



UNIVERSITI PUTRA MALAYSIA

***EFFICACY OF INACTIVATED PASTEURELLA MULTOCIDA
AGAINST THE BACTERIAL INFECTIONS IN BROILER CHICKENS***

KOH SIEN LING

FPV 2016 19

**EFFICACY OF INACTIVATED *PASTEURELLA MULTOCIDA*
AGAINST THE BACTERIAL INFECTIONS IN BROILER CHICKENS**

KOH SIEN LING

A project paper submitted to the
Faculty of Veterinary Medicine, Universiti Putra Malaysia
in partial fulfillment of the requirement for the
DEGREE OF DOCTOR OF VETERINARY MEDICINE
Universiti Putra Malaysia
Serdang, Selangor Darul Ehsan.

MARCH 2016

It is hereby certified that I have read this project paper entitled “Efficacy of Inactivated *Pasteurella multocida* Against the Bacterial Infections in Broiler Chickens” by Koh Sien Ling and my opinion it is satisfactory in terms of scope, quality and presentation as partially fulfillment of the requirement for the course VPD 4999-Project

Professor Dr. Mohd Hair Bin Bejo

D.V.M. (UPM), Ph.D (Liverpool, UK),

Professor
Faculty of Veterinary Medicine
University Putra Malaysia
(Supervisor)

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my supervisor, Prof Dr Mohd Hair Bejo for his vast knowledge, invaluable guidance and support throughout this project.

I would also express my sincere appreciation to all the serology and bacteriology laboratory staffs; Mr Indra, Mr Saipul, Mrs Mardia, Miss Krishnammah, Mr. Azri, Dr Sabri for help in completing my project.

I am also thankful to my project partner's Wendy Yong for her help, cooperation and continue support throughout this project. I would like to thank my family members who have contributed toward completion of my project.

CONTENTS

TITLE	PAGE
CERTIFICATION	i
ACKNOWLEDGEMENTS	ii
CONTENTS	iii
LIST OF FIGURES	v
ABSTRAK	vi
ABSTRACT	viii
1.0 INTRODUCTION	1
2.0 LITERATURE REVIEW	
2.1 Aetiology agent	3
2.2 Epidemiology	3
2.3 Clinical signs	4
2.4 Gross pathology	5
2.5 Pathogenesis	6
2.6 Diagnosis	7
2.7 Control and prevention	8

3.0	MATERIALS AND METHODS	
3.1	<i>Pasteurella multocida</i> isolate	9
3.2	Preparation of polyvalent bacterial vaccine for fowl cholera	9
3.3	Experimental design	11
3.4	Isolation and identification	12
3.5	Histopathology	12
4.0	RESULTS	
4.1	Clinical signs	13
4.2	Necropsy	14
4.3	Bacterial isolation	15
4.4	Histopathology findings	16
5.0	DISCUSSION	20
6.0	CONCLUSION	22
7.0	RECOMMENDATION	22
	REFERENCES	23



LIST OF FIGURES

Figure		Page
1	(a) Severe generalized congestion of the liver and (b) generalized congestion of kidneys of the dead chickens from group 1 at day 2 post challenge via intramuscular route	14
2	(a) Normal liver of the chicken from non challenge group 7 and (b) liver of scarified chickens in group 1 with mild hepatitis and mild degeneration and necrosis of hepatocytes HE. Bar = 20µm.	17
3	Severe congestion and hepatitis with severe necrosis and degeneration of hepatocytes of dead chicken from group 1 challenged via intramuscular route. HE. Bar = 20µm	17
4	(a) Normal lung of the chicken from non challenged group 7 and (b) lung of sacrificed chicken from group 1 which showed mild pneumonia with mild congestion, degeneration and necrosis of parenchyma. HE. Bar = 20µm.	19
5	Severe pneumonia with severe congestion and necrosis of lung parenchyma in dead chicken from group 1 challenged via intramuscular route. HE. Bar = 20µm.	19

ABSTRAK

Abstrak daripada kertras projek yang dikemukakan kepada Fakulti Perubatan Vetrinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999-Projek

