
**UNIVERSITI PUTRA MALAYSIA**

***PATHOGENICITY OF FOWL ADENOVIRUS ISOLATES OF MALAYSIA IN SPECIFIC PATHOGEN FREE CHICKENS***

**MAJDI BIN AHMAD**

# PATHOGENICITY OF FOWL ADENOVIRUS ISOLATES OF MALAYSIA IN SPECIFIC PATHOGEN FREE CHICKENS

BY

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It is hereby certified that I have read this project paper entitled "Pathogenicity of Fowl Adenovirus Isolates of Malaysia in Specific Pathogen Free Chickens" by Majdi Bin Ahmad and in my opinion it is satisfactory in terms of scope, quality, and presentation as partial fulfillment of the requirement for the course VPD 4999-Project.



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## ABBREVIATIONS

| | |
|---|---|
| FAdV | Fowl Adenovirus |
| IBH | Inclusion body hepatitis |
| HE | Hematoxilin and eosin |
| SPF | Specific pathogen free |
| IBDV | Infectious bursal disease virus |
| CAV | Chicken anemia virus |
| pi | Post-inoculation |
| $^{o}C$ | Degree Celsius |

## ABSTRAK

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

**PATHOGENISITI PENCILAN ADENOVIRUS AVIAN MALAYSIA DALAM AYAM BEBAS PATOGEN KHUSUS**

**Oleh**

**Majdi Bin Ahmad**

**Februari 2015**

**Penyelia : Profesor Dr. Mohd Hair Bin Bejo**

Avian adenovirus (FAdV) adalah virus tanpa kapsul dan merupakan patogen utama menyebabkan inklusi badan hepatits (IBH) pada ayam. Ayam terjejas menunjukkan kadar kematian yang tinggi dengan biasanya pucat lesi, rapuh, pendarahan dan pembesaran hati, dan pembentukkan jasad rangkuman intranuklear (INIB) dalam organ tersebut. Objektif kajian ini adalah untuk menentukan pathogenisiti pencilan FAdV Malaysia terkini dalam ayam bebas patogen khusus (SPF). Tiga puluh enam anak ayam SPF berumur sehari telah dibahagikan kepada 3 kumpulan utama iaitu kumpulan A, B dan C. Ayam kemudiannya dibahagikan kepada kumpulan kecil iaitu kumpulan

pengorbanan dan kematian. Kumpulan A dan B telah disuntik (0.5ml / ayam) dengan FAdV pencilan A (UPM11134) dan B (UPM1127), masing-masing melalui laluan dalam peritoneal. Kumpulan C telah bertindak sebagai kumpulan kawalan dan kekal tidak disuntik. Anak ayam diperhatikan dua kali sehari bagi perubahan klinikal dan kematian. Sampel hati, limpa, hempedal dan trakea dikumpulkan dan diawit dalam 10% bufer formalin untuk pemeriksaan histologi. Kajian ini menunjukkan kematian 100% masing-masing dalam kumpulan kematian A dan B dalam masa 3 dan 4 hari selepas inokulasi (pi). Walaupun dalam kumpulan pengorbanan masing-masing kumpulan A dan B menunjukkan 100% dan 90% daripada kematian telah dicatatkan dalam tempoh 4 dan 7 hari pi. Kumpulan kawalan kekal tiada kematian. Pemeriksaa lesi mata kasar pada ayam yang kematian adalah pembesaran hati dan kekuningan dengan pendarahan berbintik telah direkodkan dalam anak ayam dari kumpulan A dan B. Limpa juga menghadapi pembesaran dan kekuningan, manakala tiada lesi yang ketara dicatatkan pada hempedal dan trakea. Pemeriksaan histologi hati dan hempedal dari kumpulan A dan B mendedahkan basophilic jasad rangkuman intranuklear. Tiada lesi yang ketara dicatatkan pada trakea dan limpa. Kesimpulannya, FadV pencilan Malaysia adalah sangat patogenik kepada SPF anak ayam berusia sehari berikutan inokulasi virus melalui laluan dalam peritoneal.

***Kata kunci*:** Adenovirus avian (FAdV), ayam bebas patogen khusus (SPF), laluan dalam peritoneal, jasad rangkuman intranuklear (INIB)

## ABSTRACT

An abstract of the project paper presented to the Faculty of Veterinary Medicine in partial fulfillment of the course VPD 4999 - Project

**PATHOGENICITY OF FOWL ADENOVIRUS ISOLATES OF MALAYSIA IN SPECIFIC PATHOGEN FREE CHICKENS**

**By**

**Majdi bin Ahmad (159530)**

**February 2015**

**Supervisor: Professor Dr. Mohd Hair Bin Bejo**

Fowl Adenovirus (FAdV) is a non-enveloped virus and is a primary pathogen to cause inclusion body hepatits (IBH) in chickens. Affected chicken showed high mortality with typically lesions of pale, friable, haemorrhage and enlarged liver, and the present of intranuclear inclusion bodies (INIB) in the organ. It was the objective of the study to determine the pathogenicity of recent FAdV isolates of Malaysia in specific pathogen free (SPF) chickens. Thirty six one-day-old SPF chicks were divided into 3 major groups namely groups A, B and C. The chickens were then divided into sub-group namely the sacrificed and mortality groups. Groups A and B were inoculated

(0.5mL/chick) with FAdV isolates A (UPM11134) and B (UPM1127), respectively via intra-peritoneal route. The group C was acted as a control group and remained un-inoculated. The chicks were observed at least twice daily for any clinical abnormality and mortality. Samples of liver, spleen, gizzard and trachea were collected and fixed in 10% buffered formalin for histological examination. The study showed a 100% mortality in the mortality groups A and B within 3 and 4 days post-inoculation (pi), respectively. While in the sacrificed groups A and B, mortality of 100% and 90% respectively were recorded within 4 and 7 days pi. The control group remained no mortality throughout the whole period. On necropsy, gross lesions of enlarged and yellowish liver with petechial hemorrhages were recorded in chicks from groups A and B. The spleen was enlarged and yellowish, whilst no significant lesions were recorded in the gizzard and trachea. Histological examination of the liver and gizzard from groups A and B revealed typical basophilic intranuclear inclusion bodies. No significant lesions were recorded in the trachea and spleen. It was concluded that FAdV isolates of Malaysia is highly pathogenic to one-day-old SPF chicks following inoculation of the virus via intra-peritoneal route.

***Keywords:*** Fowl Adenovirus (FAdV), specific pathogen free (SPF) chicks, intra-peritoneal route, intranuclear inclusion body.

# 1.0 INTRODUCTION

Fowl Adenovirus (FAdV) is belong to sub-group I avian adenovirus (*Aviadenovirus*) and distributed widely throughout the world. It commonly affects domestic avian species and all ages are susceptible (McConnell and Fitzgerald, 2003). This virus infection can be asymptomatic to healthy birds or associated with a variety clinical and pathologic condition that immune-compromised the birds. It has been associated with infectious body hepatitis (IBH), hydropericardium syndrome (HPS), gizzard erosion, enteritis, pancreatitis, decreased egg production syndrome, and respiratory disease (McFerran and Adair, 1977). The viral particles accumulate in the nucleus to form intranuclear inclusion bodies.

FAdV in chickens is a primary pathogen to cause inclusion body hepatits (IBH) which then cause sudden onset and sharply increased mortality for three to four days and then usually returns to normal on day five. IBH normally seen in broiler chickens or meat producing birds at three to seven weeks of age, but can be seen as early as seven days old and as old as 20 weeks of age (Mahani and Azhar, 2006). It usually characterized by acute mortality peaking at day 3 to 4 post inoculation and stopping at day 5 pi. Mortality usually ranges between 5 to 10% but can reach up to 30%. The clinical signs that can be seen at 12 to 24 hours prior to death are depression weakness, respiratory depress, ruffled feather, inappetance, prostration, reduce body weight, and white pasty dropping adhere to the feather around cloaca. FAdV primarily affecting

liver and hemopoietic system. The main lesions of IBH are friable, pale, and enlarged liver. There also some degree of petechial to ecchymotic hemorrhages on liver. Histologically eosinophilic or basophilic, large, round intranuclear inclusion bodies are detected in the hepatocytes (McCracken and Adair, 1993).

