THE PATHOLOGY OF *SALMONELLA POTSDAM* INFECTION IN YOLK MEMBRANE AND EMBRYONATED CHICKEN EGGS

By

ZURINA BINTI RAMLI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirement for the Degree of Master of Veterinary Science

August 2005

Dedicated to,

My beloved Husband (Fahmy) and My Mom,

For your loves, support and trust,

My daughter (Nur Farahiyah Zakirah),

My son (Mohammad Zul Fikri)

Mother and father in-law (Mama & Baba),

Sisters & brothers (Zubaidah, Hawa, Zalekha, Razali, Razak, Abdul, Waheeda,

Aza , Salmi, Hisham, Azmi & Rosdi)

Thanks for everything...

Abstract of thesis presented to the Senate of Univesiti Putra Malaysia in fulfillment of the requirement for the degree of Master of Veterinary Science

THE PATHOLOGY OF *SALMONELLA POTSDAM* INFECTION IN YOLK MEMBRANE AND EMBRYONATED CHICKEN EGGS

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Chairman : Associate Professor Jasni Sabri, PhD

Faculty : Veterinary Medicine

Salmonellosis is a common and economically important disease in animal such as in poultry and a public health hazard to human. The aetiological agent involved was a S*almonella species*. A new *Salmonella serovar* isolated in Malaysia, *Salmonella potsdam* (Salmonella enterica subsp. enterica serovar Potsdam) was isolated from a case of yolk sac infection in a young ostrich and believed to be pathogenic in young poultry chick and could be zoonotic to human. This *salmonella serovar* was inoculated into day-old chicks and embryonated chicken eggs, to study its pathogenicity and the pathological effects on yolk membrane. Day-old chicks were inoculated with *S. potsdam* using different routes, namely intraperitoneal(Group 1), oral (Group 2), subcutaneous (Group 3) and navel (Group 4). The mortality was

100%, 30%, 70% and 0% in each group respectively. Death occurred 2 days post-inoculation in Group 1, 8-11 days post-inoculation in Group 2 and 2-6 days post-inoculation in Group 3. The rapid death especially in Group 1 and the pathological changes in chicks which died shortly following inoculation with this pathogenic bacteria were considered to be indicative of shock and attributed to the action of endotoxin. The affected yolk membrane was mild to severely congested. The results from the bacteriological tests showed pure growth of this bacteria from the organs sampled, especially in Group 1. Most organs sampled from chicks that were euthanised after 14 days post-inoculation, also revealed pure growth of *S. potsdam.* There was neither clinical sign nor mortality in chicks of group 4 which remained healthy like those in the control group. There was no bacteria isolated from chicks infected via the navel swab, which indicated that yolk sac infection by *S. potsdam* cannot occur from completely healed navel.

Embryonated chicken eggs were infected with *S.potsdam* using different routes, namely through yolk sac (Group 1) and shell swab (Group 2). *Salmonella potsdam* appeared to be highly pathogenic to chick embryos in Group 1, with 100% mortality and 80% mortality in Group 2. The entire affected embryo in Group 1 showed congested yolk sac. *Salmonella potsdam* was recovered from all the embryos that died during the observation periods. Hatched chicks in Group 2 revealed pure growth of *S. potsdam* isolated from vital organs which cultured on Brilliant Green agar

after placing in selenite broth indicative that these chicks could be carrier chicks.

Salmonella potsdam was observed in the lesion of the yolk membrane. Its isolation from characteristic yolk membrane lesion indicated that it was associated with yolk sac infection. Immunohistochemistry observations validated the relationship between lesion seen in yolk membrane and *S. potsdam*. Immunoperoxidase staining technique facilitated detection and localisation of *S. potsdam* for light microscopy. Specific reactions were labeled in the yolk membrane. *Salmonella potsdam* antigen was observed in endodermal cells, around and in the blood vessels of the yolk membrane. This is the first report where the immunoperoxidase staining technique has been used in the study of yolk membrane lesions in day old chicks and embryonated chicken eggs.