KEBERKESANAN *PASTEURELLA MULTOCIDA* TIDAK AKTIF TERHADAP JANGKITAN BAKTERIA DALAM AYAM PEDAGING

Oleh

Koh Sien Ling

2016

Penyelia: Prof. Dr. Mohd Hair Bejo

Pasteurella multocida adalah ejen penyebab penyakit kolera ayam. Ia menyebabkan kerugian ekonomi kepada industri poultri yang dikaitkan dengan morbiditi dan kematian yang tinggi. Objektif kajian ini adalah untuk menentukan keberkesanan *P. multocida* tidak aktif sama ada sebagai tunggal atau kombinasi serogroup A dan D daripada jangkitan bakteria dalam ayam pedaging. Lapan puluh empat ekor anak ayam telah dibahagi sama kepada tujuh kumpulan. Pada umur satu hari, setiap ayam dalam kumpulan 1 dan 4 telah disuntik dengan *P. multocida* tidak aktif serogroup A, kumpulan 2 dan 5 dengan serogroup D dan kumpulan 3 dan 6 dengan gabungan serogroup A dan D. Semua ayam telah disuntik bawah kulit

dengan 0.1mL *P. multocida* tidak aktif berkepekatan 1×10^{11} cfu/mL, kecuali kumpulan 7 sebagai kawalan. Pada umur 14 hari, booster telah diberikan kepada kumpulan 4, 5 dan 6. Pada umur 28 hari, semua ayam telah dibahagikan kepada tiga kumpulan iaitu tiada cabaran dan cabaran samaada laluan intramuskular atau intranasal. Ayam dicabar dengan *P. multocida* serogroup A berkepekatan 1×10^8 cfu/mL. Kajian menunjukkan seekor ayam dari kumpulan 1 dan 7 masing-masing didapati mati pada hari 1 dan 2 selepas cabaran. Pada hari 8 selepas cabaran, semua ayam telah dikorbankan. *P. multocida* diasingkan dari ayam kumpulan 1 dan 7 yang mati. *P. multocida* tidak diasingkan dari semua ayam lain yang dikorbankan pada hari 8 selepas cabaran. Penemuan mata kasar bagi ayam yang mati menunjukkan kongesi yang teruk dalam hati dan buah pinggang. Manakala, tiada penemuan mata kasar bagi ayam yang dikorbankan. Histopatologi untuk ayam yang mati menunjukkan kongesi, nekrosis dan degenerasi yang teruk pada sel hati. Pneumonia, kongesi dan nekrosis yang teruk pada sel peparu juga direkodkan. Sebaliknya, hepatitis yang ringan dengan nekrosis dan degenerasi yang ringan pada sel hati, dan pneumonia yang ringan dengan degenerasi dan nekrosis ringan di sel peparu direkodkan dalam semua kumpulan ayam yang dikorbankan. Kesimpulannya, gabungan *P. multocida* tidak aktif serogroup A dan D boleh memberi perlindungan yang lebih baik terhadap jangkitan *P. multocida* serogroup A berbanding serogroup tunggal A atau D dalam ayam pedaging.

Kata kunci: Keberkesanan, *P. multocida* tidak beraktif, ayam pedaging, *P. multocida* serogroup A dan D

ABSTRACT

An abstract from the project submitted to the Faculty of Veterinary Medicine in partial fulfillment of the requirement for the course VPD4999-Project

EFFICACY OF INACTIVATED *PASTEURELLA MULTOCIDA* AGAINST THE BACTERIAL INFECTIONS IN BROILER CHICKENS

By

Koh Sien Ling

2016

Supervisor: Prof. Dr. Mohd Hair Bejo

Pasteurella multocida is the causative agent of fowl cholera in chickens. It causes economic losses to the poultry industry associated with high morbidity and mortality. The objective of this study was to determine the efficacy of inactivated *P. multocida* either as single or combination of serogroup A and D against the bacterial infection in broiler chickens. Eighty-four, day-old boiler chicken were separated equally into seven groups. On Day 1, chickens from groups 1 and 4 were inoculated with serogroup A, groups 2 and 5 with serogroup D while groups 3 and 6 with combination serogroup A and D. All the chickens were inoculated subcutaneously

