

Pathological Changes and Virus Detection in Chickens Infected with Fowl Adenovirus 8b

Sohaimi NM^{1*}, Hamsa NN¹, Bejo MH², Mazlan M², Clifford UC²

¹Department of Veterinary Laboratory Diagnosis, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia.

²Department of Veterinary Pathology and Microbiology, Faculty of Veterinary Medicine, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
*Email: fitriahsohaimi@upm.edu.my

(received 4-02-2025; revised 20-07-2025; accepted 19-09-2025)

ABSTRAK

Sohaimi NM, Hamsa NN, Bejo MH, Mazlan M, Clifford UC. 2025. Perubahan patologi dan deteksi virus pada ayam yang terinfeksi oleh *fowl adenovirus* 8b. JITV 30(3):159-169. DOI:<http://dx.doi.org/10.14334/3502>.

Fowl adenovirus (FAdV) serotipe 8b adalah agen virus patogen dari hepatitis badan inklusi (IBH) pada ayam dengan mortalitas tinggi di peternakan yang terkena dampak. Tujuan dari penelitian ini adalah untuk menentukan perubahan patologis dan untuk mendeteksi FAdV dengan metode molekuler pada organ ayam bebas patogen spesifik (SPF) yang terinfeksi FAdV serotipe 8b. Isolat FAdV, UPM1901, diinokulasi pada anak ayam umur sehari melalui *route oral* diikuti dengan pengambilan sampel pada jam ke-0 (h) untuk kelompok kontrol dan selanjutnya pada jam ke-12, 24, hari ke-3 dan ke-7 pasca inokulasi (pi). Berat badan, hati dan bursa Fabricius diukur dan dilanjutkan dengan pengumpulan organ untuk pemeriksaan histopatologi dan deteksi virus menggunakan reaksi berantai polimerase (PCR). Hasil pengamatan menunjukkan bahwa ayam dalam kelompok yang terinfeksi mengalami depresi dan tidak nafsu makan sebelum kematian mendadak dengan total mortalitas 48% dalam hari ke-5pi. Berat badan rata-rata secara signifikan lebih rendah ($P<0,05$) dibandingkan kelompok kontrol pada hari ke-3 dan ke-7 pi. Pembengkakan, perdarahan, dan nekrosis hati terekam dengan banyaknya badan inklusi intranuklear basofilik di dalam hepatosit bersama dengan perdarahan limpa dan ginjal pada hari ke-3 dan seterusnya. Asam nukleat FAdV terdeteksi di sumsum tulang pada 12 jam pi diikuti oleh organ lain pada 24 jam pi di limpa, hati, dan tonsil sekum. Pada hari ke-3 dan ke-7 pi, DNA virus terdeteksi di semua organ yang dipilih. Studi ini membuktikan bahwa FAdV patogen serotipe 8b menyebabkan kematian anak ayam, perubahan patologis dengan keberadaan DNA virus pada 12 jam pi di organ limfoid sebelum didistribusikan ke organ lain dan memerlukan strategi pengendalian yang efektif terhadap penyakit tersebut.

Key Words: *Fowl Adenovirus* (FAdV) serotip 8b, Hepatitis Badan Inklusi (IBH), Organ Limfoid, Reaksi Polimerase Berantai (PCR)

ABSTRACT

Sohaimi NM, Hamsa NN, Bejo MH, Mazlan M, Clifford UC. 2025. Pathological changes and virus detection in chickens infected with fowl adenovirus 8b. JITV 30(3):159-169. DOI:<http://dx.doi.org/10.14334/3502>.

Fowl adenovirus (FAdV) serotype 8b is a pathogenic viral agent of inclusion body hepatitis (IBH) in chickens with high mortality in affected farms. The objective of this study is to determine the pathological changes and to detect FAdV by molecular method in organs of specific-pathogen-free (SPF) chickens infected with FAdV serotype 8b. FAdV isolate, UPM1901, was inoculated into day-old chicks via oral route followed by sampling at 0 hour (h) for control group and subsequently at 12h, 24h, days 3 and 7 post-inoculation (pi). Body weight, liver and bursa of Fabricius weights were measured followed by organs collection for histopathological examination and virus detection using polymerase chain reaction (PCR). It showed that chickens in the infected group were depressed and inappetence prior sudden death with 48% total mortality within day 5pi. Mean body weight was significantly low ($p<0.05$) than control group at days 3 and 7pi. Swollen, haemorrhages and necrosis of liver were recorded with numerous basophilic intranuclear inclusion bodies in the hepatocytes along with haemorrhages spleen and kidney at 3 dpi onward. FAdV nucleic acid detected in bone marrow at 12h pi followed by the other organs at 24h pi in spleen, liver and cecal tonsils. At days 3 and 7pi, viral DNA was detected in all the selected organs. This study proves that pathogenic FAdV serotype 8b caused chick's mortality, pathological changes with existence of viral DNA at 12h pi in lymphoid organ prior distributed to other organs and necessitates effective control strategies against the disease.

Key Words: Fowl Adenovirus (FAdV) serotype 8b, Inclusion Body Hepatitis (IBH), