



***FEED- BASED INFECTIOUS BURSAL DISEASE VACCINATION IN
VILLAGE CHICKEN***

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By

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**Thesis Submitted to the School of Graduate Studies, Universiti Putra
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Science**

October 2018

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirement for the degree of Master of Science

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October 2018

Chair : Azhar bin Kasim, PhD
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Infectious bursal disease (IBD), also known as Gumboro disease, is an acute, highly contagious viral infection in chickens that can be exhibited by inflammation and followed by the atrophy of the bursa of Fabricius and immunosuppression. Clinically the disease can be seen only in chickens older than 3 weeks. Vaccination represents a very useful method for IBD controlling. The timing of optimal vaccination, doses used and the administration routes represent the most important factors in controlling the disease. In addition, the immunogenicity and histopathology of chickens can be used to monitor IBD in flocks. However, the studies between certain breed of chicken such as village chicken and IBD vaccine is lacking. The current research present an overview of immunogenicity and histopathology of UPM-bred village chickens vaccinated with IBD vaccine via feed. It was the objective of this study to evaluate and determine the most effective route for IBD vaccination on UPM-bred village chicken.

In the experiment, one hundred and five day-old UPM-bred village chicks were reared in experimental house. The chicks were randomly divided into 3 groups which were; group A (feed based IBD vaccination), group B (IBD vaccination via intraocular route), and group C (control). The chickens in group A and B were vaccinated at day 14 with the IBD vaccine, administered according to the manufacturer's recommendations via feed and intraocular routes (0.1ml/chick). Five chicks in the control group were sacrificed at 1, 7, 14, 21, 28, 35 and 42 days of age. Five chickens each from the groups A and B were sacrificed at 21, 28, 35 and 42 days of age. Body weights were taken and serum samples were collected for IBD antibody detection using enzyme linked immunosorbent assays (ELISA) prior to necropsy. On necropsy, the gross lesions were recorded and the bursa of Fabricius was weighed and fixed in 10% buffered formalin for histopathological examination. The study proved that attenuated live strain IBD vaccine is safe and effective to be used. There were no clinical

signs of IBD recorded throughout the trial in all groups of chickens. There were no significant ($p>0.05$) differences in body weight between the 3 groups. The bursa weight of the chickens in groups A and B were insignificantly ($p>0.05$) different but were significantly ($p<0.05$) lower than the control group at day 21 and above. At the end of the study, it is confirmed that feed based vaccination can induce the protective level of IBD antibody as high as the intraocular route. In addition, feed based IBD vaccination does not affect the weight of the village chickens significantly compared to the intraocular route vaccination.



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Penyakit Bursa Berjangkit (PBB), juga dikenali sebagai penyakit Gumboro, adalah jangkitan virus pada ayam yang boleh dilihat pada keradangan dan diikuti oleh atrofi pada bursa Fabricius dan immunosupresi. Secara klinikal, penyakit ini hanya boleh dilihat pada ayam yang lebih tua daripada 3 minggu. Vaksinasi merupakan kaedah yang sangat berguna dalam mengawal PBB. Masa vaksin yang optimum, dos yang digunakan dan kaedah vaksinasi merupakan faktor yang paling penting dalam mengawal penyakit ini. Di samping itu, imunogenik dan histopatologi ayam boleh digunakan untuk memantau PBB dalam kawanan. Walau bagaimanapun, kajian antara baka ayam tertentu seperti ayam kampung dan vaksin IBD adalah kurang. Penyelidikan semasa membentangkan gambaran keseluruhan imunogenik dan histopatologi ayam kampung baka-UPM dengan vaksin IBD melalui makanan. Objektif kajian ini adalah untuk menilai dan menentukan kaedah yang paling berkesan dalam vaksinasi IBD pada ayam kampung baka-UPM.

Dalam eksperimen ini, seratus lima anak ayam kampung baka-UPM yang berusia satu hari dibela di rumah eksperimen. Anak ayam dibahagikan secara rawak kepada 3 kumpulan iaitu; kumpulan A (vaksin PBB berasaskan makanan), kumpulan B (vaksin PBB melalui kaedah intraokular), dan kumpulan C (kawalan). Ayam-ayam dalam kumpulan A dan B telah divaksin pada hari ke 14 dengan vaksin PBB, melalui makanan, yang diberikan mengikut cadangan pengeluar melalui kaedah makanan dan intraokular (0.1ml/anak ayam). Lima ekor ayam dalam kumpulan kawalan telah disembelih pada umur 1, 7, 14, 21, 28, 35 dan 42 hari. Lima ayam masing-masing dari kumpulan A dan B dikorbankan pada umur 21, 28, 35 dan 42 hari. Berat badan ayam diambil dan sampel serum dikumpulkan untuk pengesanan antibodi PBB menggunakan ujian immunoserapan enzim yang berkaitan (ELISA) sebelum nekropsis dilakukan. Semasa nekropsis, lesi kasar direkodkan dan bursa Fabricius ditimbang dan diletakkan ke dalam formalin tertampun 10% untuk pemeriksaan

