UNIVERSITI PUTRA MALAYSIA

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

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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
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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

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ABSTRAK

Abstrak daripada kertras projek yang dikemukan 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

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0.1mL *P. multocida* tidak aktif berkepekatan $1X10^{11}$ cfu/mL, kecuali kumpulan 7 sebagai kawalan. Pada umur 14 hari, boster 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 $1X10^{8}$ 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

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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 $1X10^{11}$

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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 reveled generalized congestion in the liver and kidneys while no significant gross lesion seen in sacrificed chickens. Histopathology findings for the dead chickens reveled 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

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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.

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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.

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2.0 Literature Review

2.1 Aetiology agent

Pasteurella multocida is the causative agent of fowl cholera. It is gram-negative, non motile, non-spore-forming, fermentative, facultatively anaerobic coccobacilli or rods organism that belongs to genus pasteurella of the family Pasteurellaceae (Carter, 1967). It is easily isolated, often in pure culture from organs such as liver, lung, spleen and bone marrow.

Pasteurella multocida strains are classified into serogroups (A, B, D, E and F) based on capsule antigens and further classified into 16 serotypes (1–16) based primarily on lipopolysaccharide antigens using the Heddleston scheme (Carter, 1995; Heddleston, 1962). The majority of acute fowl cholera cases are caused by serogroup A strains of *P. multocida*. Moreover, fowl cholera, which is generally caused by serotypes A:1, A:3 or A:4, is a severe systemic disease which occurs in domestic poultry and wild birds (Glisson and Cheng, 1991).

2.2 Epidemiology

Fowl cholera is a contagious avian bacterial disease that all types of birds are susceptible including chickens, turkeys, ducks, geese and wild birds. All ages are susceptible to fowl cholera but more often seen in mature chickens than young chickens (Heddleston, 1962). Chickens less than 16 weeks of age are more resistance. Outside the body of host, the

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organisms are very susceptible to drying and other disinfectants. Besides, this disease usually occurs when there is adverse interaction between the host, agent and environment.

This disease is to be transmitted from the carrier birds in the flock which previously exposed or recovered from the fowl cholera outbreak (Kasten *et al.,* 1997). The immune status of the host may be associated with protection against the strain of organism with which they have previously had contact. Source of infection include carrier birds, clinically diseased poultry and their excretions and carcasses of birds which have died of the infection. Air-borne infection does occur between pens but spread via water and feed troughs is of more significance. Transmission occurs most likely due to density and distribution of bacteria and birds, virulence of the bacteria agent, interaction and contact among birds, susceptibility and ecological interaction that favor the persistence of bacteria growth in the environment. Disease tends to recur on the same site. Poultry may be infected by oral, nasal, conjunctiva and cutaneous wounds (Hughes and Pritchett, 1930).

2.3 Clinical signs

Fowl cholera is a serious disease of poultry that can present in either acute or chronic forms. Acute stages present for only few hours before death while chronic stages results from low virulent of the agent and from the extension of acute infections (Fadly *et al.,* 2008). Obvious clinical signs of acute fowl cholera may not occur until very late in the infection and these include depression, ruffled feathers, fever, anorexia, mucous discharge from mouth, diarrhea and an increased respiratory rate. In the less acute stage, oedema of

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the lungs, pneumonia and perihepatitis are seen. Chronic lesions include caseous arthritis of the hock and foot joints, swelling with induration of one or both wattles and caseous exudates in the middle ear. In the terminal stages of fowl cholera, death is probably caused by massive bacteraemia and endotoxic shock (Carter, 1967).

2.4 Gross pathology

Lesions of fowl cholera are not constant but vary in type and severity. The greatest variation is related to the course of the disease, whether acute or chronic (Carter, 1967).

When the course of the disease is acute, most postmortem lesions are associated with vascular disturbances. These include general passive hyperemia and congestion throughout the carcass, accompanied by enlargement of the liver and spleen. Petechial and ecchymotic hemorrhages are common, particularly in subepicardial and subserosal locations. General hyperemia usually occurs, is most evident in veins of the abdominal viscera and in small vessels of the duodenal mucosa. Livers of acutely affected birds may be swollen and usually contain multiple small focal areas of coagulative necrosis and heterophilic infiltration. Large amounts of viscid mucus may be observed in the digestive tract, particularly in the pharynx, crop and intestine.

