
UNIVERSITI PUTRA MALAYSIA

# PATHOGENICITY AND IMMUNOGENICITY OF INFECTIOUS BURSAL DISEASE VIRUS ATTENUATED IN BGM-70 CELL LINES IN SPECIFIC PATHOGEN FREE CHICKENS

MOHAMMAD ARIF IZUAN BIN BURHAN

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**MOHAMMAD ARIF IZUAN BIN BURHAN**

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It is hereby certified that we have read this project paper entitled "Pathogenicity and Immunogenicity of Infectious Bursal Disease Virus Attenuated in BGM-70 Cell Lines in Specific Pathogen Free Chickens", by Mohammad Arif Izuan bin Burhan and in our opinion it is satisfactory in term of scope, quality, and presentation as partial fulfilment of the requirement for the course VPD 4999 – Project.

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## ABSTRAK

Abstrak kertas projek yang dikemukakan kepada Fakulti Perubatan Veterinar untuk memenuhi sebahagian daripada keperluan kursus VPD 4999 – Projek.

**PATOGENISITI DAN IMUNOGENISITI VIRUS PENYAKIT BERJANGKIT BURSA YANG TELAH DILEMAHKAN DALAM BGM-70 SEL DALAM AYAM BEBAS SPESIFIK PATHOGEN**

**Oleh**

**MOHAMMAD ARIF IZUAN BIN BURHAN**

**2017**

**Penyelia: Dr. Nik Mohd Faiz bin Nik Mohd Azmi**

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Penyakit berjangkit bursa (IBD) merupakan penyakit virus mudah berjangkit yang dicirikan dengan kadar kematian yang tinggi dan melumpuhkan sistem imuniti ayam yang boleh menyebabkan kerugian ekonomi yang teruk dalam industri ternakan ayam. Kawalan IBD adalah dengan mengamalkan biosekuriti yang baik dan program vaksinasi. Objektif kajian ini adalah untuk menentukan patogenisiti dan imunogenisiti virus penyakit berjangkit bursa yang sangat virulent (vvIBDV) yang telah dilemahkan dalam BGM-70 sel dalam ayam SPF untuk pembangunan vaksin berasaskan IBD kultur tisu daripada jangkitan vvIBDV. Ayam SPF berumur sehari dibahagikan kepada 5 kumpulan iaitu kumpulan A, B, C, D, dan E. Kumpulan A dan B telah disuntik dengan virus UPM190 IBD ($10^{9.7}$ $TCID_{50}$ / 1.0 mL) “passaged” 10 dan 15, 0.1 mL

masing – masing melalui laluan subkutaneus. Sementara itu, kumpulan C dan D telah disuntik dengan vaksin UPM0081 IBD ($10^{9.5}TCID_{50}$ / 1.0 mL) "passaged" 10 dan 15, 0.1mL masing-masing melalui laluan subkutaneus. Kumpulan E sebagai kawalan dan tiada rawatan telah diberikan. Anak ayam disediakan makanan dan minuman "*ad libitum*". Lima anak ayam dari setiap kumpulan inokulasi dikorbankan pada hari 14 dan 28. Manakala, lima anak ayam dari kumpulan kawalan dikorbankan pada hari 1, 14 dan 28. Sebelum anak ayam dikorbankan, berat badan direkodkan dan sampel serum diambil untuk menentukan titer antibodi menggunakan *enzyme linked immunosorbent assay* (ELISA). Semasa nekropsi, lesi kasar serta berat bursa direkodkan. Kajian ini menunjukkan tiada kematian dan tanda klinikal ditunjukkan di kesemua kumpulan inokulasi dan kawalan. Keputusan yang sama ditunjukkan oleh lesi kasar bursa. Nisbah bursa kepada berat badan menunjukkan perbezaan penurunan bererti ($p<0.05$) dalam kumpulan A dan D jika dibandingkan kepada kumpulan E pada umur 14 hari. Walau bagaimanapun, tiada perbezaan bererti ($p>0.05$) pada hari ke 28. Lesi bursal sepanjang eksperimen kekal normal ke ringan (skor lesi 0 hingga 1). Titer IBD antibodi daripada ujian ELISA adalah negatif untuk semua kumpulan sepanjang kajian. Kesimpulannya, vvIBDV yang dilemahkan dalam BGM-70 sel tidak berkesan dalam induksi lesi di bursa Fabricius dan titer antibodi.

**Kata kunci:** Penyakit berjangkit bursa (IBD), Virus hidup yang telah dilemahkan, BGM-70 sel kultur, Ayam SPF berumur sehari, IBD titer antibodi.

## ABSTRACT

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

**PATHOGENICITY AND IMMUNOGENICITY OF INFECTIOUS BURSAL DISEASE VIRUS ATTENUATED IN BGM-70 CELL LINES IN SPECIFIC PATHOGEN FREE CHICKENS**

**By**

**MOHAMMAD ARIF IZUAN BIN BURHAN**

**2017**

**Supervisor: Dr. Nik Mohd Faiz bin Nik Mohd Azmi**

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Infectious bursal disease (IBD) is a contagious viral disease characterized by high mortality and immunosuppression that able to cause severe economic losses to the poultry industry. Control of IBD is mainly by practicing good biosecurity and vaccination programme. It was objective of the study to determine the pathogenicity and immunogenicity of very virulent IBD virus (vvIBDV) attenuated in BGM-70 cell lines in the specific pathogen free (SPF) chickens for the development of IBD tissue culture based vaccine against vvIBDV infection. One day old SPF chicks were divided

into 5 groups namely the groups A, B, C, D, and E. The groups A and B were inoculated (0.1mL) with UPM190 IBDV isolate ($10^{9.7}$ $TCID_{50}$ / 1.0 mL) passaged 10 and 15, via subcutaneous route, respectively. Meanwhile, groups C and D were inoculated (0.1mL) with UPM0081 IBD isolate ($10^{9.5}$ $TCID_{50}$ / 1.0 mL) passaged 10 and 15 via subcutaneous route, respectively. The group E acted as the control group and was not inoculated with the virus. The chicks were provided feed and water *ad libitum*. Five chicks from each virus inoculated group were sacrificed on 14 and 28 days of age. Meanwhile five chicks from control group were sacrificed on 1, 14 and 28 days of age. Before sacrificing, the body weight was recorded and serum samples were collected to determine the antibody titer using enzyme linked immunosorbent assays (ELISA). On necropsy, gross lesions were observed and weight of bursa of Fabricius was recorded. The study showed that no mortality and clinical signs were observed in all inoculated and control group throughout the experiment. Same goes to the gross lesion of the bursal of Fabricius. The bursal to body weight ratio was significantly reduced ($p<0.05$) in groups A and D when compared to group E on 14 days of age. However, there was no significance difference ($p>0.05$) in all groups on 28 days of age. Bursal lesion score throughout the experiment remain normal to mild (lesion scoring of 0 to 1). IBD antibody titer from ELISA test was negative for all groups throughout the experiment. It was concluded that the live attenuated vvIBDV in BGM-70 cell lines was ineffective in induction of lesions in the bursa of Fabricius and IBD antibody titer.

**Key words:** Infectious bursal disease (IBD), live attenuated, BGM–70 cell lines, day old SPF chicks, IBD antibody titer.

## 1.0 Introduction

Infectious bursal disease (IBD) or Gumboro disease is one of the major concern to the poultry industry worldwide. It was first reported in Delware, Gumboro in 1957 and was first diagnose in 1962 (Cosgrove, 1962). It's a highly contagious immunosuppressive viral disease of chickens which poses significant economic losses to the industry due to high mortality and immunosuppression (Hair-Bejo *et al.,* 2004). Usually the infected chicken will show clinical signs such as anorexia, ruffled feather, watery diarrhoea, depressed, trembling and severe prostration. (Cosgrove, 1962).

