UNIVERSITI PUTRA MALAYSIA

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

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By

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

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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

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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. Tompak 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 epitelium 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 mengandungi kromatin bergugus dan sitoplasma elektron tumpat dan mempunyai banyak mitokondria. Dalam Sel Jenis II, nukleusnya berbentuk kuboid dan sitoplasmanya mengandungi 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.

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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:

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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:

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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:

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