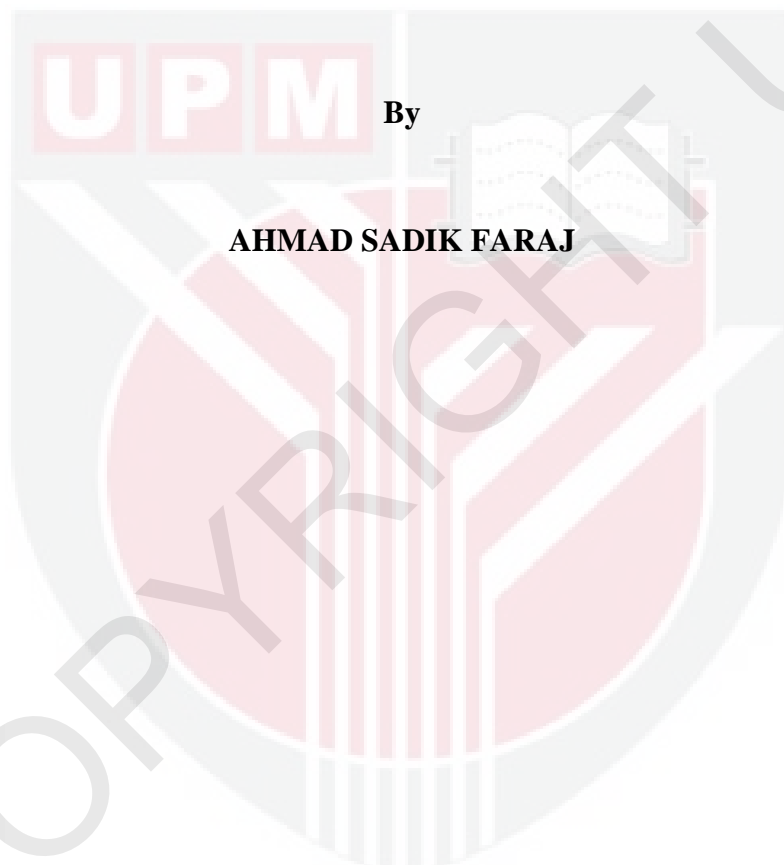


**PATHOGENICITY OF *SALMONELLA* ENTERITIDIS PHAGE TYPE 1
(MALAYSIAN ISOLATE) IN SPECIFIC-PATHOGEN-FREE CHICKENS**



**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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PATHOGENICITY OF *SALMONELLA* ENTERITIDIS PHAGE TYPE 1 (MALAYSIAN ISOLATE) IN SPECIFIC-PATHOGEN-FREE CHICKENS

By

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Salmonella enteritidis (SE) is one of the major concerns to the poultry industry throughout years. SE causes food poisoning due to consumption of poultry or poultry products contaminated by the bacteria. Many SE phage types have been prevailed around the world especially phage types 4, 8, 13a and 23 and few others with less isolation rates. The SE phage type 1 has increased in incidence to become one of the major phage types isolated from Europe, Asia and few other countries. It was the objective of this study to isolate and characterise the phage type of SE isolated from commercial poultry farm in Malaysia and to determine its pathogenicity in one-day-old and 21-day-old specific-pathogen-free (SPF) chickens, upon oral inoculation.

White Leghorn SPF one-day-old and 21-day-old chicks were divided into three groups namely the SE, Control and Mortality groups. The chicks in the SE and Mortality groups were inoculated orally with SE phage type (PT) 1 at a concentration of 1×10^8 cfu/mL. The chicks in SE and Control groups were sacrificed at 6 and 12 hrs post

inoculation (pi) and at 1, 2, 3, 5, 7, 10, 14 and 21 days pi. The chicks were observed for the clinical signs, body weight gain and gross lesions. Tissue samples were collected for bacteriological isolation, histopathological and ultrastructural analysis.