Infectious bursal disease virus (IBDV) is a non-enveloped virus measured approximately 55 to 60nm in diameter (Hirai and Shimankura, 1974) and belongs to the genus Avibirnavirus of the family Birnaviridae. (Dobos *et al.,* 1979). It contains double stranded RNA surrounded by a single T=13 icosahedral shell. The virus is made up of two segment genomes known as segment A and segment B which coded for viral protein 1 (VP1), 2 (VP2), 3 (VP3), 4 (VP4) and 5 (VP5) where VP2 contains virus neutralizing epitopes (Fahey et al., 1989). Segment A contains two open reading frames (ORFs) that encode for non-structural VP5 protein and a precursor polyprotein that can give rise to VP2 and VP3 structural proteins and VP4 protease protein. Segment B contains one ORF that encodes for VP1 protein, a RNA polymerase. IBDV was categorize into 2 serotypes namely pathogenic serotype 1 and non-pathogenic serotype 2. However, only serotype 1 IBDV has been known to cause disease in chickens while serotype 2 only recovered from turkeys. Serotype 1 have been further classified into three groups based on the pathogenicity and antigenicity of the virus namely very virulent IBDV (vvIBDV), classical IBDV (caIBDV), and variant IBDV

(vaIBDV) (Lukert and Saif, 1997). The vvIBDV have the ability to induce clinical IBD with high mortality, induce immunosuppression in all ages and complicate efficacy of other vaccination programme (Hair-Bejo, 2006).

IBDV is a sturdy virus that very resistant to environment and difficult to be removed from infected farm once the outbreak occurs. Thus, vaccination is the most effective procedure for prevention and control of IBDV outbreak in the farms. There are four major types of vaccines that available for IBD control which is live attenuated vaccines, immune-complex vaccine, live recombinant vectored vaccines expressing IBDV antigens and inactivated oil-emulsion adjuvanted vaccines (Muller *et al*., 2012). However, the accident of outbreak in the farms can still do to happen due to lack of efficacy in the vaccine (Nurulfiza *et al.,* 2006). Therefore, a highly immunogenic with low in pathogenicity of IBDV isolate need to be used for the development of IBD vaccine. Thus, will result in the production of safe vaccine that can effectively provide full protection against IBDV in the future (Hair-Bejo *et al*., 2004).

This study hypothesized that the live attenuated vvIBDV in BGM-70 cell lines is safe and could induce high IBD antibody in the SPF chickens for the development of IBD tissue culture based vaccine against vvIBDV infection.

The objectives of this study were:

1. to determine the pathogenicity and immunogenicity of vvIBDV attenuated in BGM-70 cell lines in specific pathogen free (SPF) chickens.
2. to determine the clinical signs, gross and histological lesions of chickens inoculated with the attenuated IBDV.
3. to determine the IBD antibody induced by the chickens.

## 2.0 Literature Review

### 2.1 Infectious bursal disease

Infectious Bursal disease (IBD), is a highly contagious viral infection of young chickens characterized mainly by severe lesions in the bursa of Fabricius followed by immunosuppression (Saif *et al*., 1998) which caused severe impact of economic losses to the farmers (Thangavelu *et al*., 1998). The disease was first clinically recognised in 1957 in USA (Cosgrove, 1962). Since then, IBD outbreaks have been reported worldwide, but have been relatively under control due to proper immunization programmes in poultry farms. Nevertheless, in the late 1980s, outbreak of the disease with high mortality due to vvIBDV emerged in Europe (Chettle *et al*., 1989). Many reported that vvIBDV are antigenically similar to the caIBDV (Synder 1990), but it is able to establish infection at high levels of maternal antibody (MDA), which is usually protective against caIBDV strains (Chettle *et al*., 1989). Chickens which received IBD

vaccine of caIBDV origin could not be protected against vaIBDV infections. The characterization of IBDV field isolates during disease outbreaks is vital for the right use of IBD vaccine and effective control and prevention of the disease. The first outbreak of vvIBDV infection in chickens in Malaysia was reported in 1991 (Hair-Bejo, 1992).

### 2.1.1 Susceptibility to physical and chemical agent

According to Van den Berg (2000), IBDV infections can continue to occur and spread widely nevertheless of a properly done cleaning and proper disinfection procedures in the farms. This is because, the virus is stable in nature and highly resistant to many physical and chemical disinfectants. Moreover, the virus was also found to be resistant to heat and ultraviolet irradiation (Benton *et al.,* 1967). The survivability of the virus can up to 5 hours in 56˚C (Benton *et al.,* 1967) and 90 minutes in 60˚C while it can survive up to 21 days at room temperature 25˚C (Cho and Edgar, 1969). The virus also viable for up to 60 days in poultry house litter and outside the host for at least 4 months (Baxendale, 2002). Furthermore, the IBDV are also stable in acidic and alkaline condition range from pH 2 to pH 13 (Benton *et al.,* 1967). Finally, the virus was also known to be resistant to chemical such as ether, chloroform, merthiolate, trypsin, phenol, staphene and hyamine (Benton *et al.,* 1967).

### 2.1.2 Clinical signs gross lesions

According to Cosgrove (1962), IBD infected chickens will normally develop clinical signs such as dehydration, whitish or watery diarrhoea, soiled vent feathers, depressed, anorexia, ruffled feathers, trembling, severe prostration, and finally died. The susceptibility of the chicken to develop clinical sign is associated to the size of bursa relative to the body weight which is the largest at 3 weeks of age which will allow maximum virus replication, increase virus concentration and formation of large immune complexes that contribute to the clinical disease (Ivanyi and Morris, 1976). Therefore, chicken infected with IBDV will normally develop clinical signs in between age of 3 to 6-week-old (Zaheer and Saeed, 2003). Infected chicken more than 6 weeks old rarely develop clinical signs of disease. However, they produce protective antibody to the virus. The IBD has a short incubation period usually 2 – 3 days (Saif, 1998) after which birds show clinical sign. Gross lesion can be observed in the primary target organ of the viruses, bursa of Fabricius. The organ will undergo major changes start from 3 days' post infection (pi). It enlarges twice of the normal size because of oedema and hyperaemia by 4-day pi followed by atrophy, reaching one third of its original weight by 8-day post infection (Saif, 1998).



### 2.1.3 Histological lesions

Degeneration and necrosis of lymphocytes start in the medullary area of the bursal follicles can be detected as early as day 1 pi with light microscopy (Kaufer and Weiss, 1976). B–lymphocytes in the bursal follicles, germinal follicles and perivascular sheaths of the spleen are destroyed (Henry *et al*., 1980). There was also infiltration of heterophils and hyperplasia of the reticuloendothelial cells and the interfollicular tissues in the bursa of Fabricius. As the disease subsides the corticomedullary epithelium proliferates and cystic cavities develop in the medullary area of the follicles. By day 3 or 4 pi, all of the lymphoid follicles were affected (Lasher and Shane, 1994).

**2.2 Pathogenesis and pathogenicity of IBDV**

The natural IBDV infection in chickens usually occur via oral route accompanied by gut associated lymphoid cells (Kaufer and Weiss, 1976). The virus replicates primarily in the lymphocytes and macrophages of the gut-associated lymphoid tissues (GALT) (Hair-Bejo *et al*., 1997) before it caused primary viraemia by travels to the target organ which is bursa of Fabricius via the blood circulation. Replication will occur rapidly in developing B–lymphocytes in the bursa of Fabricius (Lim *et al*., 1999). The virus can be detected as early from 4 hours pi in the macrophages and lymphoid cells of the caecum, and at 5 hours pi in macrophages and lymphoid cells of the duodenum and jejunum (Becht, 1980). By 13 hours pi, most of the bursal follicles are positive for virus and by 16 hours pi., a secondary viremia occurs. This will be followed by virus secondary replication and destruction in other organs leading to disease and death (Muller *et al.,* 1979). The cause of death in clinical IBD is mainly due to circulatory

failure as a result of severe haemorrhage and also severe dehydration due to diarrhoea and reduce in water intake (Hair-Bejo, 1993).

