UNIVERSITI PUTRA MALAYSIA

# PATHOGENESIS OF HAEMORRHAGIC SEPTICAEMIA IN ORGAN CULTURE, MICE AND CALVES

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# PATHOGENESIS OF HAEMORRHAGIC SEPTICAEMIA IN ORGAN CULTURE, MICE AND CALVES

**By**

**AMNA ELAMIN MOHAMED**

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

**December 2007**

## DEDICATION

ALLAH you are my supreme love

ALHAMDULILLAH ….thank you for everything

To the memory of my late father

My beloved mother, sisters and brothers

Thanks for your doa', love and care

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

**PATHOGENESIS OF HAEMORRHAGIC SEPTICAEMIA IN ORGAN CULTURE, MICE AND CALVES**

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**Chairman: Associate Professor. Jasni Sabri, PhD**

**Faculty: Veterinary Medicine**

Experimental intraperitoneal infection of mice with *Pasteurella multocida* serotype B:2 at the dose of $10^7$cells caused death in all infected mice between 18 to 24 hours. Microscopy showed suppurative tracheitis. The alveoli lumina were infiltrated with numerous inflammatory cells mainly neutrophils. Pink-stained fibrin and red blood cells were also present in the alveoli lumina. Blue shade of bacterial colonies were observed in the alveolar space and pulmonary vessels. In the aorta, there was degeneration of the endothelium, disruption of the intima and focal myolytic changes in the smooth muscle.

In infected mice, scanning electron microcopy (SEM) showed cilial damage and deciliation of tracheal epithelial cells. In the lung extravasation of red blood cells in the alveolar lumina was observed. Rod-shaped bacteria were seen attached to the alveolar wall causing depression on the surfaces of the cells. Changes in the aortas were characterized by rounded endothelial cells. Some rounded endothelial cells were seen about to detach and expelled into the blood vessel lumen. Bacteria were

most evident at the intercellular junction of the endothelial cells that were beginning to slough.

In infected mice, transmission electron microscopy (TEM) of the trachea showed bacterial cells attached to deformed cilia. Necrotic and apoptotic endothelial cells were seen protruded into the lumen of the affected blood vessel. Several bacteria were attached to the plasma membrane of the endothelial cells and in the blood vessel lumen. Endothelial cells showed electron dense cytoplasmic extensions around the bacteria and as attaching and effacing lesions which were interpreted as the first steps in the phagocytosis process. In the lung, there was thickening of the alveolar septa. Bacteria were seen dividing inside the neutrophils and in the lumen of the blood vessels. Some bacteria were also seen in vacuolated macrophages. Endothelial cells showed apoptosis. Fibrin was present in the alveolar lumina. In the aorta, endothelial cells were fragmented and detached from the basement membranes. Bacteria were attached to the fragmented endothelium.

Calves inoculated intranasally with 3.0 x$10^{10}$ *P. multocida* serotype B:2 following stressed with dexamethasone developed septicaemia and fibrinous pleuropneumonia. All infected calves died between 24 to 48 hours. Thrombi were observed in the nasal cavity and lung. It is therefore believed that the *P. multocida* had gained entry into the blood vessel of the nasal cavity and / or lung to various organs causing septicaemia. This is validated by the isolation of pure growth of *P. multocida* from various congested organs following intranasal infection.

The microscopic changes in infected calves were suppurative rhinitis, suppurative tracheitis, fibrinoid necrosis in the blood vessel walls, thrombosis, necrotic vasculitis, alveolar congestion and alveolitis with edema and fibrin deposition in the alveolar lumen. Scanning electron microscopy (SEM) showed severe loss of cilia and the presence of free red blood cells in the nasal mucosa and trachea. Bacteria were attached to the alveolar wall causing depression on the cell surface. In the aorta of infected calves, bacteria were most evident near the base of extruding cells.