FAdV is varying in pathogenicity due to age factor, route of inoculation, and the serotypes itself. Many studies to isolates the virus via natural routes or by direct contacts were failed, but give high pathogenicity when inoculated through parenteral route of inoculation (Saif, 2003). This might be suggested that adenovirus is pathogen to the host but need others factors or agents to allow them create pathogenicity in the natural infection. For example co-infection with infectious bursal disease virus (IBDV) and chicken anemia virus (CAV) give high pathogenicity to FAdV to create hepatitis and death (Norfitriah, 2012). So, other factors that lead to immunosuppress can trigger the pathogenicity of the virus. Infection of FAdV-9 virus alone through intraperitoneally caused 15% mortality (Erny et al., 1996) compared to subcutaneous caused 100% to one-day-old chicks (Alvarado et al., 2007)

Virus can be presented in fecal material, semen, kidney, nasal mucosa, and tracheal. Thus it can be excreted in all excretion method, but high virus titer mostly found in feces (Saif, 2003). Vertical transmission is very important route because infected hatching chicks may excrete virus in feces from the time of hatching but typically do not excrete until two to four weeks of ages due to maternal antibody. In

horizontal transmission, massive interchange of different strains can happen via interchanging flocks from different parent flocks. Horizontal occur only when infected brought to healthy chicken, thus aerial spread between farms cannot occur, but if spread by fomite between two farms can be occur. The incubation period is short about 24 to 48 hours following infections by natural route (Saif, 2003), but rate of horizontal spread through a flock is slow (McFerran, 1991).

## 1.1 Objective

The objective of this study was to determine the pathogenicity of recent FAdV isolates of Malaysia in specific pathogen free (SPF) chickens.

# 2.0 LITERATURE REVIEW

## 2.1 Adenovirus Infections in Poultry

### 2.1.1 Introduction

Adenovirus is non-enveloped DNA virus with 70 to 90 nm in diameter. The virus likely to replicate in the cell nucleus thus accumulation will then form intranuclear inclusion bodies. There are 4 strains of adenovirus which are one strain affect mammals called *Mastadenovirus* and three strains for bird species called *Aviadenovirus* (Fitzgerald, 2003). Each strains give different characteristic and 3 strains of *Aviadenovirus* are divided into serotypes based on the virus neutralization test (Ritchie, 1995). Various serotypes of *Aviadenovirus* are associated with different grouping such as quail bronchitis, inclusion body hepatitis, and hydropericardium in Adenovirus I, hemorrhagic enteritis in turkeys and marble spleen diasese of pheasants in Adenovirus II, and lastly chicken egg drop syndrome, egg drop syndrome in ducks in Adenovirus III (Ritchie, 1995). The first avian adenovirus isolated was in 1949 from a case from a lumpy skin disease in cattle and was inoculated in embryonated chicken eggs (Anjum, 1990). Whilst the first FAdV isolated from birds was from an outbreak of respiratory disease in bobwhite quails (*Colinus virginiamus*) by Olson (Akhtar *et al.*, 1992). The adenovirus is commonly recovered from persistent infection on natural asymptomatic birds. Morbidity and mortality associated with adenovirus infections are vary in different strains and the

host status. The virus cannot be detected in asymptomatic infected bird until there a series of disease infections that causing immune-compromised to the bird (Ritchie, 1995).

### 2.1.2 Fowl Adenovirus Infections

Fowl Adenovirus is belong to sub-group I avian adenovirus (*Aviadenovirus*) and distributed widely throughout the world. It commonly affects domestic avian species and all ages are susceptible (McConnell and Fitzgerald, 2003). This virus infection can be asymptomatic to healthy birds or associated with a variety clinical and pathologic condition that immune-compromised the birds. It has been associated with infectious body hepatitis (IBH), hydropericardium syndrome (HPS), gizzard erosion, enteritis, pancreatitis, decreased egg production syndrome, and respiratory disease (McFerran and Adair, 1977). With such variability of clinical pathologic condition by this virus, it is not possible to evaluate the overall economic important due to high mortality disease (McConnell and Fitzgerald, 2003).

## 2.2 Aetiology

### 2.2.1 Classification

There are five species of Fowl Adenovirus (FAdV) namely FAdV-A, FAdV-B, FAdV-C, FAdV-D, and FAdV-E. Viruses within each species are further recognized into 12 serotypes "FAdV - 1,2,3,4,5,6,7,8a, 8b, 9, 10, and 11" based on the result of cross neutralization assays (McConnell and Fitzgerald, 2003).

### 2.2.2 Morphology

The adenovirus virion in a non-enveloped and an icosahedral in structure with a diameter range from 70 to 90 nm. The densities is between 1.32 and 1.37 g/ml in calcium chloride. The nucleic acid is double–stranded DNA which comprised of only 11.3-13.5% and the remainder is the protein (Zsak and Kisary, 1984). As adenovirus has different isolates, then each isolates has different densities of DNA contents. The virion also made up of 252 capsomeres which arranged in triangular faces with six capsomeres along each edge, 240 non-vertex campsomer (hexon) and 12 vertex capsomeres (penton bases) are major structural proteins. Vertex carry projections called fiber (Russel, 2000) which then important in sequencing studies due to similar fiber length and antigenic properties in neutralization tests (McConnell and Fitzgerald, 2003).

### 2.2.3 Virus Replication

The replication involved two phases. Early phase is when the virus enter to the host with varies route inoculation whether natural or experimental. It then will transfer the virus

DNA to the nucleus which is followed by transcription and translation of early (E) genes (Russel, 2000). The early genes now responsible for a redirection of cellular function to facilitate replication of the virus DNA and consequent from next transcription and translation will form late (L) genes which a coding for viral structural proteins. Last phase is when the viral protein assemble into a complete virion in the nucleus thus at the same time disrupt the nuclear membrane to release the virus for further cell destruction (McConnell and Fitzgerald, 2003). Inclusion body is formed in the nucleus which can be demonstrated histologically by HE stain and immunofluorescence (McNulty and Smyth, 2002). In an ultrastructural studies, virus particles that accumulate in nucleus can be determine by showing a form of crystalline array (Adair *et al.*, 1979). Immunostaining method and cytochemical also can be used to detect by observing large intranuclear inclusions in infected cell (Almenesh *et al.*, 2012).

### 2.2.4 Susceptibility to Chemical and Physical Agents

All avian adenovirus tested positive are resistant to lipid solvent like ether and chloroform, sodium deoxycholate, 50% alcohol, trypsin, and 2% phenol. They also resistant to variation of pH level from 3 to 9, but can be inactivated in dilution 1 to 1000 concentration of formaldehyde. They can be inhibited by DNA inhibitors luDR and BuDR (McConnell and Fitzgerald, 2003). It is accepted that adenovirus can be inactivated in aqueous solution in reducing heat stability by divalent ions after exposed it to 56$^{o}$C for 30 minutes, but some test show variability thermo-stability whereas can

survived even after 18 hours exposure to 56$^{o}$C. These different results in showing susceptibility of adenovirus might be due to lack of technique and need to standardize the procedure.

### 2.2.5 Pathogenicity

Pathogenicity is the ability of a pathogenic agent to produce disease in a host. Fowl Adenovirus pathogenicity vary in different serotypes, or even strains of same serotypes. There is some relationship between genotype and virulence, but not serotypes and virulence. For example, FAdV-1 able to produce tumor like cell in human and hamster experimental, but not give in avian serotypes. Route of inoculation also recently give a lot different in pathogenicity whereby many study to isolates the virus via natural routes or by direct contacts were failed, but give high pathogenicity when inoculated through parenteral route of inoculation. This might be suggested that adenovirus is pathogenic to the host, but need others factors or agents to allow them create pathogenicity in the natural infection. For examples, co-infection with IBDV give high pathogenicity to FAdV (Fadly *et al*., 1976), and same as CAV that trigger ability to cause hepatitis and death (Bulow *et al*., 1986). This is different if co-infection with parvovirus that reduces the ability of adenovirus to growth in cell cultures as well as pathogenicity and oncogenicity (McFerran, 1991). In oral route inoculation of FAdV-8 in two days old chicks, the virus replicate in gastrointestinal tract epithelial to produce viraemia and reach targeted organ of liver and hemapoetic system about 24 hours pi (Saif, 2003). The

viral antigen peak was able to detect around day 2 pi and day 6 pi. The second peak antigen detection in the plasma is due to release of virus from damaged hepatic cells (Almenesh *et al.*, 2012). Other study showed that infection FAdV-9 in one-day-old chicks through intra-peritoneal cause 15% mortality and 17% reduction in body weight (Erny *et al*., 1996). Besides that, other research on infection FAdV-9 through subcutaneously caused 100% and 20% mortality to one and seven day old chicks, respectively (Alvarado *et al*., 2007). This suggests that, FAdV is varying in pathogenicity due to age factor, route of inoculation, and the serotypes itself.