Pathogenicity of the IBDV infection from different strains will depends on the tissue involvement. The surviving chicken that infected with vvIBDV, could induce high mortality and severe immunosuppression due to severe damaged of the lymphoid organs or tissues during secondary viraemia. In contrast, the lesions caused by caIBDV infection is rather less severe when compared to the vvIBDV infection and immunosuppresion recorded if the chicks were infected with the virus at below 2 to 3-weeks-old, while if the infection occurs at a later age, it could cause lower percentage of mortality compared from the vvIBDV infections. Meanwhile, the vaIBDV only causes immunosuppression and damaged of the lymphoid organs, especially the bursa of fabricius. Therefore, the infected chicken is highly susceptible to other pathogenic pathogens and died later due to secondary infection (Hair-Bejo, 1993).

**2.3 Control and prevention of IBD**

Before the development of live attenuated vaccines, IBDV was intentionally exposed to the chickens of the farms that previously having outbreak of IBD at an early age in order to control the disease (Lasher and Davis, 1997). Nevertheless, when severe immunosuppressive effects were observed even at early stage of infection, this control method was no longer effective. Moreover, sanitary precautions must be carried out rigorously to minimize the spreading of the virus. However, due to the virus is stable in nature and highly resistant to many physical and chemical disinfectants, immunization is still the best method to prevent the infection (Lukert and Saif, 1991).

In general, live attenuated vaccination is the live IBDV that have been attenuated by serial passage in tissue culture, eggs or embryo-derived tissues, with the aim to reduce the pathogenicity of the virus while increase the immunogenicity so that the vaccine can induce immune response while attenuating the ability of the vaccine virus to cause clinical disease or significant immunosuppression (Schijns *et al*., 2008) but still be able to mimic natural infection in the target host so they can replicate and induce both cellular and humoral immunity. (Hermann Muller *et al*., 2012). However, it may have the risk associated with the potential for reversion to a virulent phenotype (Muskett *et al.,* 1985). Effective vaccination programme should be considered factors such as types of vaccine given, time of vaccination, and level of maternally derived antibody (MDA) in the chicks and IBDV field strains (Hair-Bejo *et al.,* 2004).

Virulence of IBDV vaccines were subjectively classified as the "mild", "intermediate" or "hot" strain. The "mild" vaccine exhibit poor efficacy because it was unable to neutralize high level of MDA in chickens and failed to induce IBD antibody. Nevertheless, some of the "intermediate" and most of the "hot" vaccines have a much better efficacy and may break through higher level of MDA but it can cause severe bursal lesions as those observed in the field IBD outbreak (Hair-Bejo *et al*., 2000).

Early vaccination programme is recommended especially during the first 2 – 3 weeks of ages to provide protection against IBDV infection (Nunoya et al., 1992). Passive immunity can only protect the chicks until 18 to 21 days of age (Wyeth and Cullen, 1976). Chicks are susceptible to infection when the MDA level decline but the titer against IBDV not yet induced to protective level (Zaheer and Saeed, 2003). Therefore, it is important to vaccinate before MDA levels reach low sub protective levels (Sharma

*et al*., 1987). Several advantages of day old vaccination such may help in early protection for the chicks against the disease, easy handling of the chicks, less manpower needed and also reduce vaccination stress. However, MDA interfere IBD vaccine response and delay or prevent induction of humoral response (Block *et al.,* 2007). Therefore, it is vital for a vaccine to overcome or neutralize MDA and establish itself in bursa of Fabricius for the induction of high and protective level of IBD antibody in order to prevent the infection. Serological monitoring is also necessary to determine the optimal timing for vaccination (Berg and Meulemans, 1991).

The specific pathogen free (SPF) chickens serve as a good model to study the pathogenicity and immunogenicity because it is free from maternally derived antibodies (MDA) that are reported to cause interference with live IBDV when inoculated in chickens with high MDA titre. This interference will affect the validity of the data generated if non-SPF chickens are to be used for the study. Furthermore, the virus being an avian virus with chicken as the natural host for clinical disease, other animal models such as rats or mice are not suitable as models. This is justified in studies carried out by Jackwood *et al.,* (2008).

## 3.0 Materials and Methods

### 3.1 Experimental design

A total of 55-day old specific pathogen free (SPF) chickens were randomly divided into 5 groups namely the groups A (n = 10), B (n = 10), C (n = 10), D (n = 10) and E (n = 15). The chicks were kept separately under optimal condition in the Animal Research Facilities (ARF) in Faculty of Veterinary Medicine, UPM, where feed and water were provided *ad libitum*. On day 1, UPM190 IBDV passaged 10 and 15

($10^{9.7}$ $TCID_{50}$ / 1.0 mL) were inoculated into the day-old chicks in groups A and B, subcutaneously (0.1mL). The groups C and D were inoculated with UPM0081 IBDV passaged 10 and 15 ($10^{9.5}$ $TCID_{50}$ / 1.0 mL), subcutaneously (0.1mL). Meanwhile, group E acted as the control group and no treatment was given. However, 5 chicks from the control group were sacrificed for sampling during day 1 of ages. The chicks were monitored daily for any abnormal changes. On days 14 days old, 5 chicks from group A, group B, group C, group D and group E were sacrificed. The remaining chicks in group A and B were inoculated with UPM190 IBDV passaged 10 and 15 as a booster, subcutaneously (0.1mL) and the remaining chicks in group C and D were inoculated with UPM0081 IBDV passaged 10 and 15 as a booster, subcutaneously (0.1mL). Meanwhile there was no treatment given to the control group. On day 28 days old, all the remaining chicks from group A, group B, group C, group D and group E were sacrificed for sampling. Prior sacrificing, body weight was recorded and serum sample was taken for IBD antibody detection using enzyme linked immunosorbent assay (ELISA). Necropsy was then conducted to examine the bursa of Fabricius and the gross lesions observed were recorded. Bursa of Fabricius was then removed from the body, weight and fixed in 10% buffered formalin for histological examination and lesion scoring (Appendix, 1).

### 3.2 IBDV inoculum preparation

Two vvIBDV isolated obtained from IBD outbreaks in 2000 and 2004 identified as UPM0081 and UPM190, respectively and were adapted 12 times in embryonated specific pathogen free (SPF) eggs before serially passaged in BGM–70 cell line for

attenuation. Both isolates were passaged 10 times (low passage) and 15 times (high passage), respectively. During virus harvesting, the infected flasks were freeze-thawed three times, centrifuged at 1000xg for 20 minutes and the cell culture supernatant was filtered using 0.22µm syringe filter. The respective filtrates were checked for sterility by inoculating petri plates containing blood agar which were incubated at 37˚C and 25˚C for bacterial and fungal contamination for 48 hours and 72 hours, respectively. Lastly, titre of both UPM0081 and UPM190 IBDV was determined to be $10^{9.5}$ and $10^{9.7}$ $TCID_{50}$ / 1.0 mL, respectively before being used to inoculate 1 day-old SPF chicks subcutaneously at the dosage of 0.1 mL per chick.