Chronic fowl cholera is characterized by localized infections, in contrast to the septicemic nature of the acute disease (Cater, 1967). They often occur in the respiratory tract. Pneumonia is especially common lesion in birds. Infections of the conjunctiva and

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adjacent tissues occur, and facial edema may be observed. Chronic localized infections can involve the middle ear and cranial bones and have been reported to result in torticollis. Caseous arthritis and productive inflammation of the peritoneal cavity and the oviduct are common in chronic infections. A fibrinonecrotic dermatitis that includes caudal parts of the dorsum, abdomen, and breast and involves the cutis, subcutis, and underlying muscle has been observed in turkeys and broilers. Sequestered necrotic lung lesions in poultry should always raise suspicion of cholera (Olson *et al*., 1966).

2.5 Pathogenesis

Disease occurs when there is adverse interaction between agent, environment and host result in development of lesions, clinical signs and cause death in the animal. In the case of fowl cholera, the agent enters the animals through tissues of the respiratory tract but invasion of agents into bloodstream from the lungs are however poorly understood (Bojesan *et al.,* 2004). Basically, fowl cholera is a septicemic infection where bacteria can be isolated in large amount from blood of late infected birds. Death of the chickens may be result of massive bacteraemia and endotoxic shock (Rimler and Rhoades, 1987). Once the agent in the host, innate immune system namely phagocytes and complement play their role as host defense. However, *P.multocida* has mechanism that can evade the immune system which is the presence of capsule (Boyce *et al.,* 2002).

2.6 Diagnosis

Conventional detection methods for *P. multocida* are based on bacteria isolation and identification using blood agar, suspicious clones by biochemical and serological tests. These methods are time consuming and require at least 72 hours in obtaining positive or negative results. Blood agar is non selective medium for *Pasteurella* isolation which it produce non hemolytic growth. The samples that can be collected for isolation of fowl cholera are liver, spleen, kidney, lung from newly dead birds (Christensen *et al.,* 1998).

In triple sugar iron agar, contain three sugars namely dextrose, lactose and sucrose, phenol red for detecting carbohydrate fermentation and ferrous ammonium sulphate for detection of hydrogen sulphide production indicated by blackening in the butt of the tube. If the medium in the butt of the tube becomes yellow (acidic), but the medium in the slant becomes red (alkaline), the organism being tested only ferments dextrose (glucose). Gas production is indicated by splitting and crackling of the medium.

Additional biochemical tests for *Pasteurella* sp are ODC, trehalose, mannitol, d-sorbitol and dulcitol. All of these tests should give positive result except dulcitol will be negative if *Pasteurella multocida ss multocida* is being tested (Christensen *et al.,* 1998).

The morphology of colonies was described as gray in colour, non-haemolytic, non mucoid with typical sweetish smell of indole. Moreover, characteristics bipolar staining features

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under microscope were frequently observed in Wright's stain smears of fresh samples (Heddleston *et al*., 1962).

2.7 Control and prevention

Prevention of fowl cholera can be done by elimination of reservoir or source of *P.multocida* in the poultry flock. Good management practice and good sanitation with strict biosecurity in the farm can be the best way to prevent fowl cholera outbreak.

During outbreak, flock infected should be quarantined and eliminated as soon as economically feasible. Besides, all the housing and equipments must be disinfected before re-population.

Last but not least, vaccination plays an important role in combating fowl cholera in prevalent and endemic areas. Commercially produced bacterins and live vaccine are available in the market. Bacterins usually contain whole cells of serotypes 1, 3 and 4 emulsified in oil adjuvant. Live vaccine can also be used to vaccinate chickens as it stimulates immune responses and thus provide protection against multiple serotypes (Bierer, 1972). However, study shows vaccine can cause chronic fowl cholera. Therefore, bacterin is suggested to be used at 10-12 weeks and live vaccine at 18-20 weeks to minimize live vaccine induced chronic fowl cholera (Hofacre *et al.,* 1986).