histopatologi. Kajian itu membuktikan bahawa vaksin PBB strain hidup yang dilemahkan adalah selamat dan berkesan untuk digunakan. Tiada tanda-tanda klinikal PBB direkodkan sepanjang eksperimen di semua kumpulan ayam. Tiada perbezaan ($p > 0.05$) yang signifikan dalam berat badan ayam antara 3 kumpulan. Berat bursa ayam dalam kumpulan A dan B tidak banyak ($p > 0.05$) berbeza tetapi ketara ($p < 0.05$) lebih rendah daripada kumpulan kawalan pada hari ke 21 dan ke atas. Pada akhir kajian, ia disahkan bahawa vaksinasi berasaskan makanan boleh menyebabkan tahap perlindungan antibodi PBB setinggi vaksinasi kaedah intraokular. Di samping itu, vaksin PBB berasaskan makanan tidak memberikan kesan kepada berat ayam kampung dengan ketara jika dibandingkan dengan vaksinasi kaedah intraokular.



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LIST OF ABBREVIATIONS

| | |
|----------------|--|
| % | Percent |
| ANOVA | Analysis of variance |
| DOSM | Department of Statistics Malaysia |
| EID | Egg infectious dose |
| ELISA | enzyme-linked immunosorbent assay Enzyme Linked Immunosorbent Assay |
| EU | European Union |
| FAO | Food and Agriculture Organisation |
| G | Gauge |
| H&E | Hematoxylin and Eosin |
| IB | Infectious bronchitis |
| IBD | Infectious bursal disease |
| IBDV | Infectious bursal disease virus |
| MD | Marek's disease |
| MDA | Maternal derived antibodies |
| MOA | Ministry of Agriculture |
| MVP | Malaysian Vaccine and Pharmaceutical |
| nm | Nanometre |
| m ² | Metre square |
| ND | Newcastle disease |
| °C | Degree celcius |
| PBB | <i>Penyakit Bursa Berjangkit</i> |
| PBS | Phosphate-buffered saline |
| Ppm | Part per million |

| | |
|--------|---|
| PSA | Poultry Science Association |
| RNA | Ribonucleic acid |
| S | Second |
| SAS | Statistical Analysis System |
| SPF | Specific pathogen-free |
| STC | Standard challenge |
| UK | United Kingdom |
| UPM | Universiti Putra Malaysia |
| US | United States |
| USDA | United States Department of Agriculture |
| vIBD | Virulent infectious bursal disease virus |
| vv | Very virulent |
| vvIBDV | Very virulent infectious bursal disease virus |
| µg | microgram |
| µl | microliter |
| µm | Micrometre |

CHAPTER 1

GENERAL INTRODUCTION

Human population increasing at a linear progression simultaneously with rapid economy growth with effect of urban transformation, increase income and changes in consumer preference have encouraged the increment in animal protein demand (Devendra, 2006). It is estimated more than 50 billion chickens are raised per annum as a source of food, for their meat and eggs. Other than that, in the United States (US), where the world largest economic activities take place is not left out of the drastic increase in the demand for animal protein source. Each year in the US, more than 8 billion chickens are slaughtered annually to compliment the demand for the meat. In the last three decade, Malaysia has experienced a tremendous increase in demand for animal based protein source too, due to increases per capital consumption of the major meat type (Bisant, 2006).

In Malaysia where chicken is the cheapest source of protein available. It is estimated 46.6kg of poultry meat consumption per capita as reported by Department of Statistics Malaysia (DOSM) in 2015. The low feed conversion ratio of chicken compared to other sources of protein such as beef cattle, sheep and goat is one of the contributing factors. Most of the meat producing poultry are fast growing white plumage broiler chicken raised in modern highly mechanised farms operated by big cooperation. While for rural homes, rearing of *ayam kampung* or village chicken is a tradition. They are known for their hardiness and resistant to diseases compared to the commercial broiler chicken. These chickens provide important source of meat and eggs. Village chickens are usually reared free ranging thus producing more lean muscles and less fat. With the increases of demand on village chicken in Malaysia due to the awareness of its health benefit, it is expected that its production will scale up in the coming years.

Through selective breeding on the Malaysian village chicken, a few village chicken phenotypes were developed at the University of Putra Malaysia (UPM) known as UPM-bred village chicken. It grows faster, can lay 120 to 200 eggs per annum and reach market weight earlier (less than 13 weeks) and more resistant to diseases (Jawad *et al.*, 2015).

