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EFFICACY OF INACTIVATED AVIAN PATHOGENIC ESCHERICHIA COLI AGAINST THE BACTERIAL INFECTIONS IN BROILER CHICKENS

WENDY YONG WAI KHENG

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EFFICACY OF INACTIVATED AVIAN PATHOGENIC *ESCHERICHIA COLI* AGAINST THE BACTERIAL INFECTIONS IN BROILER CHICKENS

WENDY YONG WAI KHENG

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It is hereby certified that we have read this project paper entitled “Efficacy of Inactivated Avian Pathogenic *Escherichia coli* against the Bacterial Infections in Broiler Chickens”, by Wendy Yong Wai Kheng and in our opinion it is satisfactory in terms of scope, quality and presentation as partial fulfillment of requirement for the course VPD 4999-Final Year Project.

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Professor Dr. Mohd Hair Bejo
DVM (UPM), PhD (Liverpool),
Dean,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Supervisor)

_____

Assoc. Professor Dr. Zunita Zakaria
Bachelor of Science (UM),M.Sc. (UPM), PhD (UPM),
Deputy Dean,
Faculty of Veterinary Medicine
Universiti Putra Malaysia
(Co-Supervisor)

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Abstrak daripada kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar,Universiti Putra Malaysia untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek

KEBERKESANAN *ESCHERICHIA COLI* PATOGENIK AVIAN YANG TIDAK AKTIF TERHADAP JANGKITAN BAKTERIA DALAM AYAM PEDAGING

Oleh

Wendy Yong Wai Kheng

2016

Penyelia: Profesor Dr. Mohd Hair Bejo

Penyelia Bersama: Profesor Madya Dr. ZunitaZakaria

Escherichia coli Patogenik Avian (APEC) menyebabkan colibacillosis dalam ayam dan ia menunjukkan pelbagai keadaan klinikal seperti colisepticemia dan selulitis. Tujuan kajian ini adalah untuk mengenalpastikankeberkesanan APEC yang tidak aktif sama ada sebagai tunggal atau kombinasi gen berkaitan virulen (VAG) 5 dan 6 terhadap jangkitan bakteria dalam ayam pedaging. Lapan puluh empat ekor ayam berumur satu hari dibahagi sama kepada tujuh kumpulan. Pada umur satu hari, setiap ayam dalam kumpulan 1 dan 4 telah diberi suntikan bawah kulit dengan APEC yang tidak aktif dengan VAG 5. Kumpulan 2 dan 5 dengan VAG 6, dan kumpulan 3 dan 6 dengan gabungan VAG 5 dan VAG 6. Kumpulan 7 tidak diberi suntikan kerana ia adalah kumpulan kawalan. Pada umur 14 hari, boster telah diberikan kepada kumpulan 4, 5 dan 6. Pada umur 28 hari, ayam dalam setiap kumpulan telah dibahagikan kepada kumpulan dicabar dan tidak dicabar. Kumpulan dicabar

telah diinokulasi (0.1 mL) dengan VAG 6 (10^8cfu/mL) sama ada melalui intramuskular atau laluan intranasal. Pada hari ke 35, semua ayam dikorbankan. Hati dan limpa diambil untuk mengenalpastikan kehadiran bakteria, manakala hati dan trakea untuk histopatologi. Kajian menunjukkan dua ayam dari kumpulan kawalan terbantut pertumbuhan, dehidrasi dan kurang selera makan selepas dicabar dengan suntikan intramuskular, dan menunjukkan lesi kasar perihepatitis dan pericarditis. *E. coli* dapat diasingkan hanya daripada satu sampel dari kumpulan yang sama. Histologi menunjukkan hepatitis teruk dengan hepatosit nekrosis dan degenerasi yang teruk, dan tracheitis dengan nekrosis dan degenerasi yang teruk. Sebaliknya, hepatitis ringan dengan hepatosit nekrosis dan degenerasi ringan, dan tracheitis dengan nekrosis dan degenerasi ringan telah direkod dalam kumpulan ayam tanpa tanda klinikal.Berdasarkan tanda klinikal, lesi dan hasil pengasingan bakteria, APEC tidak aktif sama ada sebagai tunggal atau kombinasi dengan VAG 5 dan 6 memberikan perlindungan yang lebih baik terhadap jangkitan bakteria di dalam ayam pedaging berbanding dengan kumpulan tidak diinokulasi. Kesimpulannya, APEC tidak aktif sama ada sebagai tunggal atau kombinasi dengan VAG 5 dan 6 adalah berkesan dan mampu memberikan perlindungan dicabar dengan VAG 6.