Transmission electron microscopy (TEM) of infected calves showed spherical blebbing with fine granules on the tip of destructed cilia (bullae). Bacteria were closely adhered to the endothelial surface and some were connected by tiny surface strands to the plasma membranes. Some of the bacteria were engulfed by the endothelial cells. There was apoptotic and necrotic morphology of dying endothelial cells after contact with *P. multocida*. Some erythrocytes have passed through the vascular wall. On the basis of these findings, it is considerd that endothelial necrosis and apoptosis may be related to the disruption of endothelial barrier which then lead to vascular leakage. This may therefore contribute to the hemorrhagic complication of hemorrhagic septicemia.

*In vitro* experiments conducted to study adhesion and colonization of *P. multocida* serotype B:2 to tracheal mucosa, lung and aorta explants of calf showed that *P. multocida* B:2 can adhere to the lung, trachea and aorta. The adherence of bacteria at different time (1, 2, 3, 4, and 6 hours), is significantly different, ($P<0.0001$). For the lung and aorta, the attachment of the bacteria in the first and second hour post incubation was high, while that of 3 hours was moderate. There was no colonization

at 4 and 6 hours post infection. Attachment of the bacterium to the trachea also showed significant difference (P<0.0089) where the attachment of the bacteria in the first hour post infection was high, compared to 2, 3, 4 and 6 hours post infection which was moderate to no bacteria. The results of this study revealed that *P. multocida* B:2 was more adhesive to the lung tissues which showed severe colonization at 1 and 2 hours after incubation while aorta showed mild *P. multocida* B:2 colonization at 1 and 2 hours after incubation and trachea showed less *P. multocida* B:2 colonization. This result showed that the *in vitro* colonization decreased with time. Transmission electron microscopy showed severe damage to the epithelium. This study showed that after 1 hour of incubation, *P. multocida* was lost from the surface of the cell. Therefore, transiet time between 10 to 30 minutes is needed to detect adhered bacteria to tracheal epithelial cells and aorta endothelial cells. These studies demonstrated that *P. multocida* type B:2 was able to colonize and invade the lung, trachea and aorta explants.

Transmission electron microscopy showed degenerative features of the lung *in vivo* and *in vitro*. Bacteria were attached to the surface of the endothelial and epithelial cells. There was evidence of invasion and the bacteria appear to be concentrated at certain locations along the cellular membrane of lung which induced dramatic morphological changes on the surface of the attached cells. Some endothelial and epithelial cells form blebbing on the site of the attachment. Some internalized bacteria appeared in membranous vacuoles.

*Pasteurella multocida* was able to attach to and invade epithelial and endothelial cells *in vivo* and *in vitro*. It is therefore believed that the *P. multocida* had gained

entry into the blood vessel of the nasal cavity, trachea and lung to various organs causing septicaemia. Thus the growth of bacteria to an overwhelming number and then colonization of different organs resulted in the development of lesions in various organs and death due to vital organ failure.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
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**PATOGENESIS SEPTISEMIA BERDARAH DALAM KULTUR ORGAN, TIKUS DAN ANAK LEMBU**

Oleh

**AMNA ELAMIN MOHAMED**

**December 2007**

**Pengerusi: Profesor Madya. Jasni Sabri, PhD**

**Fakulti: Perubatan Veterinar**

Jangkitan ekperimental keatas tikus dengan *P. multocida* serotip B:2 menunjukkan pada dos sel $10^7$ adalah sangat patogenik dan kesemua tikus mati antara 18 – 24 jam pasca inokulasi. Pemeriksaan histopatologi menunjukkan trakeatis ber supuratif, lumina alveolus padat dipenuhi dengan neutrofil, fibrin terwarna eosin dan sel darah merah. Koloni bakteria terdapat dalam ruang alveolus dan salur darah pulmonari. Nyahjana endotelium dan miolisis fokal otot licin aorta jelas kelihatan.

Pemeriksaan dengan mikroskop elektron imbas menunjukkan kerosakan keatas silia dan nyahsilia sel epithelium trakea pada tikus yang dijangkiti. Berlaku ekstravasasi sel darah merah ke dalam lumina alveolus dan bakteria bentuk rod kelihatan melekat pada dinding alveolus menyebabkan sel pneumatik alveolus menjadi lekuk. Sel endotelium aorta yang tertanggal daripada dinding salur darah didapati berada dalam lumina salur darah. Bakteria juga terdapat di dalam ruang antara sel endotelium.