### 3.3 Antibody titer

Serum samples collected from chicken at different age was tested for IBD antibodies using commercial enzyme linked immunosorbent assay (ELISA). Antigen coated plate was acclimatized to room temperature prior used. Negative and positive control (100µL) were dispensed into respective wells accordingly. These followed by 100µL of 1:500 (v/v) diluted test sera into respective wells. Plate was covered and incubated at room temperature for 30 minutes. The plate was then washed 4 times with 300µL wash buffer per well. After washing, 100µL of sheep anti-chicken IgG labelled with

alkaline phosphatase was added into the well, and further incubated at room temperature for 30 minutes. The plate was washed as described previously and 100µL of substrate buffer that contain diethanolamine buffer with enzyme co-factor was added into the well. Plate was incubated for another 15 minutes at room temperature. 100µL of stop solution was dispensed into the each well to stop the reaction. Microtiter plate reader was used to record the absorbance at 405nm and the IBD antibody titer was then generated using BioCheck 2000 software.

### 3.4 Histopathology and lesion scoring

Bursa of Fabricius was removed from body during post mortem and fixed in 10% buffered formalin solution for 24 hours. Tissues samples were then trimmed to a thickness of 5mm and underwent a series of dehydration, clearing and impregnation using automatic machine (Leica ASP 300). Processed samples were then embedded within paraffin wax, trimmed and sectioned to 4µm and fixed on glass slide for haematoxylin and eosin staining. Bursa samples were examined for histological changes under light microscope (Bock and Peter, 1984). Histological changes were graded on a scale 0 to 5 whereas 0 (normal), 1 (mild), 2 (mild to moderate), 3 (moderate), 4 (moderate to severe), 5 (severe) (Chan, 2013) (Appendix, 1).

### 3.5 Statistical analysis

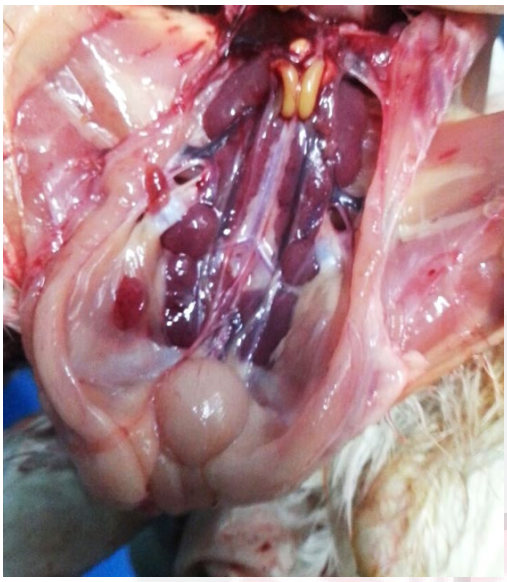
Data collected were analysed using SPSS version 22 using one-way analysis of variance (ANOVA) (Ostertagova, 2013).

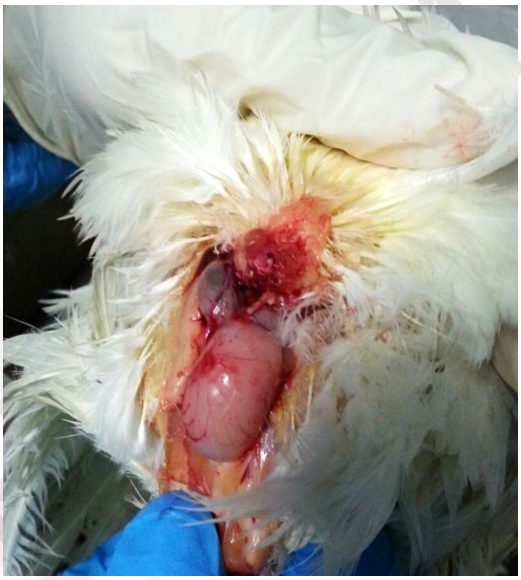
## 4.0 Results

### 4.1 Clinical signs and gross lesions

No mortality and clinical signs were observed in all inoculated and control groups throughout the experiment (Figure, 1). Furthermore, no gross lesion was also observed in all the inoculated and control groups (Figures, 2 and 3).

Figure 1: Healthy chickens in group A during 14 days of age.





**4.2 Body weight**

Body weight of all chickens increased significantly ($p<0.05$) throughout the trial from day old chicks until age 28 days of ages (Figure, 4). There was also no significance difference ($p>0.05$) for body weight between groups at 14 days of ages. However, at 28 day old, there was significance difference ($p<0.05$) for body weight between Group A (UPM190 passage 10) and B (UPM190 passage 15) was statistically higher ($P<0.05$) compared with group D (UPM0081 passage 15) (Appendix, 3).

Figures 2: Normal bursa of Fabricius in 14-day-old chicken in group C.

Figures 3: Normal bursa of Fabricius in 28-day-old chicken in group C.

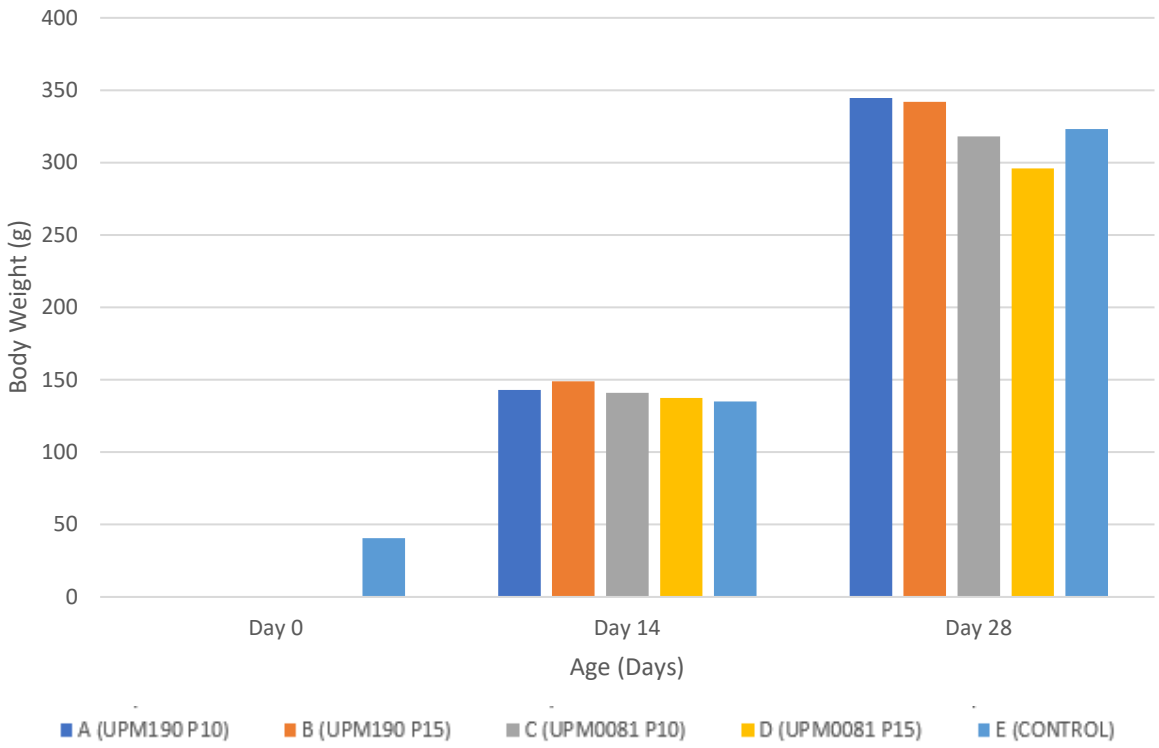



Figure 4: Body weight of chickens throughout the trial.