3.0 MATERIALS AND METHODS

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3.1 *Pasteurella multocida* isolate

Pasteurella multocida serogroup A isolate used was UPM 1231 isolate while serogroup D was UPM 1387 isolate.

3.2 Preparation of polyvalent bacterial vaccine for fowl cholera

3.2.1 Isolation and preparation of UPM 1231 and UPM 1387 inoculums

Stock culture for both serogroups were streak onto fresh blood agar plates and kept in the incubator overnight at 37°C. Biochemical tests were performed to confirm the identity of the bacterial colonies on the blood agar plates. Then, a single colony of each bacteria of interest from the blood agar plate was transferred into fresh brain heart infusion medium and incubated overnight in the shaker rotating at 200 rpm at 37°C (Mulks *et al*., 1997).

3.2.2 Inactivation and emulsification of isolated bacterial inoculums

The bacterial inoculums (1mL) and 9mL of brain heart infusion medium (BHI) were transferred into microcentrifuge tube. Next, serial dilution was performed by adding BHI

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and the turbidity of the dilution was compared with McFarland standard. The dilution that had the same turbidity with McFarland standard was chosen. Then, dilution factor was recorded and calculation was done to get the bacterial inoculums' concentration. Concentration of $1X10^8$ cfu/mL serogroup A was used for challenge, while concentration of $1X10^{11}$ cfu/mL was used for inoculation of serogroup A and D.

For inactivation, 2.5% formalin was added into the bacteria inoculums. The mixtures were mixed by vortexing and kept in the incubator at 37°C for 2 hours. Next, 100 µl of the mixtures was spread on fresh blood agar to confirm complete inactivation if there was no growth (Samina *et al*., 2013).

Finally, aluminum potassium sulphate alum and the inactivated bacterial innoculum were combined at the ratio of 1:10. The mixture was emulsified with vortex until the both innoculum and adjuvant mixed properly. The bacterial innoculum were combined at 1:1 ratio for the combined serogroup A and D innoculum.

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3.3 Experimental design

Eighty-four, day-old boiler chicken were separated equally into seven groups, namely groups 1 and 4 inoculated with inactivated serogroup A, groups 2 and 5 with serogroup

D, groups 3 and 6 with combination of serogroup A and D and group 7 as control. All the chickens from group 1 to 6 were inoculated subcutaneously with 0.1mL of $1X10^{11}$ cfu/mL of inactivated *P. multocida* on day 1. Booster was given to groups 4, 5 and 6 on day 14. The chicks were kept in battery cages with wire mesh floors. Feed and water were given *ad libitum*.

For challenge, the bacterial innoculum used was *P. multocida* serogroup A with concentration of $1X10^{8}$ cfu/mL. Prior to challenge, all the chickens were divided into three groups, namely non-challenge and challenged via intramuscular or intranasal route. It consists of four birds from each group 1 to 7 and 0.1mL of the bacterial innoculum was challenged accordingly. All the chickens were observed twice daily for any clinical signs and mortality.

At Day 8 post challenged, all the chickens were sacrificed. On necropsy, gross lesions were observed and specimens of liver and lung were collected for *P. multocida* isolation and identification and histopathology examination.

The use of chickens in the study was approved on 21 December 2015 by Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia with reference number UPM/IACUC/FYP.2015/FPV.008.

3.4 Isolation and identification

Bacterial isolation and identification were done on the samples of lung and liver. Fresh lung and liver samples were streaked onto blood agar and incubated overnight at 37°C.

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Isolates were subculture onto blood agar and grown overnight at 37 °C to harvest pure colony. Those colonies were also stained using Gram stain by looking for bipolar staining suggestive evident of *P.multocida*. Biochemical test using TSI (+ve), SIM (+ve), urease (-ve), citrate (-ve), oxidase (+ve), catalase (+ve), ODC (+ve), trehalose (-ve), mannitol (+ve), sorbitol (+ve) and dulcitol (-ve) for *P.multocida* identification was done for confirmation (Charitha *et al.,* 2012)

3.5 Histopathology

Samples of liver and lung were fixed in 10% buffered formalin for at least 24 hours. Samples were then cut, placed into cassettes and tissue processing was done. The samples were embedded with wax and tissue trimming was performed. The samples were sectioned at 4µm. The sections were floated into purified water at 45°C and picked up on glass slides, drained upright, dried using a slide warmer at 58°C for a minimum of 30 minutes and allowed to cool down for 10 minutes to improve adhesion. Staining by using hematoxylin and eosin (HE) methods was done as the final step before observed under microscope (Peters, 2002).

