bergantung kepada lokasi dan umur. Dalam anak lembu berumur 3 bulan, rekahan epithelium ditemui pada bronkiol dan tisu limfa interstis ditemui pada anak lembu berusia 8 bulan. Purata bilangan sel plasma dan IEL meningkat dengan umur. Penemuan dengan SEM mendapati kawasan sel kolumnar tidak bersilia ditemui kebanyakannya pada anak lembu berusia 8 bulan dan bentuk sel tersebut berbeza dengan lokasi dan umur. Dari

segi ultrastruktur, dua jenis sel epithelium tidak bersilia ditemui pada LPE nodul limfa. Sel Jenis I mempunyai nukleus berbentuk silinder mengandung kromatin bergugus dan sitoplasma elektron tumpat dan mempunyai banyak mitokondria. Dalam Sel Jenis II, nukleusnya berbentuk kuboid dan sitoplasmanya mengandung vesikel yang berbagai saiz, granul tumpat elektron dan mitokondria yang banyak. Permukaan berongga sel jenis II mempunyai mikrovilus yang kecil. Oleh itu dapat disimpulkan bahawa BALT pada anak lembu yang berumur 8 bulan menunjukkan organisasi dan morfologi yang tinggi berbanding dengan anak lembu berumur 3 dan 6 bulan.

ACKNOWLEDGEMENTS

All the achievements are due to Almighty GOD for his guidance and blessing over me throughout the period of this study. First, I would like to express my deepest appreciation and gratitude to Associate Professor. Md Zuki Bin Abu Bakar, Chairman of my Supervisor Committee for providing his valuable guidance, advice, continuous supervision, patience and understanding towards the completion of my study.

I wish to express my thanks and appreciation to members of the Supervisory Committee, Professor Dr. Mohd Zamri Saad, Associate Professor Dr. Abdul Rahman Omar and Associate Professor Dr. Mohd Effendy Abdul Wahid for their invaluable advice and guidance in conducting the study and encouragement.

I am also very grateful to my sponsor, GRA (Graduate Research Assistant) for providing me the financial support to enable me to pursue the degree of Doctor of Philosophy in Malaysia.

I would like to express my thankfulness to the Agricultural Ministry of Malaysia and His Excellency the Livestock and Fisheries Minister of Myanmar for offering me the opportunity to study in Malaysia. I am much obliged to the Directors General of respective Departments and Rector/Dean of both Malaysia and Myanmar for their support for award at a scholarship me.



I am very much indebted to Mr. Mohd. Jamail Samad, Dr. Md. Sabri Mohd. Yusoff, Dr. Mohd Shafarin Shamsuddin and Ms Ernie Zuraida Ali of Histopathology laboratory for their technical assistance. I wish to express my deepest gratitude to Miss Azilah Abdul Jalil, and Mr. Ho Oi Kuan of the Electron Microscopy Unit, Institute of Bioscience, Universiti Putra Malaysia and to the academic and supporting staff of the Faculty of Veterinary Medicine, Universiti Putra Malaysia for their assistance and support during the course of the study. I would like to thank all my Myanmar friends and my lecturers from Malaysia and the University of Veterinary Science, Myanmar for their encouragements.

Deepest thanks are due to my grandmothers, my father, my sisters and my nephew (Major Chan Pyae Soe) for their patience and moral support during the course of this study. I wish to express my sincere thank to Dr. Myo Thant, Professor Saw Ba Blu and Professor Aye Cho , Heads, Department of Anatomy, University of Veterinary Science, Myanmar.

Last but not the least; I would like to convey special acknowledgement to my friends Dr Hafeez Yagoub Mohamed, Ms Rozaini Bt Mohd Zohdi, Dr.Awang Hazmi and Dr.Intan Shameha Bt Abdul Razak for their kindness and hospitality.