### 4.3 Bursal of Fabricius weight

Bursa weight of all chickens increased significantly ($p<0.05$) throughout the trial from day 1 to day 28 of ages (Figure, 5). There was no significance difference ($p>0.05$) for bursa weight between groups at days 14 and 28 of ages (Figure, 5; Appendix, 4).

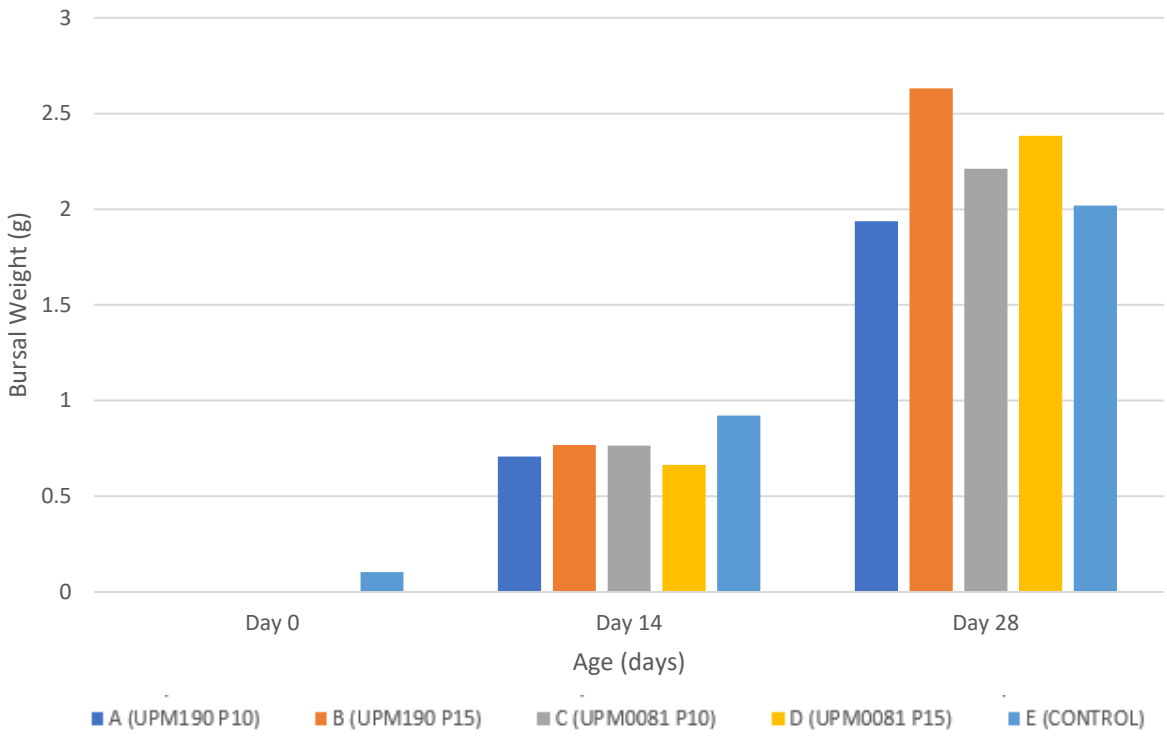


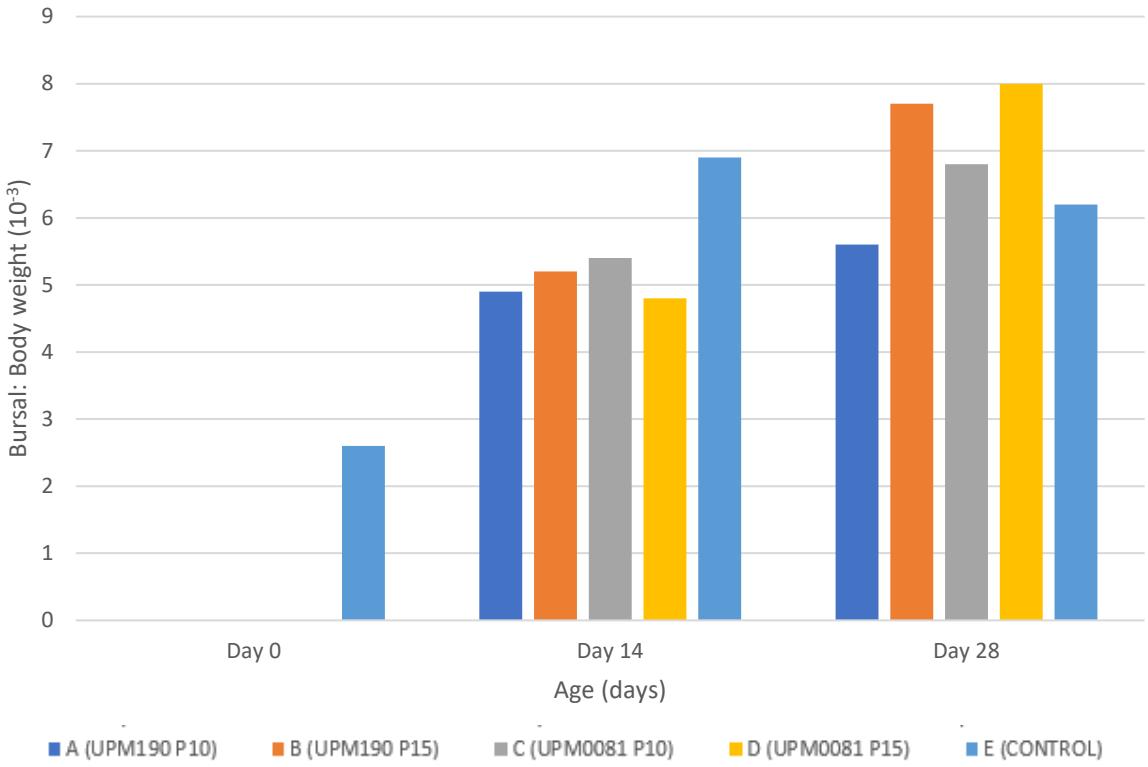

Figure 5: Bursal weight of chickens throughout the trial.



### 4.4 Relative bursal to body weight ratio

There was significance difference ($p<0.05$) for the ratio between groups at day 14 of age. Group A (UPM190 passage 10) and D (UPM0081 passage 15) was statistically lower ($p<0.05$) when compared to group E (control group) ($p<0.05$). However, no

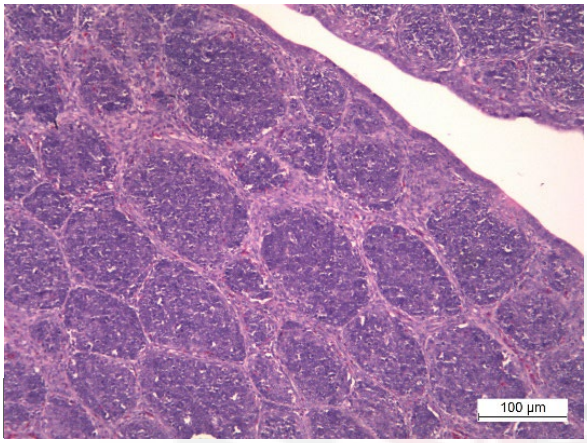
significance difference ($p>0.05$) was recorded in all day 28 of age (Figure, 6; Appendix, 5).




Figures 6: Relative bursal to body weight ratio of chickens throughout the trial.

### 4.5 *Histopathological changes*

The bursal lesions in control and inoculated groups remained normal too mild (lesion scoring of 0 to 1) throughout the experiment; at days 1, 14 and 28 of age.