4.0 RESULTS

4.1 Clinical signs

4.1.1 Non-challenge group

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No significant clinical sign was observed among the chickens from all groups 1 to 7. All the chickens were healthy, with good appetite and water intake and normal body condition throughout the trial.

4.1.2 Challenged group-intramuscular route

On Day 1 post challenge, one chicken from group 1(inoculated with inactivated serogroup A) was died. On Day 2 post challenge, one chicken from the group 7 found dead.

4.1.3 Challenged group-intranasal route

No significant clinical signs observed among the chickens of groups 1 to 7. All the chickens were healthy, with good appetite and water intake and normal body condition throughout the trial.

4.2 Necropsy

4.2.1 Non-challenge group

No significant finding was observed from the chickens.

4.2.2 Challenged group-intramuscular route

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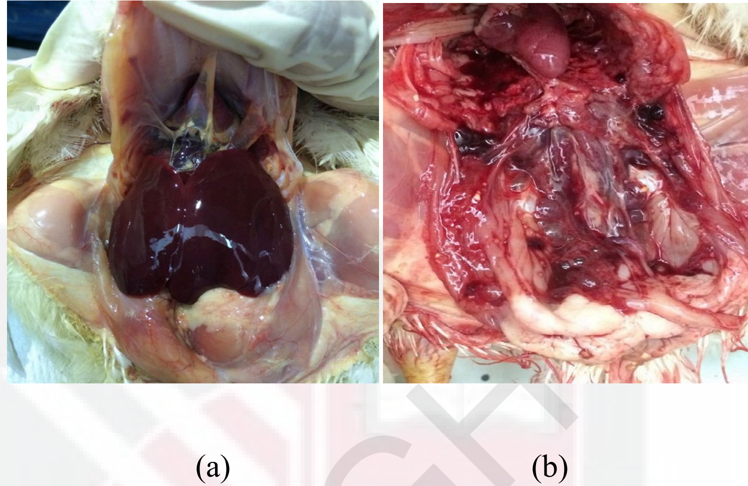
On necropsy, severe generalized congestion of liver, lungs and kidneys were recorded (Figure, 1).



(a) (b)

Figure 1: (a) Severe generalized congestion of liver and (b) generalized congestion of kidneys of the dead chickens from group 1 at day 1 post challenge via intramuscular route.

4.2.3 Challenged group-intranasal route

No significant finding was observed from the chickens.

4.3 Bacterial Isolation

4.3.1 Non-challenge group

P. multocida was not isolated from this group of chickens.

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4.3.2 Challenged group-intramuscular route

P. multocida was isolated from liver and lung of dead chickens in groups 1 and 7.

4.3.3 Challenged group-intranasal route

P. multocida was not isolated from this group of chickens.

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4.4 Histopathology findings

4.4.1 Liver Histology

Non-challenge group

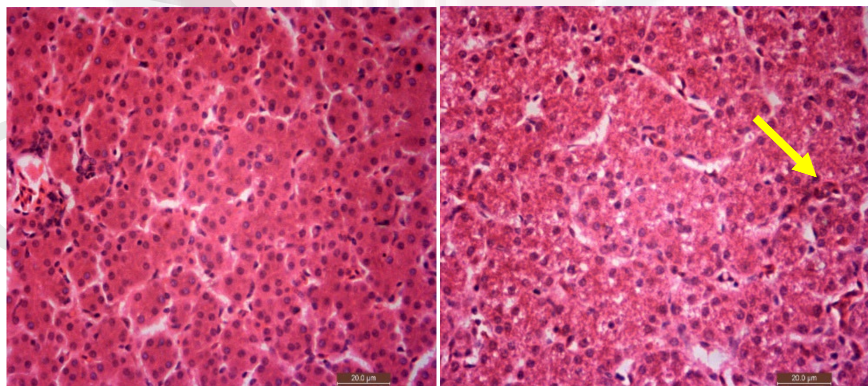
Normal to mild hepatitis with mild degeneration and necrosis of hepatocytes were recorded (Figure, 2a).