with 0.1mL of 1×10^{11} cfu/mL of inactivated *P. multocida*, except group 7 as control group. On Day 14, booster was given to groups 4, 5 and 6. On Day 28, all the chickens were divided into three groups namely non-challenge and challenge either via intramuscular or intranasal route. The study showed that one chicken each from group 1 and 7 dead at day 1 and 2 post challenge, respectively. At day 8 post challenge, all the chickens were scarified. *P. multocida* was isolated from the dead chicken in group 1 and 7. *P. multocida* was not isolated from all the other chickens scarified at day 8 post challenge. Gross lesions for the dead chickens revealed generalized congestion in the liver and kidneys while no significant gross lesion seen in sacrificed chickens. Histopathology findings for the dead chickens revealed severe congestion with severe necrosis and degeneration of hepatocytes. Severe pneumonia and severe congestion and necrosis of lung parenchyma were also recorded. In contrast, mild hepatitis with mild necrosis and degeneration of hepatocytes and mild pneumonia with mild congestion, degeneration and necrosis at the lung parenchyma were recorded in all the scarified chickens in all group. In conclusion, inactivated *P. multocida* combination of serogroup A and D could provide better protection against *P. multocida* serogroup A infection when compared to the single serogroup A or D.

Keyword: Efficacy, inactivated *P. multocida*, broiler chickens, *P. multocida* serogroup A and D

1.0 Introduction

Fowl cholera or known as Avian Pasteurellosis is a contagious bacterial disease of domesticated and wild avian species that caused by *Pasteurella multocida* and is often fatal. Among the bacterial diseases of broiler chickens, fowl cholera accounts for major economic losses to the industry worldwide through death, weight loss and condemnations. It usually appears as septicemic disease associated with high morbidity and mortality, but chronic and benign conditions often occurs (Glisson and Cheng, 1991). It typically occurs as fulminating disease with massive bacteraemia in chickens older than 16 weeks of age. The affected chickens showed clinical signs of fever, anorexia, depression, mucus discharge from mouth, diarrhea, ruffled feathers, drop in egg production, coupled with smaller eggs, increased respiratory rate and cyanosis at the time of death. For control and prevention of this disease, farms need to practice good biosecurity and effective vaccination programme. However, farmers' favourite ways to combat fowl cholera is by using various antibiotics such as sulphonamides, tetracyclines, erythromycin, streptomycin and penicilin which are given as prophylaxis or treatment to the birds (Carter, 1967). Excessive usage of antimicrobial drugs in food producing animals have gain more attention by the public due to issues such as antibiotic resistance and residues which will bring harm to human health. Therefore, fowl cholera should be controlled by using vaccine instead of antimicrobial drugs.

The hypothesis of this study was inactivated *P. multocida* combination of serogroup A and D could provide better protection against *P. multocida* serogroup A infection in chickens when compared to the single serogroup A or D. In addition, booster of inactivated *P. multocida* combination of serogroup A and D could provide better protection against *P. multocida* serogroup A infection when compared to non-booster chickens.

The objective of this study was to determine the efficacy of inactivated *P. multocida* either as single or combination of serogroup A and D against the bacterial infection in broiler chickens.

REFERENCES

- Anun, M. (2001). Molecular characterization and pathogenicity of *Pasteurella multocida* isolated in chickens with fowl cholera. MVS Thesis, University Putra Malaysia.
- Bierer, B.W and Derieux, W.T. (1972). Immunologic response of turkeys to avirulent *Pasteurella multocida* vaccine in drinking water. *Poult Sci.* 51:408-416.
- Bojesen, A.M., Petersen, K.D., Nielsen O.L., Christensen, J.P. and Bisgaard, M. (2004). *Pasteurella multocida* infection in heterophile depleted chickens. *Avian Disease*, 48:463-470.
- Carter, G.R (1967). Pasteurellosis: *Pasteurella multocida* and *Pasteurella hemolytica*. *Adv.Vet.Sci.*, 11:321-379.
- Carter, G.R (1995). Studies on *Pasteurella multocida*. I.A haemagglutination test for the identification of serological types. *Am.J.Vet.Res.*, 16:481-484.
- Christensen, J.P., Dietz, H.H. and Bisgaard, M. (1998). Phenotypic and genotypic characters of isolates of *Pasteurella multocida* obtained from back-yard poultry and from two outbreaks of avian cholera in avifauna in Denmark. *Avian Pathology*, 27:373-381.