## 2.3 Pathobiology and Epidemiology

All avian species and ages are susceptible to FAdV infection. Some viruses cause mortality in one day-old chicks, but not in 10-day-old birds (Cook, 1974). Thus pathogenicity also affect by age of the host. FAdV can be spread through horizontal and vertical transmission (Norfitriah, 2012). There is also differences in some strains can spread through either vertical and/or horizontal transmission (Winterfield, 1984). Virus can be presented in fecal material, semen, kidney, nasal mucosa, and tracheal. Thus it can be excreted in all excretion method, but high virus titer mostly found in feces (Saif, 2003). Vertical transmission is very important route because infected hatching chicks may excrete virus in feces from the time of hatching, but typically do not excrete until two to four weeks of ages due to maternal antibody. In horizontal transmission, massive interchange of different strains can happen via interchanging flocks from different

parent flocks. Spreading of virus excretion occur around four and six weeks (McFerran and Smyth, 2000). Horizontal occur only when infected brought to healthy chicken, thus aerial spread between farms cannot occur, but if spread by fomite between two farms can occur such as dust, eggs trolley, transport, and personnel (Saif, 2003). The virus appear to be latent stage when local immunity high, but during immune-compromised, the virus is unmasked and excretion reoccur (McFerran and Smyth, 2000). The incubation period is short about 24 to 48 hours following infections by natural route (Saif, 2003), but rate of horizontal spread through a flock is slow (McFerran, 1991).

## 2.4 Pathogenesis

The virus may enter the host body by inhalation or direct ingestion of contaminated feed. Then multiplies in the gastrointestinal and upper respiratory tract especially caeca and trachea, respectively (McFerran and Adair, 1977). Virus titers show peak level in feces and trachea of one-day-old chicks infected FAdV through orally and this titers still persists in feces on day 20 pi. Viraemia happened 8 hours pi and spread to most organs such as liver, spleen, caecal tonsils, lung, and bursa of Fabricius. In some cases, adenovirus can damage to bursa of Fabricius and lymphoid organ lead to immunosuppression stage. Therefore, the latent stage in virus shedding mostly occurs during stressful periods (Ritchie, 1995).

## 2.5 Clinical Signs

Inclusion body hepatitis normally seen in broiler chickens or meat producing birds at three to seven weeks of age, but can be seen as early as seven days old and as old as 20 weeks of age (Mahani and Azhar, 2006). It usually characterized by acute mortality peaking at day 3 to 4 pi and stopping at day 5 pi. Mortality usually ranges between 5 to 10%, but can reach up to 30% (McFerran and Smyth, 2000). The clinical signs that can be seen 12 to 24 hours prior to death are depression, weakness, respiratory depress, ruffled feather, inappetance, prostration, reduce body weight, and white pasty dropping adhere to the feather around cloaca (Saif, 2003).

## 2.6 Gross and Histological Lesions

FAdV primarily affecting liver and hemopoietic system. Gross lesion of IBH are friable, pale and enlarged liver. There also some degree of petechial to ecchymotic hemorrhages on liver. (McCracken and Adair, 1993). Histological examination revealed inclusion bodies in hepatocytes, eosinophilic during outbreak and basophilic from experimental in the chickens (Itakura *et al*., 1974). Hydropericardium can also be found in IBH and characterized by an accumulation of a clear straw-colored fluid in the pericardial sac (Saif, 2003). For gizzard erosion outbreak is usually happened in chicken affected by FAdV-1. The lesion showed that the keratin layer was rough, detached, and discolored by bleeding, necrosis and erosion of the mucosa (Norfitriah, 2012). Histology examination for gizzard sample revealed intranuclear inclusion bodies in glandular epithelial cells together with lesions of necrosis and degeneration of koilin layer, and

some infiltration of inflammatory reaction around lamina propria, submucosa, and muscle layers. On the respiratory disease, gross lesions always show tracheitis with excess mucus production (Saif, 2003).

## 2.7 Diagnosis

The basic suggestive diagnosis for FAdV cases can be made from the history of the farm and chickens, the clinical sign presented, mortality and morbidity percentage, gross lesions examination, and microscopic lesion examination through identify of intranuclear inclusion body. Besides that, viral particles can also be seen through standard transmission electron microscope (Norfitriah, 2012). ELISA test also can be used to detect group specific antibodies, and it is inexpensive and highly sensitive test (Mockett and Cook, 1983), but not specific due to cannot differentiate healthy and infected bird, and birds are frequently infected by many serotypes (Saif, 2003). Therefore, the gold standard procedures for confirming diagnosis of FAdV were virus isolation and identification (Norfitriah, 2012). Specimens of choice for virus isolation can be affected organ such a liver in cases of IBH, feces, pharynx, and kidney (Saif, 2003). Then the tissue samples produced into virus suspension to be inoculated in chicken embryo liver (CEL) or kidney (CEK), but the most sensitive medium for virus isolation is the embryonated chickens eggs with injection through yolk sac route. Virus neutralization test also can be used as standard procedure in identify the serotypes.

Lastly PCR are now widely applied to confirm the isolates to species and to serotypes by confirming the primers (Saif, 2003).

## 2.8 Intervention Strategies

### 2.8.1 Management procedures

Latent stage of adenovirus will start to be activated when immune status of chicken is compromised, thus any factors contributed to stress factor should be control and removed such as IBDV and CAV disease. As the virus in resistant to many inactivation agents, thus control importing the virus into farm is crucial. Therefore, must start at the primary breeder that free from adenovirus infection thus can prevent vertical and horizontal transmissions (Saif, 2003).

### 2.8.2 Vaccination

Fadly and Winterfield (1975) has used the Tipton isolates passed 74 times in chick embryo to produce IBH vaccination. The result revealed that vaccinated chicks are less susceptible to subcutaneous inoculation challenge than non-vaccinated. In addition, subcutaneous vaccination gave higher neutralization index than eye drop vaccination. Since IBH has many serotypes, thus vaccine productions have to incorporate into several serotypes. In cases of IBH-HPS disease, vaccine using an autogenous formalin-

inactivated vaccine was given an effective protection when given at dose 0.25mL at 10 to 15 days of age. In addition, dual vaccination of breeders using FAdV-4 and CAV are able to give effective protection to the chicken progeny (Saif, 2003). The vaccines production show effective in preventing mortality either natural outbreaks or experimental challenges, but development of safe vaccine that can transmit strong passive immunity and protect the chickens throughout the growing period may be established in the future (Almenesh *et al.*, 2012).

# 3.0 MATERIALS AND METHODS

## 3.1 FAdV Isolates

Two different FAdV isolates were used in this study and was kindly obtained and prepared by Professor Dr. Mohd Hair Bejo from recent field outbreaks of FAdV in Malaysia. The first isolate was UPM 11137 FAdV isolate originated from 24 days old broiler chickens. There was no clinical signs reported, but on the necropsy examination revealed gross lesions on pale, enlarged, multifocal area of necrosis and mild hemorrhage of the liver. On experimental trial in SPF embryo eggs via chorioallantoic membrane (CAM) route of inoculation, 50% mortality was recorded within 10 days post pi. This isolate was positive for FAdV with H1/H2 and H3/H4 of 1219bp and 1319bp, respectively (Juliana *et al.*, 2014).

The second isolate was originated from 18 days old broiler chickens. The farm history reported that mortality started from day 7 of age with mortality rate of 0.5% to 1.0% per day. On necropsy, pale, enlarged, yellowish, and mild multifocal area of necrosis and hemorrhages of liver was recorded. Mortality of 60% within 10 days pi was recorded in SPF embryonated chicken eggs. The isolate was confirmed as FAdV using PCR test.

## 3.2 SPF Chicks

One-day-old SPF chickens were obtained from the Malaysian Vaccine Pharmaceutical Sdn. Bhd. (MVP), Puchong, Selangor.

### 3.3 Experimental Design

Thirty six one-day-old SPF chicks were divided into 3 major groups namely groups A, B and C. The chickens were then divided into sub-group namely the sacrificed and mortality groups. Groups A and B were inoculated (0.5mL/chick) with FAdV isolates A (UPM11134) and B (UPM1127), respectively via intra-peritoneal route (Figure 1). The group C was acted as a control group by remained un-inoculated and was isolated in different building from the inoculation chickens. The chicks were observed at least twice daily and any clinical abnormality and mortality were recorded throughout the trial. Three chicks were sacrificed at day 0 pi from group C for necropsy and histological examination. Three chicks each from the sacrificed groups A, B, and C were randomly selected, weighed, and sacrificed by cervical dislocation at days 7 pi and 14 pi. On necropsy, the gross lesions were recorded and samples of liver, spleen, gizzard and trachea were collected and fixed in 10% buffered formalin for histological examination. The mortality percentage of the chicks was recorded daily in the mortality groups A, B, and C until day 14 pi.