I certify that an Examination Committee has met on 9 June 2006 to conduct the final examination of Saw Po Po on her Doctor of Philosophy thesis entitled “Morphology of Gut-Associated and Bronchus-Associated Lymphoid Tissues of Calves in Relation to Age ”in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

Tengku Azmi Tengku Ibrahim, PhD

Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Abdul Hamid Abdul Rashid, PhD

Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Noordin Mohd Mustapha, PhD

Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Internal Examiner)

Ian.A.Silver

Emeritus Professor
School of Veterinary Science
University of Bristol, UK
(External Examiner)

HASANAH MOHD. GHAZALI, PhD

Professor / Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:

This thesis submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of the Doctor of Philosophy. The members of the Supervisory Committee are as follows:

Md Zuki Abu Bakar @ Zakaria, PhD

Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Chairman)

Mohd Zamri Saad, PhD

Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

Abdul Rahman Omar, PhD

Associate Professor
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Member)

AINI IDERIS, PhD
Professor/ Dean
School of Graduate Studies
Universiti Putra Malaysia

Date:



DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.

SAW PO PO

Date:

TABLE OF CONTENTS

		Page
DEDICATION		ii
ABSTRACT		iii
ABSTRAK		vii
ACKNOWLEDGEMENTS		xii
APPROVAL		xiv
DECLARATION		xvi
LIST OF TABLES		xxi
LIST OF FIGURES		xxii
LIST OF ABBREVIATIONS		xxxviii
CHAPTER		
1	INTRODUCTION	1.1
2	LITERATURE REVIEW	2.1
	2.1 Gut-Associated Lymphoid Tissue (GALT) of Small Intestine	2.1
	2.1.1 Macroscopic Structure of Organized Lymphoid Tissue (Peyer's patches) in Small Intestine	2.5
	2.1.2 Microscopic Structure of Peyer's patches of Small Intestine	2.6
	2.1.2.1 Follicles	2.7
	2.1.2.2 Interfollicular Area (IFA)	2.9
	2.1.2.3 Dome Area	2.10
	2.1.2.4 Follicle Associated Epithelium (FAE)	2.10
	2.1.2.5 Involution of Peyer's patches in Ileum in Small Intestine	2.13
	2.2 Gut- Associated Lymphoid Tissue (GALT) of Large Intestine	2.13
	2.2.1 Macroscopic Structure of GALT of Large Intestine	2.13
	2.2.2 Microscopic Structure of GALT of Large Intestine	2.15
	2.3 Diffuse Lymphoid Tissues of Small and Large Intestine	2.17
	2.3.1 Intraepithelial Lymphocytes (IELs)	2.18
	2.3.2 Lamina Propria Lymphocytes	2.20
	2.4 Bronchus-Associated Lymphoid Tissue (BALT) of Lung	2.21
	2.4.1 Microscopic Structure of BALT	2.22
	2.4.1.1 Occurrence of BALT	2.22
	2.4.1.2 Distribution of BALT	2.23
	2.4.1.3 Lymphoid Aggregates	2.26
	2.4.1.4 Nodular Lymphoid Tissues	2.28
	2.4.1.5 Plasma Cells	2.31
	2.4.2 Electron Microscopic Structure of BALT and lymphoepithelium (LPE)	2.32