Pemeriksaan trakea tikus dengan mikroskop elektron transmisi menunjukkan bakteria terlekat pada silia yang cacat bentuk. Kromatin nukleus sel endotelium

kelihatan tumpat dan mengecil menunjukkan ciri nukleus yang tipikal. Sel endotelium nekrotik dan apoptotik menonjol ke dalam lumen salur darah. Kerap terdapat bakteria yang terlekat pada membran sel endotelium. Bakteria juga kelihatan berada dalam neutrofil dan dalam makrofaj bervakuol. Septa alveolus kelihatan lebih tebal sementara terdapat fibrin di dalam lumen alveolus. Pada aorta, sel endotelium didapati terpisah daripada membran asasnya sementara nukleus sel tersebut kelihatan terpecah dan bakteria melekat kepada serpihan nukleus tersebut.

Anak lembu yang diinokulat intranasum dengan 3.0 x $10^{10}$ *P. multocida* serotip B:2 ekoran tekanan yang dipengaruh oleh deksametason membentuk septisemia dan pleuropneumonia berfibrin. Kesemua anak lembu terinfek mati dalam tempoh 24 – 48 jam. Kajian ini merupakan kejayaan pertama sampar berdarah jangkitan ekperimental intanasum dalam anak lembu menggunakan *P.multocida* B:2. Anak-anak lembu menunjukkan gejala pneumonia dan septisemia dengan kehadiran trombus dalam rongga nasum dan paru paru. Adalah dipercayai *P. multocida* berjaya memasuki salur darah rongga nasum dan/atau paru paru dan sampai ke lain-lain organ dan menyebabkan berlakunya septisemia. Andaian ini dibuktikan dengan pengasingan pertumbuhan kultur tulin *P. multocida* daripada beberapa organ ekoran jangkitan intranasum.

Pemeriksaan dengan mikroskop cahaya menunjukkan rhinitis dan trakeatis ber supuratif, nekrosis fibrinoid pada dinding salur darah, trombosis, nekrosis vaskulitis, kongesi, alveolitis serta edema dan deposisi fibrin. Mikroskopi elektron imbas anak lembu terjangkit menunjukkan kehilangan silia yang teruk dan kehadiran sel darah merah pada mukosa nasum dan trakea. Bakteria didapati terlekat pada dinding

alveolus menyebabkan kelekukan pada permukaan sel dalam aorta. Bakteria jelas kelihatan pada bes endotelium yang menonjol. Mikroskopi elektron transmisi menunjukkan kehadiran bleb mengandungi granul halus pada penghujung silia yang telah rosak. Unjuran sitoplasma daripada permukaan sel endotelium bersambung kepada bakteria. Unjuran ini juga didapati mengelilingi bakteria yang boleh diinterpretasikan sebagai proses fagositosis sel endotelium ke atas bakteria. Sel endotelium yang apoptotik dan nekrotik kelihatan bersentuhan dengan *P. multocida.* Berasaskan cerapan ini, bolehlah dianggap bahawa sel endotelium yang nekrotik dan apoptotik menyumbang kepada perubahan patologi vesel. Dengan yang demikian apotosis dan nekrosis boleh dikaitkan kepada keruntuhan rintangan endotelium yang membawa kepada ketirisan vessel. Patofisiologi ini menyumbang kepada komplikasi pendarahan septisemia berdarah.