The study in one-day-old chicks showed weight loss in the SE group starting from day 2 pi and until the end of the trial reaching its peak at day 21 pi when the SE group weight was 39% less compared to the Control group. Diarrhoea, pasty vents, inappetance and stunted chicks were recorded starting at 12 hrs pi and until the end of the trial throughout the trial. No mortality was recorded in the Mortality group. However two chicks from the SE group died at days 3 and 5 pi. Grossly the chicks exhibited mild air sacculitis, peritonitis and mild congestion of the liver and kidney starting at day 2 pi until the end of the trial. No abnormal gross changes or clinical sign were recorded in the Control group throughout the trial. The bacteria were isolated in the SE group as early as 6 hrs pi from all organs which include the liver, spleen and cloacal swabs (66%), caecum and caecal tonsils (100%) and small intestine content and blood (33%). At day 14 pi, SE was isolated only from the liver, spleen and caecal tonsils (33%). Histopathological observed were enteritis in the duodenum, jejunum, ileum and caecum and necrosis of the enterocytes at the apical part of the intestinal villi with the presence of necrotic debris and bacterial clusters in the lumen starting at 12 hr pi until the end of the trial. Hepatitis with coagulative necrotic foci was recorded starting at 3 days pi until the end of the trial. The splenic tissue showed an increased heterophilic infiltration and mild congestion with few focal necrotic areas at 2 days pi until the end of the trial. While in the bursa of Fabricius congestion, heterophilic infiltration and necrosis of the lymphoid follicles were recorded starting day 5 pi until the end of the trial. The villi

height showed a significant ($p < 0.05$) decreased in the SE group when compared to the Control group starting day 1 pi until the end of the trial in the duodenum and jejunum, while in the ileum it started at 6 hrs pi until the end of the trial. However, crypts depth measurement remained unchanged throughout the trial. The bacterial engulfment by macrophages and penetration to the villi wall through areas of necrotized microvilli surface was recorded starting 6 hrs pi and until the end of the trial under SEM in the SE group.

The study in 21-day-old chickens showed no mortality in all groups. However the body weight was constantly reduced in the SE group starting at day 7 pi until the end of the trial, reaching a peak at day 14 pi when the body weight in SE group measured 26% lower than the Control group. Diarrhoea was recorded in the SE group starting from day 2 pi until day 7 pi. Grossly mild air sacculitis, peritonitis and mild congestion of liver were observed from day 2 pi to day 5 pi. The bacteria was first isolated from the caecum (66%) at 12 hrs pi and remained positive until day 3 pi (33%) where they were also isolated from the liver (33%), blood (33%), spleen (33%) and small intestine content (33%). In addition, SE were also isolated from the spleen (33%) and caecal tonsils (66%) at days 1 and 2 pi, respectively. In contrast, SE were not isolated from the cloacal swabs throughout the trial. Histological examination revealed hepatitis with coagulative necrotic foci in the liver at day 2 pi and until the end of the trial. Enteritis with necrosis and sloughing of the enterocytes in the apical part of the duodenum, jejunum, ileum and caecal villi was observed starting at day 1 pi and until the end of the trial. Increased heterophilic infiltration and congestion were observed in the spleen at day 1 pi and until the end of the trial. Congestion, heterophilic infiltration and areas of focal coagulative

necrosis in the lymphoid follicles were observed in the bursa of Fabricius starting at day 3 pi until the end of the trial. The intestinal villi in the duodenum in the SE group was lower than those of the Control group throughout the trial, while in the jejunum it was lower in the SE group starting at day 2 pi until the end of the trial, and in the ileum starting at day 14 pi and until the end of the trial. Intestinal crypts depth remained unchanged in the SE group when compared to the Control group. The bacteria were seen to be engulfed by the macrophages in the caecal tonsils and also making their way into the enterocytes at the villi surface through the necrotized and sloughed areas of the microvilli starting at day 1 pi and until the end of the trial.

It was concluded that the pathogenicity of SE PT 1 isolate of Malaysia is a mild to moderate one-day-old and mild in and 21-day-old SPF chickens. The SE isolate was able to cause systemic infection, body weight loss and possibly transmitting the infection from chicken to chicken.