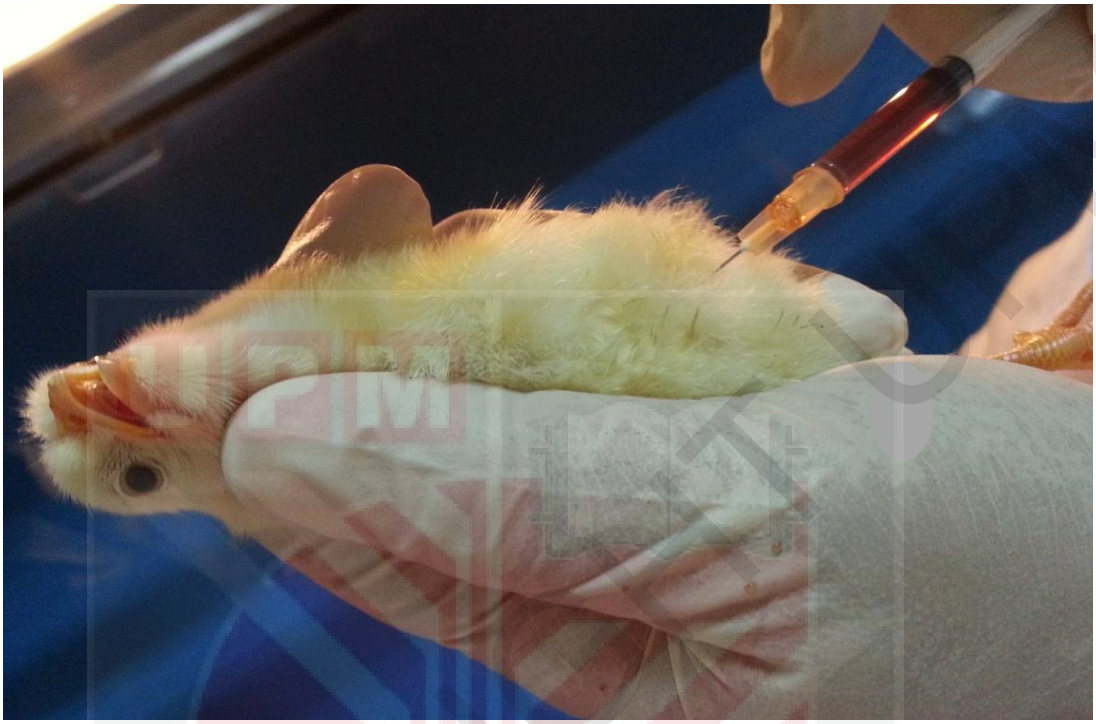

**Figure 1:** FAdV inoculation via intra-peritoneal route (0.5mL/chick) with UPM 11134 isolate for group A.

### 3.4 Clinical signs

Clinical signs were observed twice a day for any abnormality.

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### 3.5 Gross lesions

On necropsy, all gross lesions were recorded.

### 3.6 Histopathology

### 3.6.1 Hematoxylin and eosin stains

Samples of liver, gizzard, spleen, and trachea from mortality and sacrificed chicks were fixed in 10% buffered formalin for at least 48 hours. The samples were trimmed to 0.5cm thickness in block size and subsequently dehydrated in a series of alcohol, clean with xylene, and embedded in paraffin wax using an automated tissue processor (Leica). Tissues were then trimmed and section about 5µm and 4µm, respectively using microtome (Leitz) and mounted on glass slides. Next, the mounted tissue dewaxed by series of alcohol and stained with hematoxylin and eosin (HE) (Bancroft e*t al.*, 1996). Lastly the stained tissues were examined under x20, x40, and x100 objectives and histological changes were recorded.

# 4.0 RESULTS

## 4.1 Clinical signs

Weakness, inappetance, depression, and ruffled feathers were observed in chicks 12 – 24 hours prior to death (Figure 2 a,b,c). There was increased in production of pasty white fecal material that can be seen adhered to feather around the cloaca region and also present low body score condition. There were no abnormal clinical signs seen for group C and the chicks remained healthy until end of the trial. The chicks that survived at day 7 showed reduction in body weight.

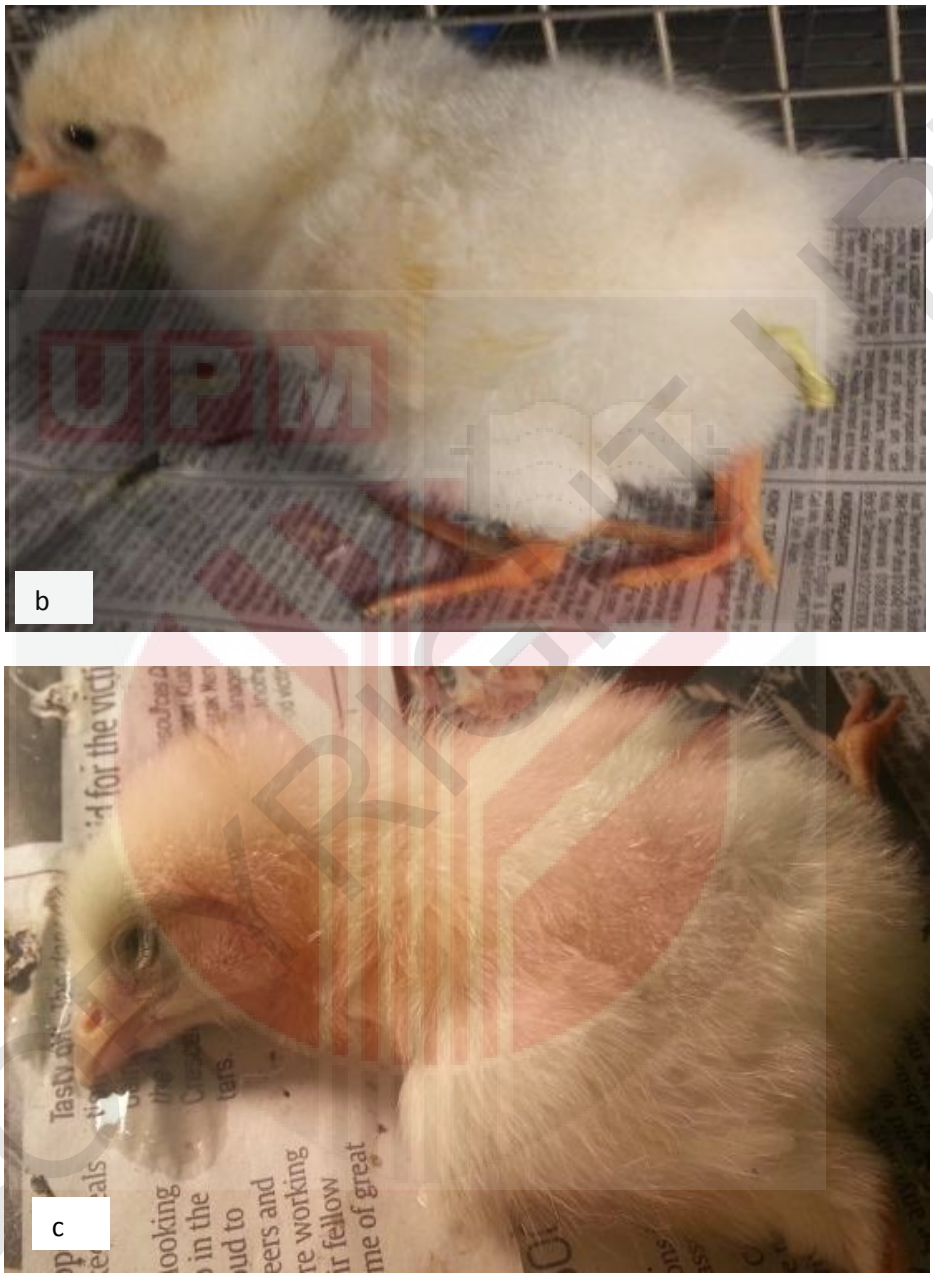


**Figure 2:** Clinical signs showed from both groups A and B within 12 to 24 hours prior to death were (a) inaapetance, reduce weight gain, depression, weakness, and position prostration, (b) white pasty dropping adhered to the feather around cloaca, and (c) watery nasal discharge, and ruffled feathers.