Just like any practices, village chicken farming also need protection from diseases to prevent massive loss for the farmers. Therefore, disease prevention is necessary and vaccination is one of the important step taken to prevent diseases to spread among flocks. Vaccines that regularly used in chickens are Marek's Disease (MD), Newcastle Disease (ND), Infectious Bronchitis (IB), and Infectious Bursal Disease (IBD). Until now, IBD has become one of the main enemy for production in the poultry sector worldwide. IBD lead to major production damages causing from great mortality, impaired

growth, excessive carcass condemnation and intense immunosuppression leading to increase vulnerability to other pathogens and interfere with the efficiency of vaccination against other highly virulent diseases. IBD or also known as Gumboro disease is an acute infectious viral infection in chickens displayed by inflammation, followed by atrophy of the bursa of Fabricius, and immunosuppression. Clinically the infection is seen only in chickens older than 3 weeks. The feathers around the vent are usually tainted with faeces comprising plenty of urates. IBD is caused by Birnavirus, a double stranded RNA virus that has a bi-segmented genome and belongs to the genus Avibirnavirus of family Birnaviridae. The virus is about 50 to 55nm in diameter with a single shell. IBD virus (IBDV) is very stable to chemical and physical agents and it can remain for long in a contaminated environment.

In Malaysia, outbreak of IBD due to very virulent IBDV (vvIBDV) was first reported in 1991 (Hair-Bejo, 1993; Phong *et al.*, 2003). Since then, the disease spread throughout the country haunting the vaccinated and non-vaccinated chickens. There are variety of vaccines available in the market that can be found in Malaysia. They are subjectively classified into the “mild”, “intermediate” and “hot” strains of attenuated live IBD vaccines based on their pathogenicity and immunogenicity. “Mild” vaccines are low in their invasiveness of the bursa of Fabricius and may easily be neutralized by high maternally derived antibody (MDA) (Hair-Bejo *et al.*, 2014). “Intermediate” and “hot” vaccines are high in invasiveness even with the presence of MDA (Haffer, 1982). The standard and ideal criteria for safety and potency of attenuated live IBD vaccine have been suggested previously (Thorton and Pattison, 1975). Normally, there would be no clinical effects caused by the vaccine when administered at day old and deliberate protection within 10 days of vaccination. The vaccine also usually cause no more than slight and brief reduction in the bursa to body weight ratio with partial and temporary lymphocytes depletion in the bursa with no significant immunosuppression effects.

Usually, intraocular and oral routes via drinking water are the common routes for IBD vaccination in village chickens. Subcutaneous or vent-drop administration was also been reported to be effective against IBDV challenged (Winterfield and Thacker, 1978). The effectiveness of in-ovo vaccination in 18-day-old chicken eggs with embryos, at time the eggs are routinely transferred to hatching tray, was also reported previously (Hair-Bejo *et al.*, 2000). In-ovo vaccination can eliminate the need for post hatch vaccination that is stressful to the chicks. In free-range chickens, feed based vaccine is believed to be the best route of vaccination. Food pellet Newcastle disease vaccine was successfully developed previously (Aini, 1989). However, the safety and immunogenicity of the IBD vaccine for UPM-bred village chicken through feed is unknown. The characteristic of IBDV which is non envelope virus, very stable to chemical and physical agents and can remain for long in environment are of advantages when used as feed based vaccine.

Smallholder farmers with lack of resources are the one whom usually rearing village chicken in small quantity. Vaccination programme forces these farmers

to invest money in terms of labour that also consumes a lot of time. For feed based vaccination, farmers only need to mix the vaccine and feed together and give it to the chickens. In free-range chickens, feed based vaccine is believed to be the best route of vaccination. It is because lack of resources are needed with little effort compared to intraocular vaccination route which required more workers to drop the vaccine into the eyes of chickens, one by one. Thus, feed based vaccination can be of choice and more practical when compared to other conventional routes of vaccination for IBD in the control prevention Of IBDV infection in free-range chickens such as in village chickens. This is strengthened by the proof that feed pellet Newcastle disease vaccine was successfully developed previously (Aini, 1989). With the feed based IBD vaccination on village chicken study, it is hope that farmers can start replacing the conventional route of vaccination thus thriving for a better productivity.

1.1 Objectives

- I. To evaluate and determine the most effective route for IBD vaccination on UPM-bred village chicken.
- II. To compare two different routes of IBD vaccination.
- III. To determine the growth responses of commercial IBD vaccine given to UPM-bred village chicken via feed.
- IV. To evaluate the histological changes of IBD vaccinated UPM-bred village chicken.
- V. To study the effectiveness of vaccination through antibody titer of IBD vaccinated UPM-bred village chicken flocks using Enzyme Linked Immunosorbent (ELISA) test.

1.2 Hypothesis

Feed based IBD vaccination at 14-day-old UPM-bred village chickens using attenuated live IBD vaccine can successfully induce protective level of IBD antibody similarly to intraocular IBD vaccination.

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