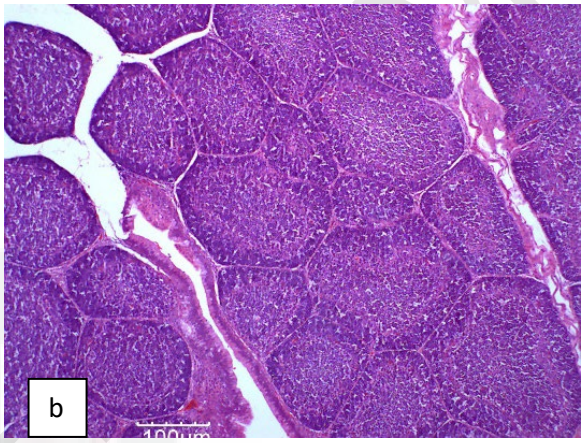
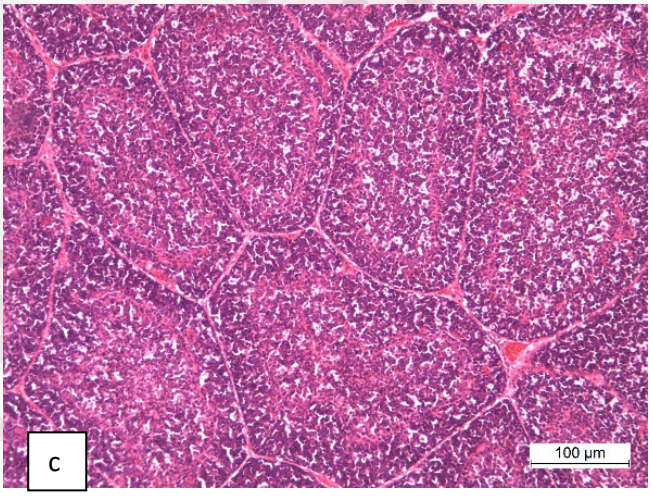
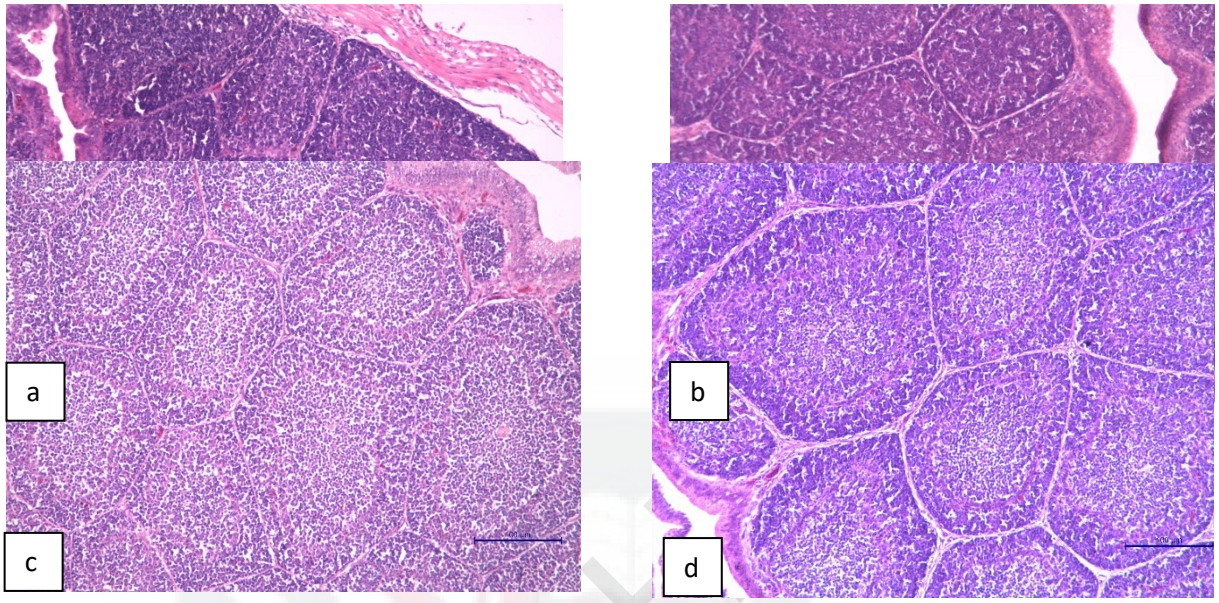
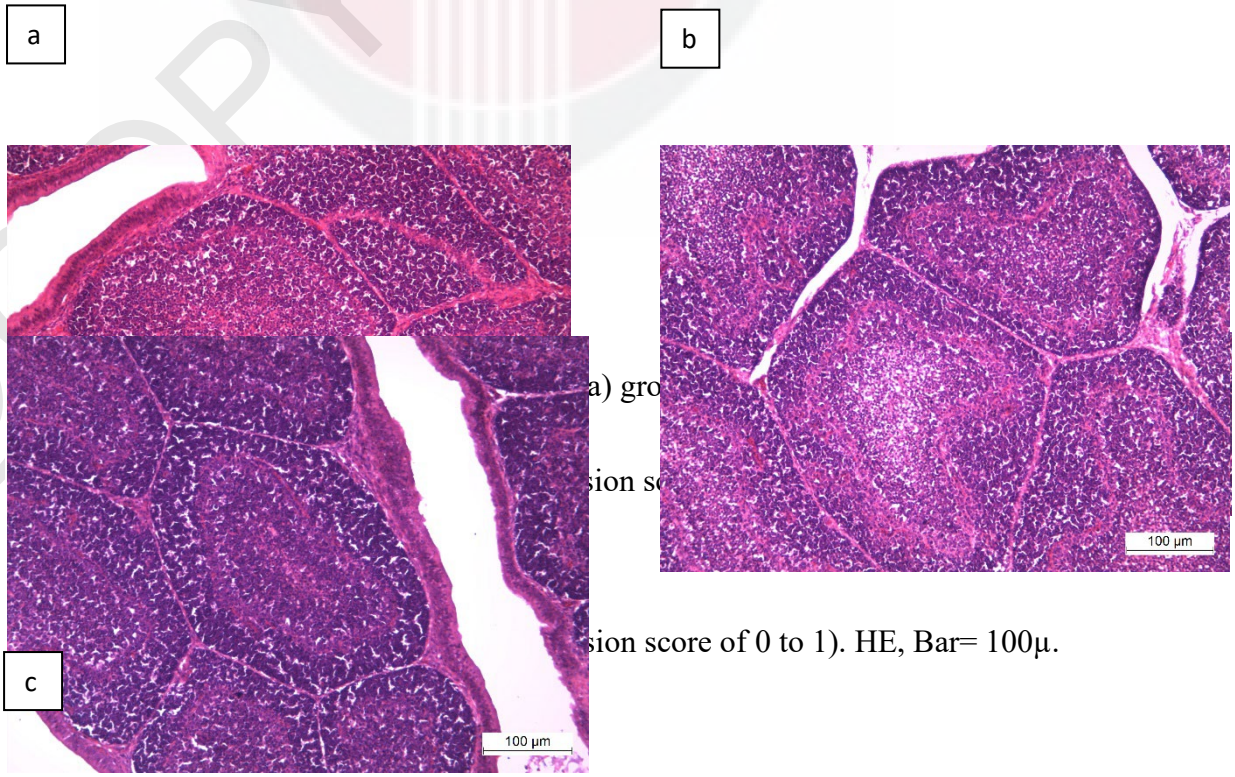




Figure 7: Bursa of Fabricius of chicken in group E (a) at 1 day of age, (b) 14 days of




age and (c) 28 days of age (lesion score of 0 to 1). HE, Bar= 100µ.




a) gro

sion s

sion score of 0 to 1). HE, Bar= 100µ.