## 4.2 Body weight

There were no body weight measurement from group A as all chicks died before day 7, but only 1 body weight measurement was taken from chick group B (49g) and 3 chicks group C (80.333 ± 1.247g) on day 7.

## 4.3 Mortality

### 4.3.1 Cumulative Mortality (%)

UPM 11134 and UPM 1127 FAdV isolates caused high mortality to both the mortality and sacrificed groups with 100% and 90.9% mortality in groups A and B, respectively recorded at day 4 pi (Figure 3). Cumulative mortality from group A started at day 2 pi (54.54%), day 3(90.9%), and achieved 100% at day 4 pi. The cumulative mortality from group B started at day 2 pi (45.45%), day 3 pi (81.82%), and 90.9% at day 4 pi. No death was recorded in group C throughout the experimental period.

### 4.3.2 Sacrifice group

There was no sampling conducted from group A because all chicks died (100%) before day 7 pi. At day 7 pi, 1 and 3 chicks were sacrificed from groups B and C, respectively.

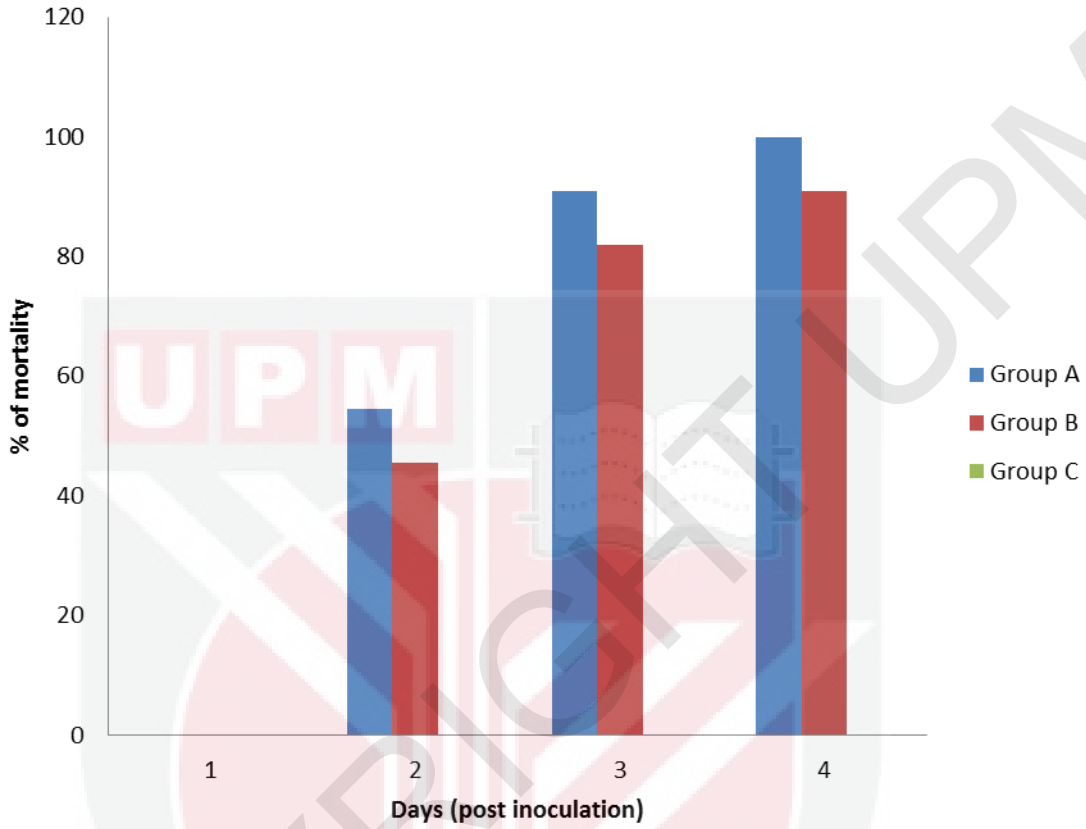


**Figure 3:** Cumulative mortality of the SPF chicks in groups A, B, and C throughout the trial.

## 4.4 Gross Lesions

### 4.4.1 Group A

Subcutaneous jaundice, hydropericardium with straw colour fluids, and empty crop were recorded in chicks which died within days 1 and 4 pi from groups A and B. The livers of

chicks were enlarged, shiny, friable, yellowish at the peripheral, and present of petechial hemorrhages throughout the trial (Figure 4 a, Figure 5 a,b). The spleen was pale and enlarged (Figure 4b).

### 4.4.2 Group B

Subcutaneous jaundice, hydropericardium with straw colour fluids, empty crop, and enlarged liver with petechial hemorrhage were observed in the dead chicks at day 2 pi (Figure 6a). The spleen was slight enlarged with pale and yellowish at day 2 and 3 pi (Figure 6b). The lesions at day 3 and day 4 pi showed the same lesions on liver, but with more severe hemorrhage and jaundice (Figure 7a,b). The liver showed dark yellow with hemorrhage at day 7 pi.

### 4.4.3 Group C

The liver, spleen, gizzard, and trachea were remained normal throughout the trial.

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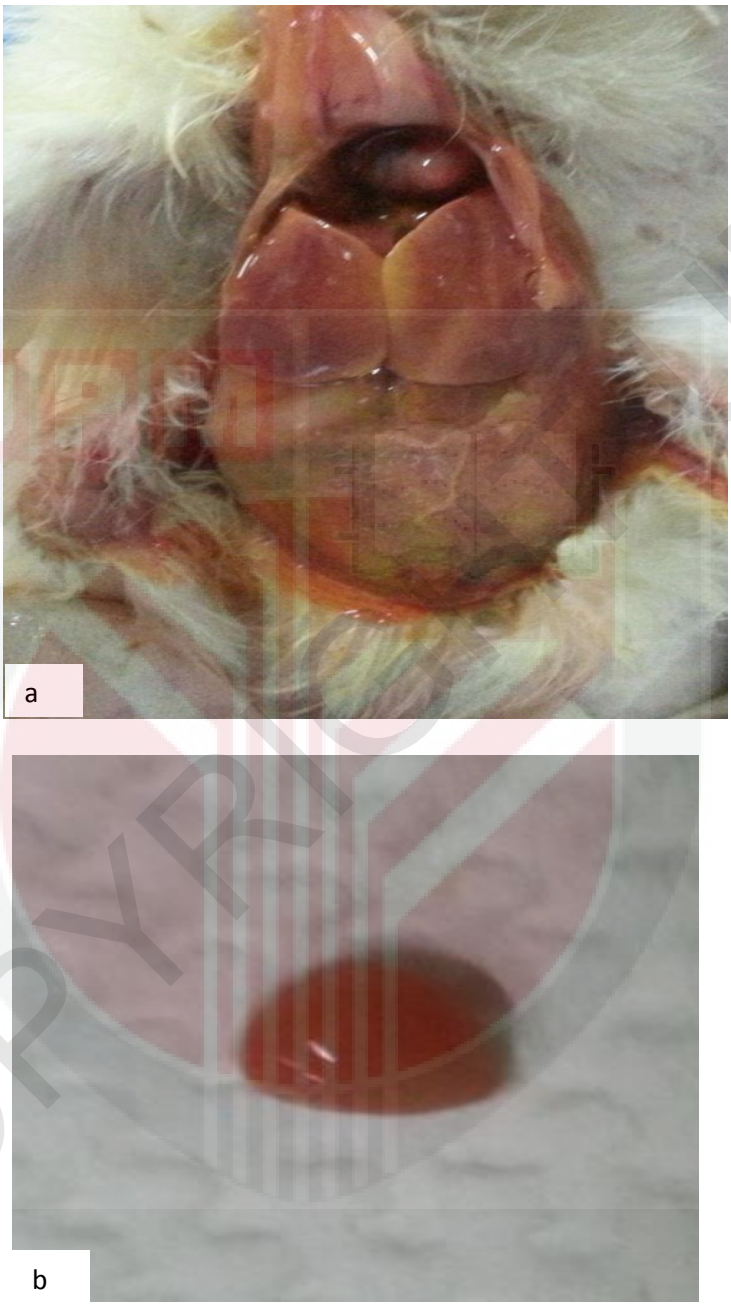


**Figure 4:** Group A. (a) Friable, pale, enlarged, yellowish and petechial hemorrhage of liver, and (b) spleen was markedly enlarged at day 2 pi.