3	GENERAL MATERIALS AND METHODS	3.1
3.1	Animals	3.1
3.2	Sampling	3.1
3.3	Gross Examination	3.2
3.3.1	Macroscopic Examination of GALT of Small Intestine	3.2
3.3.2	Macroscopic Examination of GALT of Large Intestine	3.2
3.4	Microscopic Examination	3.3
3.4.1	Sample Collection, Processing and Staining	3.3
3.4.2	Immunohistology	3.4
3.5	Electron Microscopic Examination	3.5
3.5.1	Sample Collection and Processing for Scanning and Transmission Electron Microscope (SEM and TEM)	3.5
3.6	Morphometry Study	3.7
3.7	Statistical Analysis	3.8
4	MORPHOLOGICAL EVALUATION OF GALT OF SMALL INTESTINE IN CALVES AT DIFFERENT AGES	4.1
4.1	Introduction	4.1
4.2	Materials and Methods	4.2
4.2.1	Animals and Morphometry for Macroscopic Evaluation	4.2
4.2.2	Small Intestine Samples and Processing and Staining for Light Microscopic Evaluation	4.3
4.2.3	Small Intestine Sampling and Processing for Scanning and Transmission Electron Microscopic Evaluation	4.3
4.2.4	Morphometry and Statistical Analysis	4.3
4.3	Results	4.3
4.3.1	Macroscopic Evaluation of GALT in Small Intestine	4.3
4.3.2	Microscopic Evaluation of GALT in Small Intestine	4.5
4.3.2.1	Follicles	4.5
4.3.2.2	Interfollicular Area (IFA)	4.17
4.3.2.3	Dome Area	4.20
4.3.2.4	Follicle-Associated Epithelium (FAE)	4.31
4.3.3	Microscopic Evaluation of Diffuse Lymphoid Tissues of Small Intestine	4.35
4.3.3.1	Intraepithelial Lymphocytes	4.35
4.3.3.2	Lamina Propria Lymphocytes	4.39
4.3.4	Electron Microscopy Evaluation of PP of Small Intestine	4.47
4.3.4.1	SEM Structure of Follicle and Interfollicular Area	4.47
4.3.4.2	SEM Structure of Dome Area	4.53
4.3.4.3	SEM Structure of Follicle-Associated Epithelium (FAE)	4.53

	4.3.4.4	TEM Structure of Lymphoid Cell Compartments	4.58
	4.3.4.5	TEM Structure of Follicle-Associated Epithelium	4.64
	4.3.5	Involution of Ileal Peyer`s patches of Small Intestine in 8 month-old calves	4.70
	4.3.5.1	Macroscopic Findings	4.70
	4.3.5.2	Light, Scanning and Transmission Electron Microscopic Findings	4.71
4.4		Discussion	4.92
5		MORPHOLOGICAL EVALUATION OF GALT OF LARGE INTESTINE IN CALVES AT DIFFERENT AGES	5.1
5.1		Introduction	5.1
5.2		Materials and Methods	5.2
	5.2.1	Animals and Morphometry for Macroscopic Evaluation	5.2
	5.2.2	Large Intestine Samples and Processing For light Microscopic Evaluation	5.2
	5.2.3	Large Intestine Sampling and Processing for Scanning and Transmission Electron Microscopic Evaluation	5.2
	5.2.4	Morphometry and Statistics Analysis	5.3
5.3		Results	5.3
	5.3.1	Macroscopic Evaluation of GALT in Large Intestine	5.3
	5.3.2	Microscopic Evaluation of GALT in Large Intestine	5.8
	5.3.2.1	Lymphoglandular Complex (LGC)	5.8
	5.3.2.2	Lamina Propria Lymphoid Nodule	5.17
	5.3.2.3	Lymphoid Follicles of LGC and Lamina Propria Nodules	5.22
	5.3.2.4	Interfollicular Area (IFA)	5.27
	5.3.2.5	Dome	5.30
	5.3.2.6	Follicle-Associated Epithelium (FAE)	5.37
	5.3.3	Microscopic Evaluation of Diffuse Lymphoid Tissues of Large Intestine	5.37
	5.3.3.1	Intraepithelial Lymphocytes (IELs)	5.37
	5.3.3.2	Lamina Propria Plasma Cells	5.39
	5.3.3.3	Lamina Propria Macrophages	5.42
	5.3.3.4	Lamina Propria B Lymphocytes	5.44
	5.3.4	Electron Microscopy Evaluation of Large Intestine	5.45
	5.3.4.1	SEM Structure of Follicle and Interfollicular Area of Lymphoglandular Complexes	5.49
	5.3.4.2	SEM Structure Dome Area	5.52
	5.3.4.3	SEM Structure of Follicle-Associated Epithelium	5.53
	5.3.4.4	TEM Structure of FAE	5.54

