UNIVERSITI PUTRA MALAYSIA

PATHOGENICITY OF SALMONELLA TYPHIMURIUM AND SALMONELLA STANLEY ISOLATES IN CHICKENS

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PATHOGENICITY OF *SALMONELLA* TYPHIMURIUM AND *SALMONELLA* STANLEY ISOLATES IN CHICKENS

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It is hereby certified that I have read this project paper entitled "Pathogenicity of *Salmonella* Typhimurium and *Salmonella* Stanley Isolates in Chickens", by BalqisbintiRazali and in my opinion, it is satisfactory in terms of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 - Project.

_____

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ABBREVIATIONS

cfu	colony forming unit
pi	post inoculation
ELISA	Enzyme-linked immunoabsorbent assay
HE	Haematoxylin and Eosin
BPW	buffered peptone water
RV	Rappaport-Vassiliadis
XLD	xylose lysine deoxycholate agar
TSA	trypticase soy agar
TSI	triple sugar iron agar

ABSTRAK

Abstrak daripada kertas projek yang dikemukakan kepada Fakulti PerubatanVeterinar, Universiti Putra Malaysia untuk memenuhi sebahagian daripada keperluan kursus VPD4999 – Projek.

PATOGENISITI *SALMONELLA* TYPHIMURIUM DAN *SALMONELLA* STANLEY ISOLAT DALAM AYAM

oleh

BalqisbintiRazali

Mac 2018

Penyelia: ProfesorDr. Mohd Hair bin Bejo

Objektif kajian ini adalah untuk menentukan patogenisiti isolat*Salmonella* dalam ayam. Tujuh puluh enam anak ayam dibahagikan kepada tiga kumpulan iaitu A, B dan C. Ayam dari kumpulan A (24 ekor) diinokulasi dengan *S.* Typhimurium dan ayam dari kumpulan B (24 ekor) diinokulasi dengan *S.* Stanley dengan 0.1x108 cfu melalui intraperitoneal. Ayam kumpulan C (28 ekor) kekal tidak diinokulasi dan dijadikan kumpulan kawalan. Lapan ekor ayam daripada setiap kumpulan diletakkan dalam kumpulan kematian. Sebelum inokulasi, 4 ekor anak ayam dari kumpulan kawalan dikorbankan. Pada hari 1, 4, 7, dan 14 selepas inokulasi (pi) 4 ekor anak ayam dari setiap kumpulan dikorbankan sebagai pensampelan. Sebelum necropsi, berat badan dan sampel darah diambil untuk pengesanan *Salmonella* antibody dengan teknik ELISA. Lesi mata kasar direkodkan semasa necropsi. Sampel hati, limpa, tonsil usus dan swab kloaka diambil untuk pemencilan *Salmonella.* Sampel hati diawet dalam 10% bufer

formalin untukpemeriksaanhistologi. Kajian menunjukkan berat badan meningkat dalam semua kumpulan sepanjang ujian. Perbezaan ketara ($p < 0.05$) dalam berat badan antara kumpulan pada hari 1 pi namun tidak terdapat perbezaan yang signifikan dalam berat badan ($p > 0.05$) pada hari 4, 7 dan 14 pi antara kumpulan. Tanda klinikal dilihat dari anak ayam dari kumpulan A pada hari 8 pi adalah kematian secara tiba-tiba manakala anak ayam dari kumpulan B pada hari 6 pi adalah lemah yang mati pada hari berikutnya. Namun, kumpulan C tidak menunjukkan sebarang tandaklinikal. Kadar kematian bagi kumpulan A dan kumpulan B adalah 12.5% setiap satu dan tiada kematian untuk kumpulan C. Nekropsi mendedahkan splenomegali pada anak ayam dalam kumpulan B pada hari 4, 7 dan 14 pi. Akan tetapi, tiada lesi mata kasar dilihat dalam kumpulan lain dan anak ayam yang mati. Lesi histologi pada hati menunjukkan tiada penemuan penting dalam semua kumpulan. *Salmonella*dipencilkan dari kumpulan A, pada hari 1 pi dari limpa (25%) dan hari 7 pi dari hati (25%), tetapi tidak pada hari 4 pi dan 14 pi. Manakala, untuk kumpulan B pada hari 1 pi daripada hati (25%) dan tonsil usus(25%); hari 4 pi dari hati (50%), tonsil usus (25%) dan juga limpa (25%); hari 7 pi dari hati (50%), limpa (50%), tonsil usus (50%), swab kloaka (50%); tetapi hari 14 pi tiada. Namun, tiada pemencilan dari kumpulan C. *Salmonella* dipencilkan dari hati, limpa, tonsil usus dan swab kloaka dari anak ayam mati dari kumpulan A. Sementara itu kumpulan B dari hati, limpa dan tonsil usus.Titer antibodi *Salmonella*menurun dalam semua kumpulan sepanjang ujian. Tiada perbezaan yang signifikan ($p > 0.05$) dalam titer antibodi antara kumpulan pada hari 1 pi dan 7 pi. Walau bagaimanapun, perbezaan yang signifikan ($p < 0.05$) dilihat pada hari 4 pi dan 14 pi. Kesimpulannya, *S.* Typhimurium dan*S.* Stanley isolatyang digunakan dalam