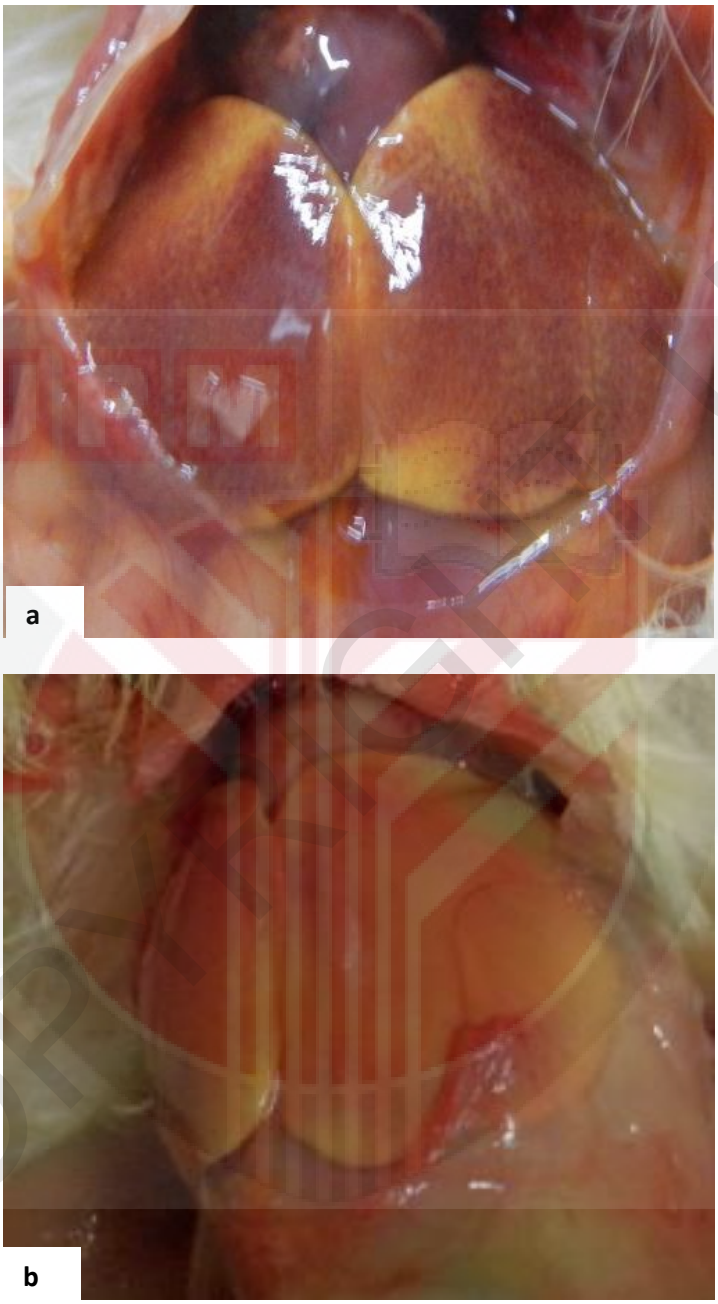


**Figure 5:** Group A. Friable, shiny, enlarged, yellowish, and petechial hemorrhage of liver at (a) day 3 pi and (b) day 4 pi, respectively.

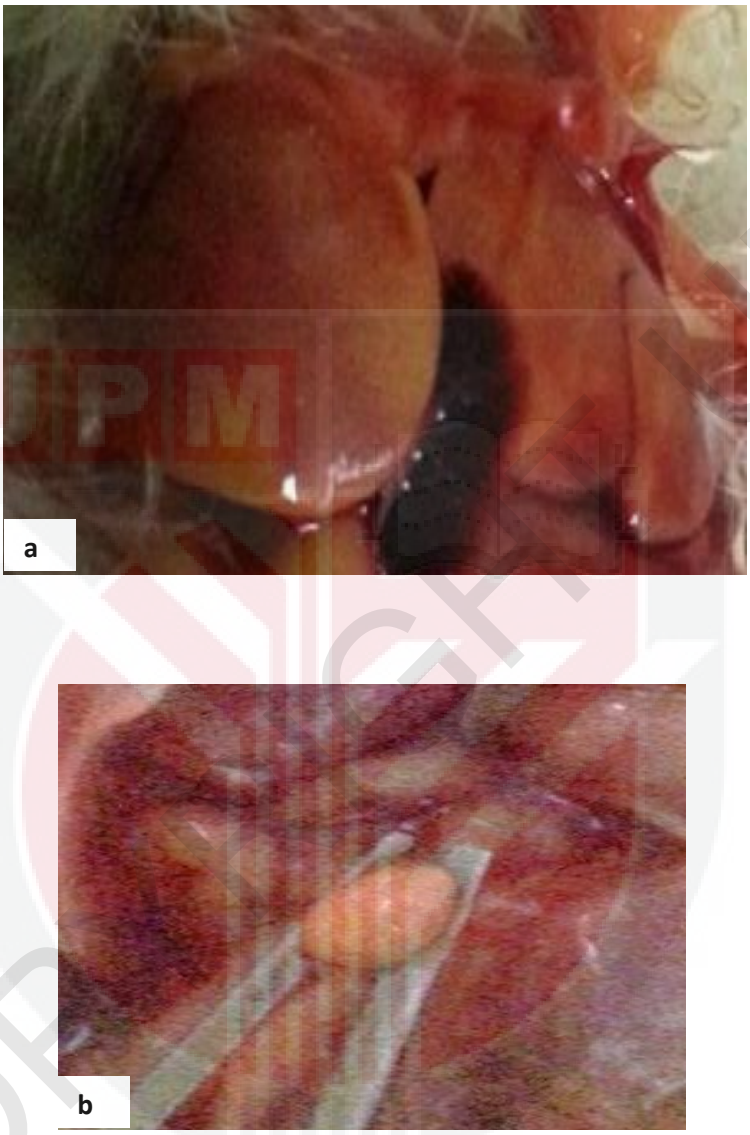


**Figure 6:** Group B. (a) Friable, pale, enlarged, yellowish and mild petechial hemorrhage of liver, and (b) spleen was markedly enlarged and pale color at days 2 and 3 pi.


**Figure 7:** Group B. Friable, shiny, enlarged, yellowish, and petechial hemorrhage of liver at (a) day 3 pi, and (b) day 4 pi.

## 4.5 Histolopathology

### 4.5.1 Group A

**Liver**

A few basophilic intranuclear inclusion bodies were observed in the hepatocytes started at day 2 pi until day 4 pi (Figure 8a, and Figure 9a,b).

**Gizzard**

Few basophilic intranuclear inclusion bodies were found at day 2 pi in the glandular epithelium of gizzard (Figure 8b).

**Trachea**

No significant finding was recorded throughout the period.

**Spleen**

No significant finding was recorded throughout the period.

### 4.5.2 Group B

**Liver**

A few basophilic intranuclear inclusion bodies were observed in the hepatocytes started at day 2 pi until day 7 pi (Figure 10a,b and Figure 11).

**Gizzard, spleen and trachea**

No significant finding was recorded throughout the trial.

### 4.5.3 Group C

The liver, gizzard, spleen and trachea were remained normal throughout the trial.