Kata kunci: *Escherichia coli* patogenik avian (APEC), tidak aktif, gen berkaitan virulen (VAG), ayam pedaging

ABSTRACT

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

EFFICACY OF INACTIVATED AVIAN PATHOGENIC *ESCHERICHIA COLI* AGAINST THE BACTERIAL INFECTIONS IN BROILER CHICKENS

By

Wendy Yong Wai Kheng

2016

Supervisor: Professor Dr. Mohd Hair Bejo
Co-supervisor: Assoc. Professor Dr. ZunitaZakaria

Avian Pathogenic *Escherichia coli* (APEC) causes colibacillosis in poultry, which it manifests various clinical conditions such as colisepticemia and cellulitis. This objective of this study was to determine efficacy of inactivated APEC either as single or combination with virulent associated gene (VAG) 5 and 6 against the bacterial infection in broiler chickens. Eighty-four day-old broiler chickens were equally divided into seven groups. On day 1, groups 1 and 4 were inoculated subcutaneously with inactivated APEC with VAG 5. Groups 2 and 5 with VAG 6 and groups 3 and 6 with combination of VAG 5 and VAG 6. Group 7 was not inoculated as it was acted as control group. On day 14, booster was given to groups 4, 5 and 6. On day 28, the chickens in each group were divided into the challenge and non-challenge groups. Chickens in the challenge groups were inoculated (0.1 mL) with VAG6 (10^8 cfu/mL) either via intramuscular or intranasal routes. On day 35, all the chickens were sacrificed. The livers and spleens were collected for bacterial identification, while livers and trachea for

histopathology. The study showed that two chickens from the control group were stunted growth, dehydrated and inappetence after challenged intramuscularly, and showed gross lesions of perihepatitis and pericarditis.*E. coli* was isolated from only one sample of the same group.Histologically, severe hepatitis with severe necrosis and degeneration of the hepatocytes, and severe tracheitis with severe necrosis and degeneration were recorded. In contrast, mild lesions of hepatitis with mild necrosis and degeneration of hepatocytes, and mild tracheitis with mild necrosis and degeneration were recorded in groups of chickens without clinical signs.Based on the clinical signs, lesions and bacterial culture, the inactivated APEC either as single or combination with VAG 5 and 6 gives better protection against the bacterial infection in broiler chickens compared to non-inoculated group. In conclusion, inactivated APEC either as single or combination with VAG 5 and 6 was effective and could provide protection against VAG 6 challenged.

Keywords: Avian Pathogenic *Escherichia coli* (APEC), inactivated, virulent associated gene (VAG), broiler chickens

1.0 INTRODUCTION

Avian Pathogenic *Escherichia coli* (APEC) belongs to the extraintestinal pathogenic group of *E. coli* (ExPEC), and it is the etiologic agent of colibacillosis. Colibacillosis, includes multiple extra–intestinal diseases often respiratory, leading to localized or systemic infection such as colisepticaemia, coligranuloma, chronic respiratory disease, coliform cellulitis, peritonitis, salphingitis, panophthalmitis, yolk sac infection and enteritis (Barnes*et al*., 2008).Localized or systemic infections depending on the strain of *E. coli*, age and the gender of the chicken, as well as the immunologic status and the presence of predisposing environmental conditions (Dizva & Stevens, 2008). Colibacillosis is therefore responsible for significant economic losses to the poultry industry due to decrease in growth rate and egg production, condemned in carcasses, mortality and cost of treatment and production.

According to Moriel *et al.* (2010), avian colibacillosis is difficult to control because of two major issues which are limited reliable methods to identify the causative strains of *E. coli* and lack of effective vaccine. These factors are due to variable characteristics of APEC strains preventing the identification of common properties, which could be the basis for diagnostic methods and vaccination. Diagnostic methods such as serotyping and genotyping are used to identify APEC strains. Although serotyping remains the most frequently used method in laboratories, it only identifies limited number of APEC strains (Schouler *et al*.,

2012). While virulence genotyping is to detect genes encoding virulence factors by using PCR amplification.

In the last decade, there is a growing concern of the use of antimicrobial drugs in veterinary medicine especially in the food production animals. Excessive and prolonged usage of antimicrobial drugs may compromise human health if resistant bacteria develop in food production animals are transferred to humans via food chain or the environment (Vuuren, 2003). Therefore, instead of using antimicrobial drugs, bacterial infection can be controlled by using a different approach which is vaccine. One of the earliest attempts to control colibacillosis by vaccination was by Gross (1957), where he used formalin inactivated O2:K1 and O78:K80 serotypes of *E. coli* as vaccines (Gross, 1957). Overall, the inactivated vaccines provided protection against homologous challenge only. Several factors were shown to play a role in determining the efficacy of an inactivated APEC vaccine namely the serogroup or serotype of the *E. coli* included in the vaccine, the type of adjuvant used, the method used to inactivate the bacteria, the route and frequency of administration, and the age of the chickens at the time of vaccine administration (Ghunaim *et al*., 2014).

Hence, the hypothesis of this study were inactivated APEC could provide protection against APEC challenge. Inactivated APEC combination of VAG 5 and VAG 6 could provide better protection against APEC challenge when compared to the single VAG 5 or VAG 6. Booster of inactivated APEC either with VAG 5, 6

or combination of VAG 5 and 6 could provide protection against APEC challenge infection when compared to non-booster group.

The objective of this study wasto determine the efficacy of inactivated APEC either as single or combination of APEC with VAG 5 and VAG6 against the bacterial infection in broiler chickens.

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