Key words: Salmonellosis, *Salmonella enteritidis*, phage type 1, pathogenicity, SPF chicks.

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

PATOGENISITI SALMONELLA ENTERITIDIS FAG JENIS 1 (MALAYSIAN ISOLAT) PADA AYAM BEBAS PATOGEN KHUSUS

Oleh

AHMAD S FARAG

Oktober 2010

Pengerusi: Profesor Mohd Hair Bejo, PhD

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Salmonella enteritidis (SE) telah mendapat perhatian utama dalam industri unggas selama ini. SE menyebabkan kes keracunan makanan disebabkan oleh memakan daging ayam atau produk ayam yang tercemar oleh bakteria ini. Banyak jenis fag (PT) SE telah berleluasa di seluruh dunia termasuk PT 4, 8, 13a dan 23 dan beberapa PT lain dengan kadar isolasi yang kurang. Insiden SE PT 1 telah meningkat kebelakangan ini dan menjadi salah satu daripada PT yang utama di Eropah, Asia dan negara lain. Tujuan kajian ini adalah untuk mengasing dan mencari PT SE yang diperolehi dari ladang unggas komersial di Malaysia dan menentukan patogenisitinya pada ayam bebas patogen khusus (SPF) berumur satu hari dan 21 hari.

Ayam SPF White Leghorn berumur satu hari dan 21 hari dibahagikan kepada tiga kumpulan iaitu kumpulan SE, kawalan dan kumpulan kematian. Anak ayam kumpulan SE dan kematian diinokulasi melalui mulut dengan SE PT 1 dalam kepekatan 1×10^8 cfu/mL. Anak ayam dalam kumpulan SE dan kumpulan kawalan dikorbankan pada 6 dan 12 jam pasca inokulasi (pi) dan pada 1, 2, 3, 5, 7, 10, 14 dan 21 hari pi. Anak ayam

diperhatikan untuk perubahan berat badan, gejala klinikal dan, lesi mata kasar dan sampel diambil untuk isolasi bakteria, perubahan histopatologi dan ultrastruktur.

Kajian pada anak ayam kumpulan SE berumur satu hari menunjukkan penurunan berat badan bermula dari 2 hari pi dan kemudian mencapai puncaknya pada 21 hari pi di mana penurunan berat badan adalah 39% bila dibandingkan dengan kumpulan kawalan. Cirit birit, kekurangan nafsu makan dan bantut direkodkan bermula dari 12 jam pi dan selanjutnya di sepanjang kajian walaupun kematian tidak tercatat dalam kumpulan kematian. Namun satu anak ayam dari kumpulan SE mati (6%) pada 3 dan 5 hari pi. Anak ayam mempamerkan lesi mata kasar seperti airsakulitis, peritonitis dan kongesi yang ringan pada hati dan ginjal mulai 2 hari pi dan selanjutnya. Kumpulan kawalan tidak menunjukkan perubahan luarbiasa atau tanda klinikal di sepanjang kajian. Bakteria diasingkan dalam kumpulan SE seawal 6 jam pi dari pada semua organ termasuk hati, limpa dan swab kloaka (66%), sekum dan tonsil sekum (100%) dan kandungan usus kecil dan darah (33%). Pada 14 hari pi, SE diasingkan hanya dari pada hati, limpa dan tonsil sekum (33%). Perubahan histopatologi termasuk enteritis pada duodenum, jejunum, ileum dan usus dan nekrosis pada enterosit pada bahagian apikal vili usus dengan kehadiran nekrotik sel dan kluster bakteria dalam lumen bermula 12 jam pi dan seterusnya. Hepatitis dengan fokus nekrotik koagulasi bermula pada 3 hari pi dan seterusnya. Tisu limpa menunjukkan peningkatan selular dan kongesi ringan dengan beberapa tumpuan nekrosis pada 2 hari pi dan seterusnya. Sementara di bursa Fabricius, kongesi, infiltrasi sel inflamasi dan nekrosis pada folikel limfoid dicatat bermula pada 5 hari pi dan seterusnya. Ketinggian vili menunjukkan penurunan yang jelas dalam kumpulan SE bila dibandingkan dengan kandungan kawalan bermula dari 1 hari pi dan