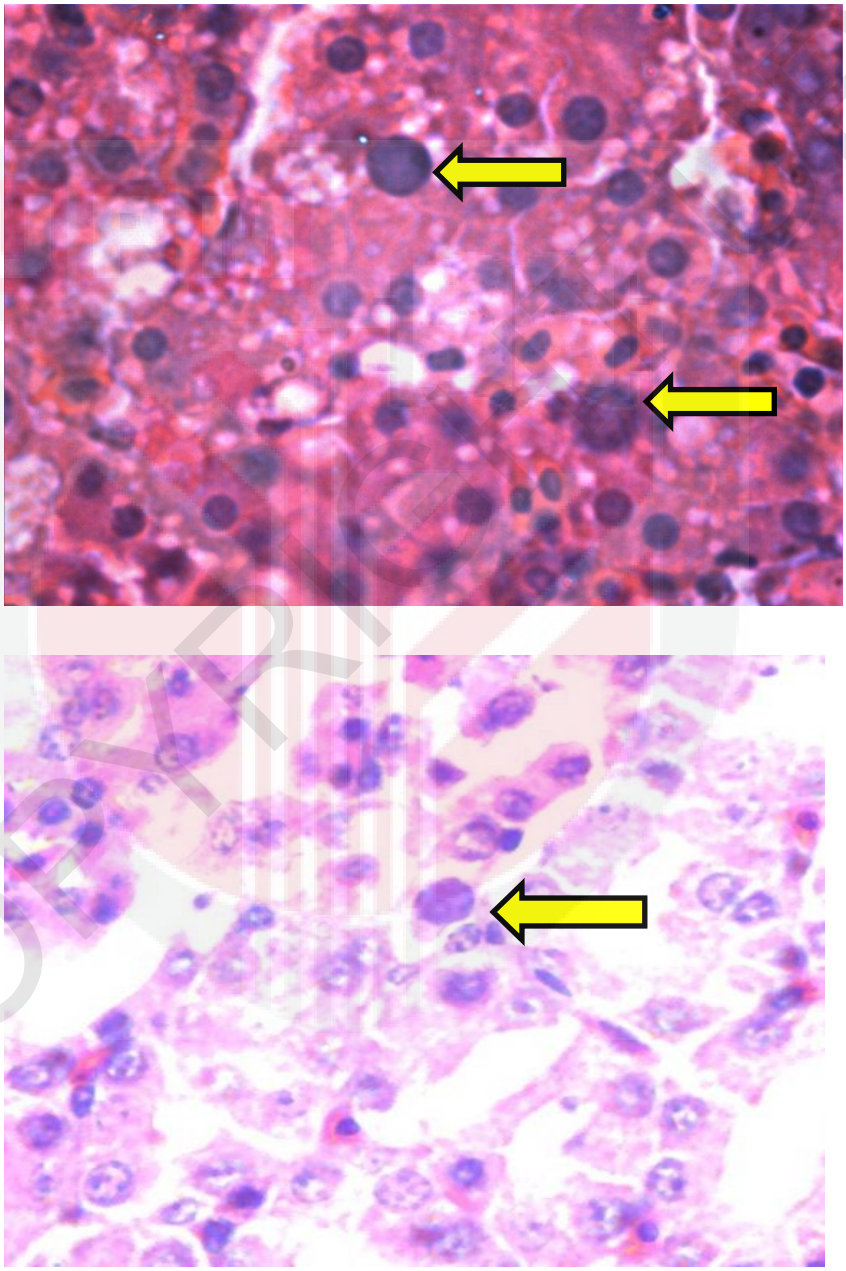

**Figure 8:** Group A. (a) A few basophilic intranuclear inclusion bodies in hepatocytes, and (b) in glandular epithelium of gizzard at day 2 pi. HE, x1000.

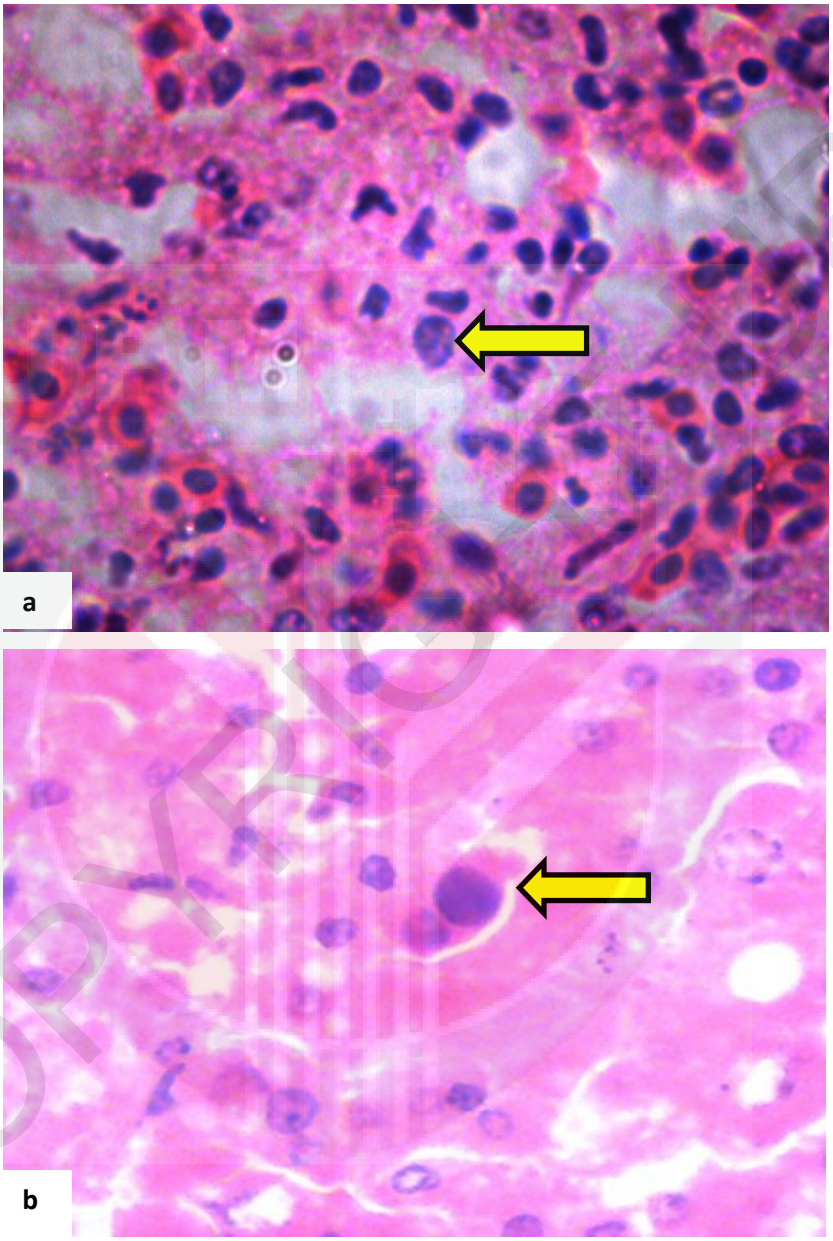


**Figure 9:** Group A. (a) Large basophilic intranuclear inclusion body in hepatocytes at day 3 pi (b) and day 4 pi. HE, x1000.

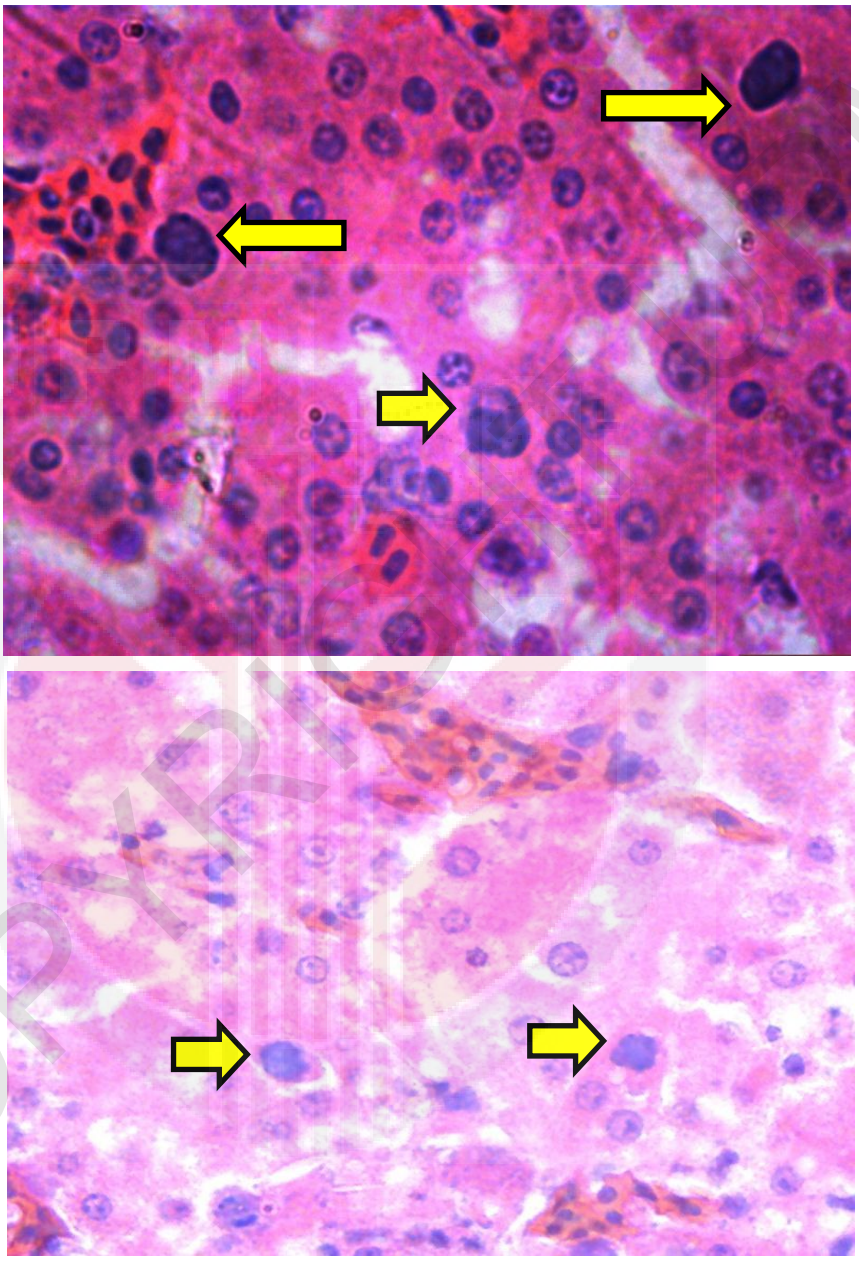

**Figure 10:** Group B. (a) A few basophilic intranuclear inclusion bodies in hepatocytes at day 2 pi and (b) day 3 pi. HE, x1000.