Kajian *in vitro* yang dijalankan adalah untuk mengkaji perlekatan dan pengkolonian *P. multocida* serotip B:2 kepada eksplan segar permukaan mukosa paru-paru dan aorta. Perlekatan bakteria pada selang masa yang berlainan (1,2,3,4 dan 6 jam) menunjukkan perbedaan signifikan ($P<0.0001$) pada paru-paru dan aorta. Perlekatan bakteria dalam tempoh 1 dan 2 jam yang pertama pasca inokulasi adalah tinggi berbanding dengan 3 jam pasca inokulasi dengan perlekatan yang sederhana. Tidak terdapat pengkolonian pada 4 dan 6 jam pasca inokulasi. Perlekatan bakteria kepada mukosa trakea juga menunjukkan perbedaan yang signifikan ($P<0.0089$) pada selang masa berlainan dimana dalam tempoh 1 jam pertama pasca inokulasi adalah tinggi berbanding dengan 2,3,4 dan 6 jam pasca inokulasi yang sederhana atau ketiadaan bakteria. Meningkatkan tempoh inkubasi menurunkan insiden perlekatan bakteria. Cerapan ini menunjukkan kecenderungan *P. multocida* untuk melekat kepada tisu

paru-paru adalah lebih tinggi seperti yang ditunjukkan oleh pengkolonian bakteria yang tinggi dalam 1 dan 2 jam pasca inkubasi sementara pengkolonian oleh *P. multocida* B:2 pada aorta dalam tempoh yang sama adalah sederhana. Insiden pengkolonian *P. multocida* B:2 ke atas eksplan trakea adalah kurang berbanding dengan aorta. Kajian ini menunjukkan pengkolonian *in vitro* menurun dengan penurunan masa untuk menjadi sederhana dalam tempoh 3 jam pasca inkubasi dan selepas 4 dan 6 jam tidak terdapat sebarang bakteria pada tisu tersebut. Cerapan di atas memberi gambaran *P. multocida* lebih mudah menyerang endotelium trakea dan aorta berbanding dengan paru-paru.

Kerosakan yang teruk ke atas epitelium seperti yang dapat dilihat dengan mikroskopi elektron transmisi memberi gambaran bahawa lebih tinggi kerosakan ke atas epitelium maka lebih tinggi bilangan sel sasaran yang terdedah. Ini jelas dapat dilihat 1 jam selepas inkubasi, *P. multocida* tidak kelihatan lagi di permukaan sel. Dengan ini jangkamasa yang singkat antara 10-30 minit adalah diperlukan untuk mengesan bakteria yang melekat pada sel epitelia trakea dan sel endotelium aorta. Kajian ini menunjukkan bahawa *P. multocida* serotip B:2 berupaya mengkoloni dan menyerang paru-paru, trakea dan aorta.

Mikroskopi elektron transmisi menunjukkan perubahan paru-paru *in vivo* dan *in vitro.* Bakteria kelihatan terlekat pada permukaan sel epitelium dan sel endotelium. Kelihatan terdapat bukti serangan oleh bakteria yang tertumpu pada lokasi tertentu di sepanjang membran sel paru-paru yang menyebabkan perubahan marfologi yang teruk di permukaan sel tersebut. Beberapa sel endotelium dan epitelium membentuk

bleb pada tapakan perlekatan. Bakteria juga kelihatan di dalam vakuol bermembran sel-sel tersebut.

*P. multocida* berupaya menyerang sel epitelium dan endotelium dalam kajian *in vivo* dan *in vitro* tersebut di atas. Dengan ini adalah dipercayai *P. multocida* berjaya memasuki ke dalam salur darah rongga nasum, trakea, paru-paru dan pelbagai organ lain yang menyebabkan septisemia. Pembiakan bakteria kepada bilangan yang amat besar dan pengkoloniannya pada organ-organ tersebut berakhir dengan pembentukan lesi di beberapa organ yang berakhir dengan kematian haiwan disebabkan oleh kegagalan organ penting.

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I certify that an Examination Committee has met on -14 december----------2007 to conduct the final examination of Amna Elamin Mohamed on her Doctor of Philosophy thesis entitled "Experimental Pathogenesis of Haemorrhagic Septicemia in Organ Culture, Mice and Calves" 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 was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Doctor of Philosophy. The members of Supervisory Committee were as follows:

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Date: 3 March 2008

DECLARATION

I declare that the thesis is my original work except for quotation and citation which have been duly acknowledged. I also declare that it has not been previously and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institution

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**AMNA ELAMIN**

Date: 20 February 2008

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