5.4	Comparison the Morphological Structure of GALT of Small and Large Intestine	5.57
5.5	Discussion	5.64
6	MORPHOLOGICAL EVALUATION OF BALT OF LUNG IN CALVES AT DIFFERENT AGES	6.1
6.1	Introduction	6.1
6.2	Materials and Methods	6.2
6.2.1	Animals, Lung Sampling and Processing for Light Microscopic Evaluation	6.2
6.2.2	Lung Sampling and Processing for Scanning Transmission Electron Microscopic Evaluation	6.2
6.2.3	Morphometry for Light Microscopic Evaluation	6.3
6.2.4	Statistics Analysis	6.3
6.3	Results	6.3
6.3.1	Microscopic Evaluation of BALT	6.3
6.3.1.1	Occurrence of BALT	6.3
6.3.1.1.1	Lymphoid Nodules	6.4
6.3.1.1.2	Aggregated Lymphoid Tissues	6.21
6.3.2	Scanning Electron Microscopic Evaluation of Bronchus-Associated Lymphoid Tissue in Lung	6.41
6.3.2.1	Epithelium of Lymphoid Nodules	6.41
6.3.2.2	Epithelium of Lymphoid Aggregates	6.42
6.3.3	Transmission Electron Microscopic Evaluation of Bronchus-Associated Lymphoid Tissue in Lung	6.49
6.3.3.1	Epithelium of Lymphoid Aggregates	6.49
6.3.3.2	Lymphoepithelium of Lymphoid Nodules	6.49
6.3.3.3	Lymphoid Cell Compartments	6.52
6.4	Discussion	6.58
7	GENERAL DISCUSSION AND CONCLUSION	7.1
7.1	Macroscopic Evaluation of GALT in Small Intestine	7.1
7.2	Microscopic and Electron Microscopic Evaluation of GALT in Small Intestine	7.3
7.3	Morphological Changes of Ileal PP during Involution	7.8
7.4	Macroscopic Evaluation of GALT in Large Intestine	7.10
7.5	Microscopic Evaluation of GALT in Large Intestine	7.10
7.6	Microscopic Evaluation of BALT in Lung	7.12
	REFERENCES	R.1
	APPENDICES	A.1
	BIODATA OF THE AUTHOR	B.1

LIST OF TABLES

Table		Page
4.1	The length and width of dPP in duodenum of individual calves of the same age and at different ages (Mean± SE)	4.7
4.2	The length and width of dPP in jejunum of individual calves of the same age and at different ages (Mean± SE)	4.7
4.3	The length, width and distance of dPP of small intestine at different ages. (Mean± SE)	4.7
4.4	The number of dPP in different parts of small intestine of calves at the same age and at different ages.	4.8
4.5	The number, length and width of cPP in ileum of calves at different ages. (Mean± SE)	4.8
5.1	The number of PP in different parts of large intestine at different ages.(Mean± SE)	5.7
5.2	The length of PP in different parts large intestine at different ages. (Mean± SE)	5.7
5.3	The width of PP in different parts of large intestine at different ages. (Mean± SE)	5.7
5.4	The number, length width and distance of dPP between the small and large intestine in calves at different ages. (Mean± SE)	5.70