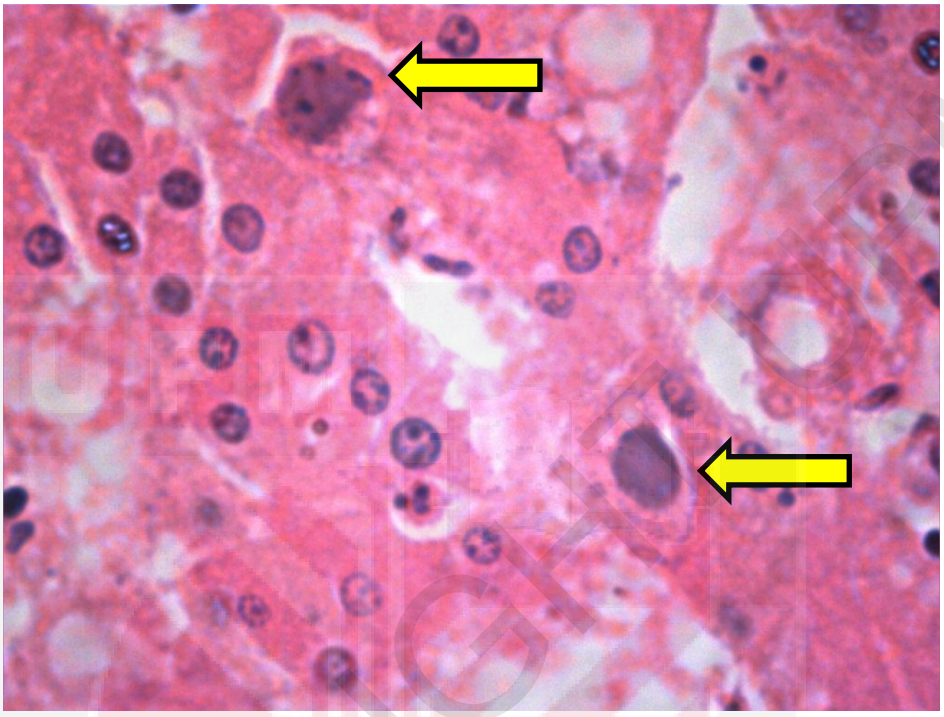

**Figure 11:** Group B. A few basophilic intranuclear inclusion bodies in hepatocytes at day 4 pi. HE, x1000.

## 5.0 DISCUSSION

Pathogenicity of FAdV in SPF chicks was determined by clinical signs, mortality percentage, gross lesions and histopathological examination. The result from this experiment showed that both FAdV isolates UPM11134 and UPM1127 from recent FAdV outbreaks in Malaysia is highly pathogenic to one-day-old SPF chicks when inoculated through intra-peritoneal route. This is in contrast to previous finding that showed infection of FAdV-9 in one-day-old chicks through intraperitoneally caused 15% mortality (Erny *et al*., 1996). Infection of FAdV-9 through subcutaneously was reported to cause 100% and 20% mortality to one and seven day old chicks, respectively (Alvarado *et al*., 2007). This suggests that, FAdV is varying in pathogenicity due to different route of inoculation and perhaps different virus strains (Erny *et al*., 1996).

The present study showed that the FAdV infected one day old SPF chicks caused clinical signs of weakness, depression, reduce body weight, ruffled feathers and white pasty dropping adhered to the feather around cloaca. The gross lesion caused by both isolates was showed almost the same lesions, but different degree of severity of hepatitis. The histological examination showed present of basophilic intranuclear inclusion bodies in the hepatocytes. In group A, 100% cumulative mortality was achieved by day 4 pi, and in group B 90% cumulative mortality was recorded by day 4 pi. In groups A and B, mortality started at day 2 pi. The liver was swollen, pale with pethechial hemorrhage. It suggests that infection by intra-peritoneal route gives 24 to 48

hours incubation period for the virus to replicate and multiply in the primary target organ of liver. This will lead to secondary viremia and separation of the virus into other organ such as gizzard and spleen. The finding of gizzard erosion from field outbreak is different from the experimental might be due to acute mortality happened to the chicks before it can develop lesion in the gizzard. But, only one sample from isolates UPM11134 able to show basophilic intranuclear inclusion body in glandular epithelium at day 2 pi.

Adenovirus is a potential virus to cause high mortality, although it may act as secondary pathogen due to immune-suppressed factor or agent such as IBDV and CAV infections. The SPF chicks are the most suitable animal to be used in the study due to absence of maternal derived antibody and highly susceptible to the disease (Norfitriah, 2012). A study of Nakamura *et al*., (2000) showed that one-day-old chicks infected with FAdV-2 or FAdV-8 via intra-muscular route caused 100% mortality and gross lesions, whereas when three weeks old SPF chicks were used, no mortality, gross lesion and microscopic lesions of IBH was observed. This may showed that, age of chicks affect the pathogenicity of FAdV. Development of immune system started from embryonic period until hatching. The first 7 days of life, maternal antibodies play an important role of defense mechanism. Next, primary immune organ of thymus and bursa of Fabricius together with secondary immune organ such as spleen act as the defense mechanism in the chicken. In addition, it also depends on natural killer cells, antigen-presenting cells, epithelial cell, and lymphoid cells. Young chickens that less exposed to environmental

antigen had undeveloped germinal centers and as a consequent to that, young chicken was not immune-competent as the older chickens.

## 6.0 CONCLUSION

FAdV isolates of Malaysia from recent field outbreaks of IBH are pathogenic in one day old SPF chicks via intra-peritoneal route injection. FAdV isolates UPM11134 and UPM1127 caused 100% and 91% mortality in the chicks, respectively. The FAdV isolates caused gross lesions typical of inclusion body hepatitis with basophilic intranuclear inclusion body in hepatocytes.

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## APPENDIX 1

**Table 1: Experimental design for pathogenicity study of two FAdV isolates.**

| Group | Day pi – Sacrifice group | | | Mortality (%) |
|---|---|---|---|---|
| | 0 | 7 | 14 | |
| **A** | 0 | 3 | 3 | 5 |
| **B** | 0 | 3 | 3 | 5 |
| **C** | 3 | 3 | 3 | 5 |
| **Total SPF chicks is respective group** | | | 21 | 15 |
| **Total SPF chicks were used** | | | 36 | |

**Group A :** FAdV isolate UPM11134.

**Group B :** FAdV isolate UPM1127.

**Group C :** Control.

## APPENDIX 2

**Table 2: Cumulative mortality of SPF chicks in group A inoculated with UPM11134 FAdV isolate.**

| Day of pi | Mortality | Cumulative mortality | Percentage (%) |
|---|---|---|---|
| 2 | 6 | 6 | 54.54 |
| 3 | 4 | 10 | 90.9 |
| 4 | 1 | 11 | 100 |

**Table 3: Cumulative mortality of SPF chicks in group B inoculated with UPM1127 FAdV isolate.**

| Day of pi | Mortality | Cumulative mortality | Percentage (%) |
|---|---|---|---|
| 2 | 4 | 4 | 45.45 |
| 3 | 4 | 8 | 81.81 |
| 4 | 1 | 10 | 90.9 |

~ One SPF chick in the sacrifice group B remained normal and was sacrificed on day 7.

~ The SPF chicks in the control group remained normal throughout the trial.