- Chung, J.Y., Wilkie, I., Boyce, J.D and Adler, B. (2001). Role of capsule in the pathogenesis of fowl cholera caused by *Pasteurella multocida* serogroup A. *Infect Immun.*, 69:2487-2492.
- Charitha, D., Harshavardan, P. and Kumar, J.K (2012). Isolation and partial characterization of *Pasteurella multocida* from poultry farm around Tirupati. *J.of Microbiology and Biotechnology Research*, 2(3):393-395.
- Dick, J.W. and Johnson, J.W. (1985). Fowl cholera immunity in broiler breeder chickens determined by the enzyme-linked immunoabsorbent assay. *Avian Disease*, 29(3):706-714.
- Dziva, F., Muhairwa, A., Bisgaard, M. and Christensen H. (2007). Diagnostic and typing options for investigating diseases associated with *Pasteurella multocida*. *Veterinary Microbiology*, 128:1-66.
- Fadly, A.M., Glisson, J.R., McDougald, L.R., Nolan, L.K. and Swayne, D.E. (2008). Pasteurellosis and other respiratory bacterial infections. In: Disease of Poultry. Saif. Y.M. 12th ed., Blackwell Publishing Professional., Iowa. Pp. 739-753.
- Fegan, N., Blackall, P.J. and Pahoof, J.L. (1995). Phenotypic characterization of *Pasteurella multocida* isolates from Australia poultry. *Vet Microbiol.*, 47: 281-286.
- Fujihara, Y., Onai, M., Koizumi, S., Satoh, N. and Sawada, T. (1985). An outbreak of fowl cholera in wild ducks in Japan. *Jpn.J.Vet.Sci.*, 48(1):35-43.

- Glisson, J.R., and Cheng H.N. (1991). In vivo antigen expressing by *Pasteurella multocida*. *Avian Disease*, 35:392-396.
- Heddleston, K.L. (1962). Studies on pasteurellosis. V. Two immunogenic types of *Pasteurella multocida* associated with fowl cholera. *Avian Disease*, 6:315-321.
- Hofacre, C.L., and Glisson, J.R. (1986). A serotypic survey of *Pasteurella multocida* isolated from poultry. *Avian Disease*, 30: 632-633.
- Hunter, B. and Wobester, G. (1980). Pathology of experimental avian cholera in mallard duck. *Avian Disease*, 24:403-414.
- Ilieve, T.R., Arsov, E., Iovcey E. and Girginov, G. (1963). Role of swine in the epidemiology of fowl cholera. *Nauchni Tr. Vissh. Vet. Med. Inst. Sofia.*, 11:289-293.
- Kubatzky, K.F. (2012). *Pasteurella multocida* and immune cells., *Microbiology and immunology*, 361:53-72.
- Moore, M.K., Cubs, L.C. and Gates, R.J. (1993). A new selective enrichment procedure for isolating *Pasteurella multocida* from avian and environmental samples. *Avian Disease*, 38:317-324.
- Mulks, S.R., Williamston, T., and Thacker, .B. (1997). Methods for producing a bacterial vaccine and novel vaccines produced thereby. *United State Patent:* 005688682.

- Olson, L.D., McCune, E.L., and Moseley, B.L. (1966). Gross and histopathological description of the cranial form of chronic fowl cholera in turkeys. *Avian Disease*, 10(4): 518-529.
- Powel, P.C. (1987). Immune mechanisms in disease of poultry. *Veterinary Immunology and Immunopathology*, 15:87-113.
- Peters, S.R. (2002). The art of embedding tissue for frozen section. Part I: A system for precision dice down cryoembedding of tissue using freezing temperature. *J. of Histopathology*, 26(1): 1-19.
- Rhoades, K.R. (1964). The microscopic lesions of acute fowl cholera in mature chickens. *Avian Disease*, 8:658-665.
- Rimler, R.B. and Rhoades, K.R. (1987). Serogroups F, a new capsule serogroup of *Pasteurella multocida*. *J. Clin. Microbiol.*, 25:615-618.
- Samina, Mohammad, Islam and Rahman (2013). Isolation and identification of *Pasteurella multocida* from chicken for the preparation of oil adjuvanted vaccine. *Microbes and Health*, 2(1):1-4
- Sood, S. and Verma, P.C. (2006). Pathology of *Pasteurella multocida* infection in chickens.
- Tamura, S. and Kurata, T. (2004). Defences mechanisms against influenza virus infection in the respiratory tract muca. *Jpn. J. Infect. Dis.*, 57:236-247.

Tsuji, M. and Matsumoto, M. (1989). Pathogenesis of fowl cholera: Influences of encapsulation on the fate of *Pasteurella multocida* after intravenous inoculation in turkeys. *Avian Disease*, 33: 238-247.