kemudian di dudenum dan jejunum sedangkan di ileum ia bermula 6 jam pi dan seterusnya. Namun pengukuran kript tetap tidak berubah sepanjang kajian. Penelanan bakteri oleh makrophage dan penembusan ke dinding mikrovili melalui kawasan permukaan mikrovili yang mati pada kumpulan SE dicatatkan bermula 6 jam pi dan seterusnya dipamerkan di bawah SEM.

Kajian pada anak ayam berumur 21 hari tidak menunjukkan kematian pada semua kumpulan, namun kumpulan SE menunjukkan penurunan berat badan mulai hari ke-7 dan selanjutnya, dan mencapai kemuncaknya pada 14 hari pi apabila berat badan kumpulan SE adalah 26% lebih rendah berbanding kawalan kumpulan. Kumpulan SE menunjukkan cirit birit bermula 2 hari pi sehingga 7 hari pi. Lesi seperti peradangan pada beg udara dan selaput perut, dan kongesi ringan hati diperhati dari 2 hari pi hingga 5 hari pi. Bakteria pertama kali diasingkan dari sekum (66%) pada 12 jam pi dan tetap positif sampai hari ke 3 pi (33%) di mana ia juga diasingkan dari hati (33%), darah (33%), limpa (33%) dan isi usus kecil (33%) pada 3 hari pi. Selain itu, SE juga diasingkan dari limpa (33%) dan sekal tonsil (66%) masing-masing pada hari 1 dan 2 pi. Sebaliknya, SE tidak dapat diasingkan dari swab kloaka di sepanjang kajian. Pemeriksaan histologi menunjukkan hepatitis dengan fokus nekrotik koagulasi dalam hati pada 2 hari pi dan seterusnya. Enteritis dengan nekrosis pada sel villi di bahagian apikal dudenum, jejunum, ileum dan sekum diperhatikan mulai 1 hari pi dan seterusnya. Peningkatan sel dan kongesi didapati pada limpa pada 1 hari pi dan seterusnya. Kongesi, sel-sel inflamasi dan nekrosis dalam folikel limfoid diperhati dalam bursa Fabricius bermula dari 3 hari pi dan seterusnya. Ketinggian dudenum vili untuk kumpulan SE adalah lebih rendah berbanding dengan kumpulan kawalan di sepanjang kajian

sementara pada jejunum vili kumpulan SE menjadi lebih rendah bermula 2 hari pi dan seterusnya, dan pada ileum ia bermula dari 14 hari pi dan seterusnya. Kedalaman kript usus tetap tidak berubah dalam kumpulan SE jika dibandingkan dengan kumpulan kawalan. Bakteria ditelan oleh makrophage di tonsil sekum dan memasuki enterosit di permukaan mikrovili melalui mikrovili yang mati dan mereput bermula 1 hari pi dan seterusnya.

Kesimpulannya, patogenisis SE isolat PT 1 dari Malaysia adalah ringan sehingga sederhana keatas anak ayam SPF berumur 1 hari dan ringan keatas anak ayam SPF berumur 21 hari. Isolat SE boleh menyebabkan jangkitan sistemik, keturunan berat badan dan kemungkinan penyeberan jangkitan daripada ayam ke ayam dan manusia.

Kata kunci: Salmonellosis, *Salmonella enteritidis*, PT 1, patogenisiti, ayam SPF.

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I certify that a Thesis Examination Committee has met on 19 October 2010 to conduct the final examination of Ahmad S Faraj on his thesis entitled “Pathogenicity of Salmonella enteritidis Phage Type 1 (Malaysian Isolate) in Specific-Pathogen-Free Chickens” in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Veterinary Science.

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DECLARATION

I declare that the thesis is my original work except for quotations and citations 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 other institutions.



AHMAD SADIK FARAJ

Date: 19th October 2010

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