### 4.6 IBD Antibody Titer

The IBD antibody titer from ELISA test was negative for all group throughout the trial; at days 1, 14 and 28 of age chickens.

**5.0 Disscusion**

The attenuation of vvIBDV in BGM-70 could result in loss of virus pathogenicity and inability of the virus to replicate in the bursa of Fabricius. However, the relative bursal to body weight ratio between groups A and D on 14 days of age were statistically lower ($p<0.05$) compared from group E (control group) may indicating mild virus replication in the bursa of Fabricius in the inoculated group. Neither clinical signs, gross and histological lesions were recorded throughout the study. Furthermore, the inoculum was also proven unable to induce immunity to the SPF chickens. This might be due to factor such as the use of continuous cell lines of mammalian origin and the route of inoculation.

BGM-70 is derived from baby grivett monkey kidney, a continuous cell lines of mammalian origin and it's also have low risk of contamination by avian viruses. The advantages of the BGM-70 cell lines are it easy to handle and maintain the for isolation and propagation of the virus compared to primary cell culture such as chicken embryo fibroblast (Lim, 2016). In 2001, Saif *et al*. reported the adaptation of vaIBDV to BGM-70 cell culture resulted in a significant reduction in the ability of the virus to replicate in the bursa of Fabricius. Hassan, (2004) reported vvIBDV couldn't be adapted in BGM-70 cell lines after 6 passages. It could be the reason why vvIBDV passaged to 10 and 15 times in this experiment resulted in loss of virus pathogenicity and inability of the virus to replicate in the bursa of Fabricius.

In 2015, MyHatch UPM93 IBD vaccine from UPM93, vvIBDV strain was successfully developed. The vaccine proven that live attenuated vvIBDV strain can be

delivered via subcutaneous injection at 1 day old chicks in the hatchery. Haddad *et al.,* (1997) reported, subcutaneous route for inoculation of Infectious Bursal Disease Virus – Bursal Derived Antibody (IBDV – BDA) complex vaccine in day old chicks has been shown to be safe and effective when there are still present of MDA. Different strains of live attenuated vvIBDV UPM0081 and UPM910 used in this experiment might not compatible with the vaccination via subcutaneous route. The development of new vaccines from different strain is critical to overcome the possible risk of reversion to more virulent IBDVs and emergence of new IBDV strains with increased virulence due to possible genomic recombination between vaccine and wild IBDV strains (Tamiru, 2013). However, the epidemiological process for the emergence of new IBDV strains with an increased virulence is still poorly understood.

## 6.0 Conclusion

It was concluded that the vvIBDV attenuated in BGM-70 cell lines was ineffective in induction of lesions in the bursa of Fabricius and antibody titer in the SPF chickens.

## 7.0 Recommendations

1. Larger number of chickens can be used to represent the overall population.
2. Test the inoculum strain using different route of inoculation other than subcutaneous route.
3. The vaccine trial can be performed to test the efficacy of vaccine challenge with pathogenic vvIBDV.
4. The pathogenicity and immunogenicity of the early passage (below passage 10) should be determined.
5. Application of cationic liposomes can be used to enhance the deliver IBD vaccine to the bursa of Fabricius and induce high and protective level of IBD antibody titre with mild bursal lesion in the hatchery vaccination.

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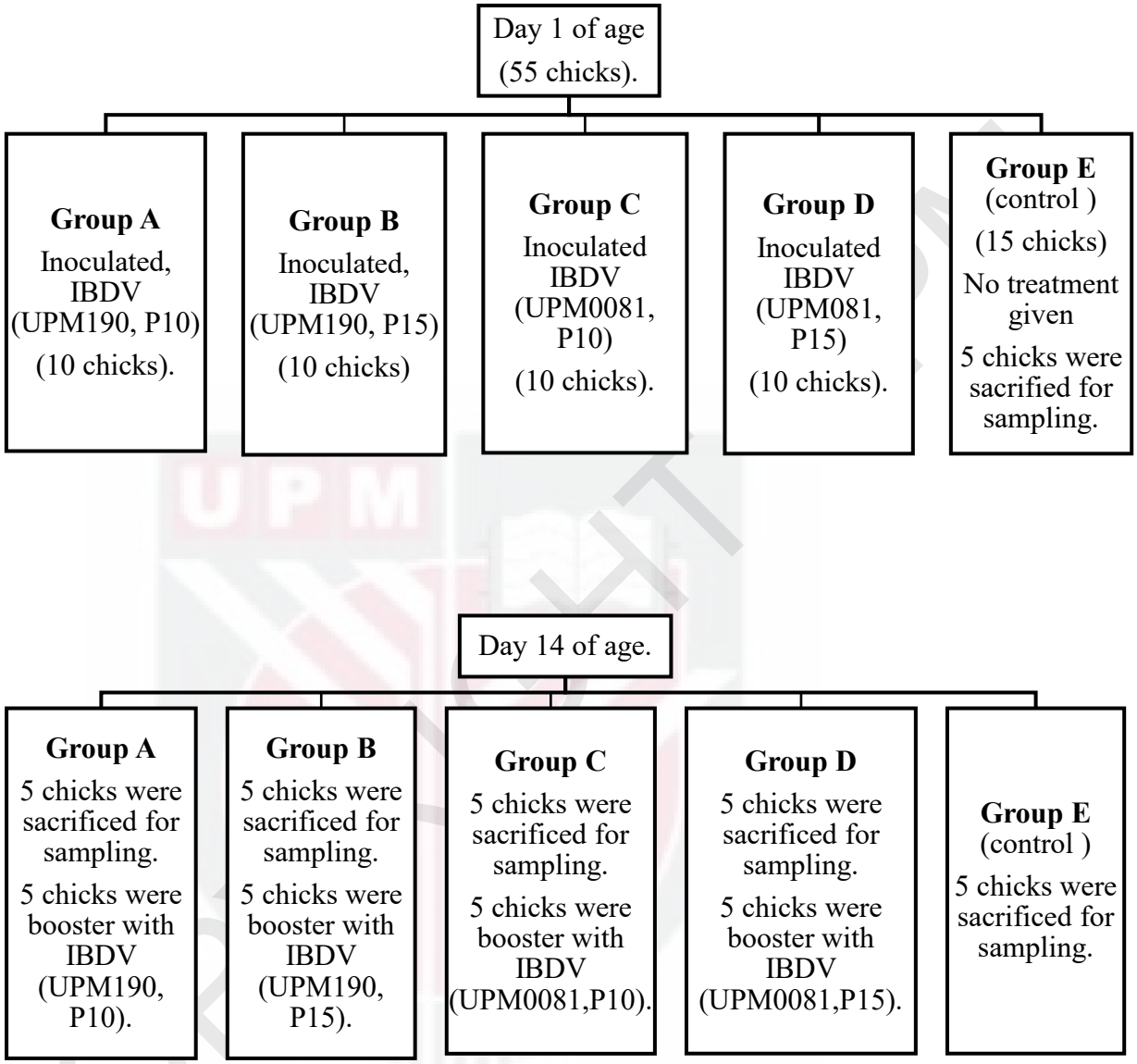
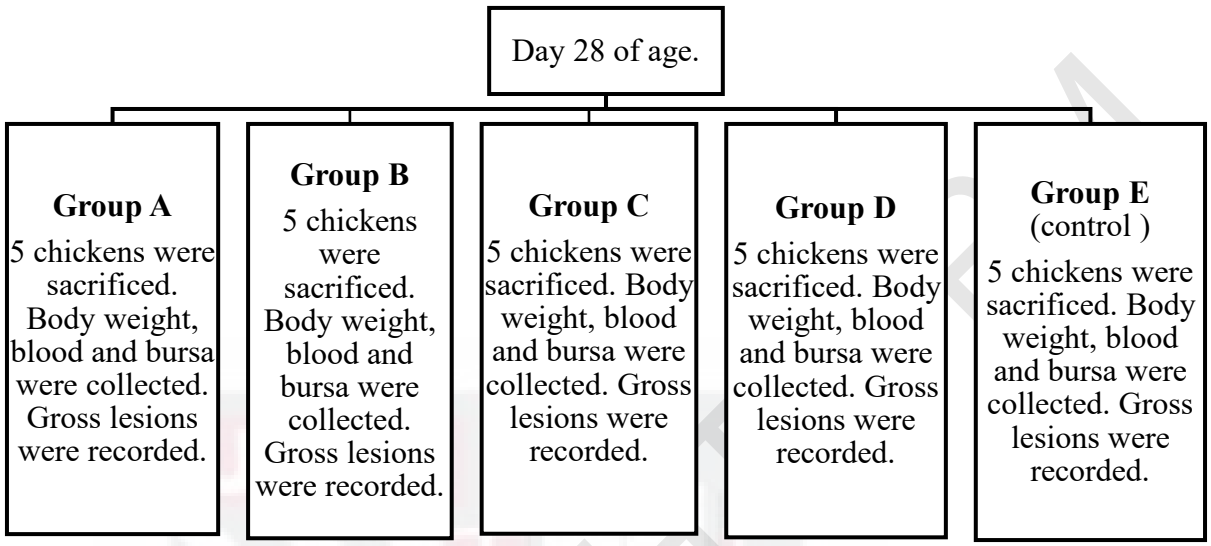
## 10.0 Appendices