LIST OF FIGURES

Figure	Page	
4.1	Photographs of the small intestine of calves immediately upon removing the intestine from the body. The dPP in the duodenum of 3 month-old calf (A), the jejunum of 6 month-old calf (B) and the cPP in the ileum of 6 month-old calf (C).	4.6
4.2	Photographs of the luminal surface of small intestine of calves show the different shapes of dPP in duodenum of 3 month-old calves (A), in jejunum of 6 month-old calves (B), and in jejunum of 8 month-old calves (C).	4.6
4.3	Photographs of the luminal surface of small intestine show the elongated-shaped cPP found in ileum of 3 month-old calf (A), 6 month-old calf (B) and 8 month-old calf (C)	4.6
4.4	Histological structures of Peyer`s patches of the small intestine of the 3 month-old calves show the shape of lymphoid follicles of PP in the duodenum (A) in the jejunum(B) in ileum (C). H&E	4.13
4.5	Histological structures of Peyer`s patches of the small intestine of 6 month-old calves show the lymphoid follicles of PP in duodenum (A), jejunum (B) and ileum (C). H&E	4.14
4.6	Histological structures of Peyer`s patches of the small intestine of 8 month-old calves show shape of lymphoid follicles of PP in the distal part of the duodenum (A), jejunum (B) and ileum (C). H&E	4.15
4.7	The number of lymphoid follicles in Peyer`s patches in different parts of small intestine in 3 (A), 6 (B) and 8 (C) month-old calves.	4.16
4.8	The length of lymphoid follicles of Peyer`s patches at different parts of small intestine in 3 (A), 6(B) and 8 (C) month-old calves.	4.16
4.9	The width of lymphoid follicles in Peyer`s patches at different parts of the small intestine in 3 (A), 6 (B) and 8 (C) month-old calves.	4.16
4.10	The mean number of lymphoid follicles in Peyer`s patches of small intestine at different ages.	4.18
4.11	The mean length of lymphoid follicle of Peyer`s patches in small intestine at different ages.	4.18
4.12	The mean width of lymphoid follicle of Peyer`s patches in small intestine at different ages.	4.18

4.13	Histological structures of the lymphoid aggregated tissue of small intestine in all calves (A) and cryptopatches of small intestine in the 3 month-old calves (B). H&E	4.19
4.14	Histological structures of the interfollicular area (IFA) of Peyer`s patches in duodenum (A), jejunum (B), and ileum(C) in the 3 month-old calves. H&E	4.21
4.15	Histological structures of the interfollicular area (IFA) of Peyer`s patches in duodenum (A), jejunum (B), and ileum(C) in the 6 month-old calves. H&E	4.22
4.16	Histological structures of the interfollicular area (IFA) of Peyer`s patches in duodenum (A), jejunum (B), and ileum(C) in the 8 month-old calves. H&E	4.23
4.17	The width of the interfollicular areas of lymphoid follicles of Peyer`s patches at different parts of the small intestine of in 3 (A), 6 (B) and 8 (C) month-old calves.	4.24
4.18	The mean width of the interfollicular areas of Peyer`s patches in different parts of small intestine in calves at different ages.	4.24
4.19	Histological structures of the Peyer`s patches of small intestine show the dome-associated villi (A) and the dome-associated crypts (B). H&E	4.26
4.20	Histological structures of the domes of dPP in the duodenum and jejunum of the 3 (A), 6 (B) and 8 (C) month-old calves. H&E	4.27
4.21	Histological structures of the dome of cPP in the ileum of the 3 (A), 6 (B) and 8 (C) month-old calves H&E.	4.28
4.22	Histological structures of the ileal PP in the 8 month-old calves show the interfollicular invagination of epithelium (A), double villi encircle the dome (B) and dome-associated crypts (C). H&E	4.29
4.23	The number of domes of Peyer`s patches at different parts of the small intestine in 3 (A), 6 (B) and 8 (C) month-old calves.	4.32
4.24	The mean number of domes of Peyer`s patches in the small intestine of calves at different ages.	4.32
4.25	The length of domes of Peyer`s patches at different parts of small intestine in 3 (A), 6 (B) and 8 (C) month old calves.	4.32
4.26	The width of domes of Peyer`s patches at different parts of small intestine in 3 (A), 6 (B) and 8 (C) month old calves.	4.33