Challenged group-intramuscular route

Normal to mild hepatitis with mild degeneration and necrosis of hepatocytes were recorded in sacrificed chickens (Figure, 2b). However, the dead chickens showed severe congestion and hepatitis with severe necrosis and degeneration of hepatocytes (Figure, 3).

Challenged group-intranasal route

Normal to mild hepatitis with mild degeneration and necrosis of hepatocytes were recorded.



(a) (b)

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Figure 2: (a) Normal liver of the chicken from non challenges group 7 and (b) liver of sacrificed chickens from group 1 with mild hepatitis and mild degeneration and necrosis of the hepatocytes. HE. Bar = 20µm.

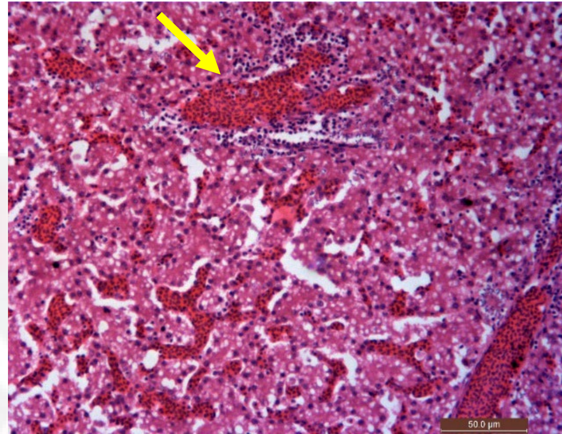

Figure 3: Severe congestion and hepatitis with severe necrosis and degeneration of hepatocytes of dead chicken from group 1 challenged via intramuscular route. HE. Bar = 50µm.

4.4.2 Lung Histology

Non-challenge group

Normal to mild pneumonia with mild congestion, degeneration and necrosis of the lung parenchyma were recorded (Figure, 4a).

Challenged group-intramuscular route

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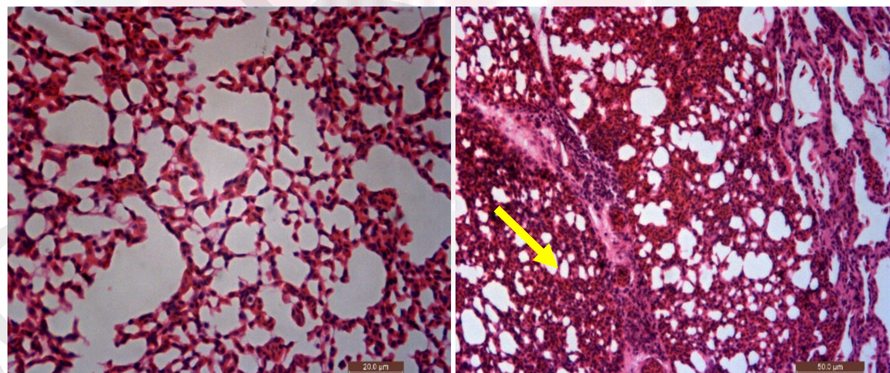
Mild pneumonia with mild congestion, degeneration and necrosis of the lung parenchyma in sacrificed chickens were recorded (Figure, 4b). Dead chickens showed severe pneumonia with severe congestion and necrosis of lung parenchyma (Figure, 5).

Challenged group-intranasal route

Mild pneumonia with mild congestion, degeneration and necrosis of the lung parenchyma were recorded.



(a) (b)

Figure 4 (a): Normal lung of the chicken from non-challenged group 7 and (b) lung of the sacrificed chicken from group 1 which showed mild pneumonia with mild congestion, degeneration and necrosis of the parenchyma. HE. Bar = 20µm.