Appendix 1: Experimental design for pathogenicity and immunogenicity of live attenuated vvIBD in SPF chickens.


Day 1 of age
(55 chicks).
Group A
Inoculated, IBDV (UPM190, P10)
(10 chicks).
Group B
Inoculated, IBDV (UPM190, P15)
(10 chicks)
Group C
Inoculated IBDV (UPM0081, P10)
(10 chicks).
Group D
Inoculated IBDV (UPM081, P15)
(10 chicks).
Group E (control )
(15 chicks)
No treatment given
5 chicks were sacrified for sampling.
Day 14 of age.
Group A
5 chicks were sacrificed for sampling.
5 chicks were booster with IBDV (UPM190, P10).
Group B
5 chicks were sacrificed for sampling.
5 chicks were booster with IBDV (UPM190, P15).
Group C
5 chicks were sacrificed for sampling.
5 chicks were booster with IBDV (UPM0081,P10).
Group D
5 chicks were sacrificed for sampling.
5 chicks were booster with IBDV (UPM0081,P15).
Group E (control )
5 chicks were sacrificed for sampling.


Day 28 of age.
Group A
5 chickens were sacrificed. Body weight, blood and bursa were collected. Gross lesions were recorded.
Group B
5 chickens were sacrificed. Body weight, blood and bursa were collected. Gross lesions were recorded.
Group C
5 chickens were sacrificed. Body weight, blood and bursa were collected. Gross lesions were recorded.
Group D
5 chickens were sacrificed. Body weight, blood and bursa were collected. Gross lesions were recorded.
Group E
(control )
5 chickens were sacrificed. Body weight, blood and bursa were collected. Gross lesions were recorded.

Appendix 2: Lesion Score of the bursa of Fabricius (Chan, 2013).

| Lesion scoring | Description |
|---|---|
| 0<br>(Normal) | Normal or undetectable. |
| 1<br>(Mild) | Mild degeneration and necrosis especially at the medullary region of lymphoid follicles. |
| 2<br>(Mild to moderate) | Mild to moderate degeneration and necrosis of lymphoid cells in some lymphoid follicles especially in the medulla. The interstitial connective tissues became oedematous and filled with inflammatory cells. |
| 3<br>(Moderate) | Moderate necrotised follicles involving both the cortex and medulla. Pyknotic nuclei were scattered in follicles. The interstitial space was obvious and presence of heterophils, macrophages, a few erythrocytes and fibroblast. Epithelial lining was thickened and vacuolated in some area. |

| | |
|---|---|
| 4<br><br>(Moderate to severe) | Moderate to severe depletion of lymphoid cells in the follicles. Lymphoid cell aggregation found in the cortex of some follicles. Necrotic cells and cysts were present in some follicles especially in the medulla. The interstitial space infiltrated with inflammatory cells and well packed with fibrous connective tissues. The intra and extra follicular areas might be hyperaemic and haemorrhagic. Epithelium was thickened, corrugated and vacuolated in some areas. |
| 5<br><br>(Severe chronic) | Severe follicular atrophy, with cysts formation within the follicles and epithelial lining off the organ. Remarkable infiltration of fibroblast in the interstitial area. Lymphocytes and monocyte infiltration were commonly observed<br><br>OR |
| 5<br><br>(Severe acute) | There was moderate to severe atrophy of the bursal follicles with cellular degeneration and necrosis involving both the cortex and medulla. Follicular cysts with fibrinous exudate and cells debris were frequently observed. The interstitial connective tissues were obvious, oedematous and infiltrated with mild to moderate inflammatory cells. The epithelial lining of the bursa was thickened and vacuolated. |

Appendix 3: Body weight of chickens throughout the trial.

| **Age (Days)** | **Body Weight (g) ± SEM** | | | | |
|---|---|---|---|---|---|
| | **Group A** | **Group B** | **Group C** | **Group D** | **Group E** |
| 1 | 40.60±0.51 | | | | |
| 14 | 143.00±4.53[a] | 149.00±8.11[a] | 141.60±9.01[a] | 137.40±7.34[a] | 135.00±10.67[a] |
| 28 | 344.60±10.00[b] | 342.00±9.12[b] | 318.20±15.49[a,b] | 296.00±12.45[a] | 323.20±9.70[a,b] |

Each value is mean ± standard error of mean of 5 chickens.

Different superscript ([a,b]) showing significance ($p<0.05$) at different point of time.

Appendix 4: Bursal weight of chickens throughout the trial.

| **Age (Days)** | **Bursal Weight (g) ± SEM** | | | | |
|---|---|---|---|---|---|
| | **Group A** | **Group B** | **Group C** | **Group D** | **Group E** |
| 1 | 0.103±0.007 | | | | |
| 14 | 0.707±0.067[a] | 0.768±0.039[a] | 0.765±0.074[a] | 0.664±0.078[a] | 0.922±0.079[a] |
| 28 | 1.937±0.027[a] | 2.632±0.336[a] | 2.211±0.411[a] | 2.384±0.347[a] | 2.019±0.255[a] |

Each value is mean ± standard error of mean of 5 chickens.

Different superscript ([a,b]) showing significance ($p<0.05$) at different point of time.

\

Appendix 5: Relative bursal to body weight ratio throughout the trial.

| Age | Bursal: Body Weight Ratio ($10^{-3}$) ± SEM | | | | |
|---|---|---|---|---|---|
| | **Group A** | **Group B** | **Group C** | **Group D** | **Group E** |
| 0 | 2.6±0.2 | | | | |
| 14 | 4.9±0.4[a] | 5.2±0.4[a,b] | 5.4±0.2[a,b] | 4.8±0.4[a] | 6.9±0.5[b] |
| 28 | 5.6±0.2[a] | 7.7±0.9[a] | 6.8±1.0[a] | 8.0±1.0[a] | 6.2±0.8[a] |

Each value is mean ± standard error of mean of 5 chickens.

Different superscript ([a,b]) showing significance ($p<0.05$) at different point of time.