kajian ini adalah patogenik kepada ayam kerana ia menyebabkan kematian dan lesi mata kasar, dan dapat diasingkan dari organ.*Salmonella* Stanley isolat lebih patogenik pada ayam berbanding *S.* Typhimurium.

Kata kunci: *Salmonella* Stanley, *Salmonella* Typhimurium, ayam, patogenisiti

ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine, Universiti Putra Malaysia in partial fulfilment of the course VPD 4999 – Project.

PATHOGENICITY OF *SALMONELLA* TYPHIMURIUM AND *SALMONELLA* STANLEY ISOLATES IN CHICKENS

by

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Supervisor: Professor Dr.Mohd Hair bin Bejo

The objectives of this study were to determine pathogenicity of *Salmonella* isolates in chickens and to isolate the agent from the organs. Seventy-six-day-old chicks were divided into three groups namely; group A, inoculated with *S.* Typhimurium (24 chicks); group B, inoculated with *S.* Stanley (24 chicks); and group C, was left uninoculated, and acted as the control group (28 chicks). The chicks in groups A and B were inoculated intraperitoneally with 0.1 ml of $1x10^8$ colony forming unit (cfu) of *S.* Typhimuriumand *S.* Stanley, respectively at day old. Eight chicks from all groups were separated and monitored for mortality. Chicks were provided with feed and water *ad libitum* throughout the trial, and monitored for abnormal clinical signs and mortality at least twice daily. Prior to bacterial inoculation, four chicks from group C were sacrificed. On days 1, 4, 7 and 14 post inoculation (pi), four chicks were sacrificed from each group. Body weights and blood samples were collected for detection of *Salmonella* antibody using ELISA technique prior necropsy. On necropsy, gross lesions were recorded and samples of liver were collected and fixed in 10% buffered formalin for histological examination. Samples of liver, spleen,

caecal tonsils and cloacal swabs were collected for bacterial isolation and identification. The study showed that body weight of chickens in all groups increased throughout the trials. There was significant difference ($p<0.05$) in body weight between groups on day 1 pi, although no significant difference in body weight ($p>0.05$) on days 4, 7 and 14 pi between groups. Clinical signs seen from a chick in group A on day 8 pi was sudden death meanwhile a chick from group B on day 6 pi was weakness that was found dead on the next day. However, group C showed no abnormal clinical signs throughout the trial. Mortality rate for groups A and B were 12.5% each and none for group C. Necropsy revealed splenomegaly on chicks in group B on days 4, 7 and 14 pi. Nevertheless, no lesion was seen in other groups and the dead chicks. Histology of liver revealed no significant findings in all groups. *Salmonella* wasisolated from group A, on day 1 pifrom spleen (25%) and on day 7 pi from liver (25%), but none on days 4 and 14 pi. However, for group B on day 1 pi *Salmonella* was isolated from liver (25%) and caecal tonsil (25%); on day 4 pi from liver (50%), caecal tonsil (25%) and also spleen (25%); on day 7 pi from liver (50%), spleen (50%), caecal tonsil (50%), and cloacal swab (50%); but day 14 pi nil. *Salmonella* was not isolated from group C. *Salmonella* isolation from dead chicks from group A was from liver, spleen, caecal tonsil and cloacal swab meanwhile, group B was from liver, spleen and caecal tonsil. *Salmonella* antibody titre declined in all groups throughout the trials. There was no significant difference ($p>0.05$) in antibody titre between groups on days 1 and 7 pi. However, significant difference ($p<0.05$) was seen on days 4 and 14 pi. In conclusion, *S.* Typhimurium and*S.* Stanley isolates usedin the study were pathogenic to chickens as it caused death and gross lesion, and were able to be isolated from the organs.