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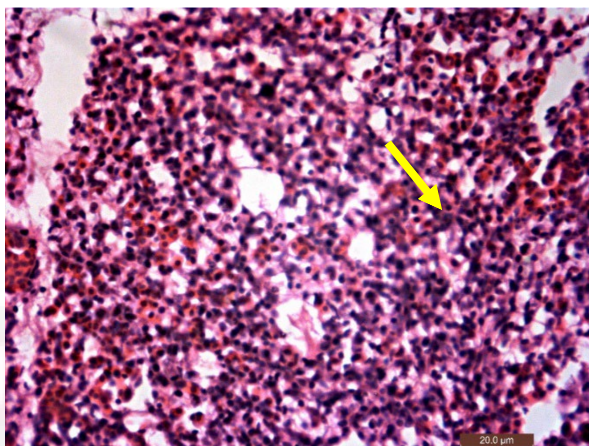

Figure 5: Severe pneumonia with severe congestion and necrosis of lung parenchyma of dead chicken from group 1 challenged via intramuscular route. HE. Bar = 20µm.

5.0 Discussion

The study indicated that vaccination of inactivated *P. multocida* combination of serogroup A and D with booster could provide better protection (100%) against *P. multocida* challenge when compared to single dose (75%) following intramuscular route of *Pasteurella* challenge. It also notice that respiratory organs such as lung and liver were affected which lead to cause of death to the infected chickens as result of *P. multocida* infection. *P. multocida* was not isolated in the lung and liver of all the chickens that scarified after day 8 pi but was isolated in the dead chickens. It suggests that cause of death for the chickens can be due to invasion of *P. multocida* in the organs (Powel, 1987). Moreover, the higher mortality rate in chickens through intramuscular route of challenge

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related to possibilities cause of deaths; results of respiratory failure from pneumonia and congestion of lung or as a result of massive bacteremia and endotoxic shock due to *P. multocida* (Rimler and Rhoades, 1987). All this indicates that systemic invasion of *P. multocida* occurs during 24 hours post inoculation.

Basically, *P. multocida* begins as respiratory problem as indicated by various studies. In this study, chickens were challenged via intranasal and intramuscular route. Most studies generally employ infection routes by passing through the upper respiratory tract and reveled mortality rate ranging from 60%-100%. Meanwhile, in this study no mortality observed in chickens inoculated via intranasal route which previously vaccinated with inactivated *P. multocida* combination of serogroup A and D. This indicates that first defense mechanism of the chickens at mucosal and vaccination seem to prevent invasion of the agent to host.

Mortality was observed through intramuscular route challenge indicate that the organism was rapid enter into blood and once the organism enter, they multiply in liver and spleen before release to the blood stream and spread to the whole body (Tsuji and Matsumoto, 1989). Therefore, severe haemorrhages and congestion observed in all parts of organs as massive bacteremia occurred in dead chickens.

In the case of dead chickens, *P. multocida* was isolated from both lung and liver samples. However, *P. multocida* was no isolated from all the chickens that scarified at day 8 pi. This could be due to role of IgA in elimination if the organism 3 days onward after reinfection (Tamura and Kurata., 2004). In addition, the gross lesions such as generalized

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congestion in liver, lung and spleen were observed in dead chickens while no significant lesion noticed in life chickens indicate systemic spread and toxic effects of the organism have developed bacteremia before causing death to the chickens (Kubatzky, 2012).

The histopathology findings showed there was responses towards the inoculation which indicated by infiltration of inflammatory cells in the liver and lung. There was also degeneration, congestion and necrosis of the lung and liver as the result of *P. multocida* infection.

6.0 Conclusion

It was concluded that vaccination together with booster of inactivated *P. multocida* combination of serogroup A and D provide better protection than single serogroup against fowl cholera infection in broiler chickens.

7.0 Recommendation

Experiment trial could be conducted in specific pathogen free (SPF) chickens in order to prevent the interference of maternal derived antibodies and other infections that can influence the outcomes. Enzyme-linked immunosorbent assay (ELISA) could be conducted to determine the antibodies titre in the chickens.

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