This study has revealed the pathogenesis of *S. potsdam* in chicks and embryonated chicken eggs.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk Ijazah
Master Sains Veterinar

PATOLOGI *Salmonella potsdam* YANG MENGJANGKITI PADA MEMBRAN YOLK DAN TELUR AYAM BEREMBRIO

Oleh

ZURINA BINTI RAMLI

Ogos 2005

Pengerusi : Profesor Madya Jasni Sabri, PhD

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Salmonellosis merupakan sejenis penyakit yang biasa berlaku dan penting dari segi ekonomi pada haiwan seperti ayam dan memudaratkan pada manusia dari segi kesihatan umum. Etiologi yang terlibat ialah *Salmonella species*. Sejenis *Salmonella serovar* yang baru diisolat di Malaysia, iaitu *Salmonella potsdam* (Salmonella enterica subsp. enterica serovar Potsdam) yang diisolat daripada membran yolk anak burung unta di Universiti Putra Malaysia (UPM) dan dipercayai patogenik pada anak ayam dan zoonosis pada manusia. *Salmonella serovar* ini telah dikaji pada anak ayam berumur 1 hari dan telur ayam berembrio untuk menjelaskan kepatogenan dan patologi pada membran yolk. Anak ayam dijangkiti dengan *S. potsdam* melalui cara yang berbeza iaitu intra peritoneum (kumpulan 1), mulut (kumpulan 2), di bawah kulit (kumpulan 3) dan pada pusat (kumpulan 4). Pada setiap kumpulan, kadar kematian iaitu 100%, 30%, 70% dan 0% telah berlaku.

Kematian berlaku selepas 2 hari dijangkiti pada kumpulan 1, di antara 8-11 hari pada kumpulan 2 dan di antara 2-6 hari pada kumpulan 3. Kematian yang cepat terutama pada kumpulan 1 dan perubahan patologi pada anak ayam selepas dijangkiti oleh bacteria yang patogenik ini dipercayai disebabkan oleh kejutan dan kesan endotoxin. Membran yolk menunjukkan kongesi dari sedikit sehingga sangat teruk. Keputusan daripada ujian isolat bakteria menunjukkan hanya bakteria ini sahaja yang diisolat daripada sampel organ terutama dari kumpulan 1. Anak ayam yang telah dimatikan selepas 14 hari dijangkiti, juga menunjukkan hanya *S. potsdam* sahaja yang diisolat. Pada kumpulan 4, tiada tanda-tanda klinikal dan kematian di mana anak-anak ayam ini kelihatan sihat seperti anak ayam pada kumpulan kawalan. Tiada bakteria yang diisolat pada kumpulan ini dan ini menunjukkan jangkitan membran yolk tidak boleh berlaku pada pusat ayam yang baik sepenuhnya.

Eksperimen pada kumpulan telur ayam berembrio yang dijangkiti dengan *S. potsdam* telah dikaji melalui cara inokulasi yang berbeza iaitu melalui kantung yolk (kumpulan 1) dan sapuan pada kulit telur (kumpulan 2). Jangkitan oleh *Salmonella potsdam* menunjukkan kesan yang sangat patogenik pada embrio anak ayam dimana bakteria ini telah membunuh kesemua embrio dengan 100% kematian pada kumpulan 1 dan 80 % kematian pada kumpulan 2. Pada keseluruhannya, embrio yang telah dijangkiti dengan *Salmonella potsdam* menunjukkan kongesi pada kantung

yolk. *Salmonella potsdam* telah diisolat pada semua embrio ini sepanjang tempoh eksperimen. Anak ayam yang telah menetas dari kumpulan 2 ini menunjukkan hanya *S. potsdam* sahaja yang diisolat dari media agar brilliant green selepas dipencilkan dalam media selenite. Ini menunjukkan anak ayam ini boleh menjadi pembawa jangkitan bakteria ini.

Salmonella potsdam dapat dilihat pada lesi membran yolk. Pemencilan bakteia ini daripada lesi membran yolk menunjukan bahawa lesi ini berkaitan dengan kehadiran bakteria ini. Pemeriksaan secara imunohistokimia menyokong kejadian lesi membran yolk berkaitan dengan kehadiran bakteria *S. potsdam*. Pewarnaan "immunoperoxidase" telah digunakan untuk mengesan dan memcari lokasi *S. potsdam* menggunakan mikroskop cahaya. Satu reaksi yang spesifik dilabel di membran yolk. Antigen *S. potsdam* yang dilabel dapat dilihat dalam ruang sel endoderm, di sekeliling dan di dalam salur darah pada membran yolk. Teknik perlabelan "immunoperoxidase" ini adalah pertama kali digunakan untuk menkaji lesi membran yolk pada anak ayam dan telur ayam berembrio dengan menggunakan mikroskop cahaya.

Kajian ini telah mendedahkan patogenesis *S. potsdam* pada anak ayam dan telur ayam berembrio.

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I certify that an Examination Committee met on 28 June 2005 to conduct the final examination Zurina Binti Ramli on her Master of Veterinary Science thesis entitled "The Pathology of *Salmonella potsdam* Infection in Yolk Membrane and Embryonated Chicken Eggs" 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 follows:

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This thesis is submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the degree of Master of Veterinary Science. 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 Universiti Putra Malaysia or other institutions.

ZURINA BINTI RAMLI

Date: 8 AUG 2005

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