*Salmonella*Stanley isolate is more pathogenic in chickens when compared to *S.* Typhimurium.

Keywords: *Salmonella* Typhimurium, *Salmonella* Stanley, commercial broiler chicken, pathogenicity

1.0 INTRODUCTION

1.1 Background of the Study

Salmonella Typhimurium and *Salmonella* Stanley are also known as *S. enterica* subspecies*enterica* serovar Typhimurium and *S. enterica* subspecies*enterica* serovar Stanley, respectively. It is gram negative rods of the Enterobacteriaceae family. *Salmonella* consist of two major species which are *S. enterica* and *S. bongori*(WHO, 2017). *Salmonellaenterica* is further divided to six subspeciescomprise of over 2500 serovar(OIE, 2008).

Common cause of human foodborne disease is *S. enterica* with variety of animals being identified as reservoirs (Hendriksen et al., 2012). *Salmonella* Typhimurium can be found in a large number of different animals as reservoir without specific source, whereas duck is the only reservoir where *S.* Stanley is found in high frequency (Bangtrakulnonth et al., 2004). Besides, the potential risk for exposure to *Salmonella* via contaminated foodrises with growth in consumption of meat and poultry product (Foley et al., 2011).

Geographical distribution is a factor contributing to variation of *Salmonella*serovar between country. In developed countries, *S.* Typhimurium is second most common cause of human salmonellosis (Hendriksen et al., 2012), and its importance is rising in Southeast Asia (Bangtrakulnonth et al., 2004). Bangtrakulnonth et al. (2004) also added that in 1995, *S.* Stanley infections were among the 15 most common serovars in 12 out of 104 countries. A recent study in Thailand showed that from 2002 to 2007, the second most common serovar causing human salmonellosis cases is due to

*S.*Stanley. In 2008, *S.* Stanley was among the most prevalent cause of human Salmonellosis in a few other countries (Hendriksen et al., 2012).

Salmonella Typhimuriumoften presence in poultry asymptomatically (Bjerrum et al., 2003) either subclinical infection or as a healthy carrier (Antunes et al., 2016) although Al-Abadi & Al-Mayah (2013) found clinical signs shown by infected chickens such as depression, anorexia, huddling together and mild to severe diarrhoea with mortality rate of 10% and lesions of enlargement of liver and spleen and enteritis. However, not much is known regarding *S.* Stanley infection in poultry.

Salmonellosisis suggested to simply transmit to humans through poultry meat through healthy animals as the disease is not detected earlier during processing (Antunes et al., 2016). As less is known about *Salmonella*infection in poultry in Malaysia, thus a study is needed to learn more about *Salmonella* infection for the prevention and control of the infection in chicken farms and prevention of the agent to enter the food chain, and transmission to human.

1.2 Hypotheses

The hypotheses of the study were:

1. H_0**:** There is no significant difference of pathogenicity and *Salmonella* antibody titre between *S.* Typhimurium and *S.* Stanley inoculated chickens and the control group

H_A**:** There is significant difference of pathogenicity and *Salmonella* antibody titre between *S.* Typhimurium and *S.* Stanley inoculated chickens and the control group

2. H_0: There is no significant difference in the bacteria isolation in organs of chickens between *S.* Typhimurium and *S.* Stanley inoculated chickens and the control group

   H_A: There is significant difference in the bacteria isolation in organs of chickens between *S.* Typhimurium and *S.* Stanley inoculated chickens and the control group

1.3 Objectives

The objectives of this study were:

1. to determine the clinical signs and mortality percentage of chickens inoculated either with *S.* Typhimuriumor *S.* Stanleyisolate
2. to determine the gross and histological lesions, and *Salmonella* antibody titre of chickens inoculated with *S.* Typhimurium or *S.* Stanley isolate
3. to isolate and identify *S.* Typhimurium or *S.* Stanley in organs of the chickens

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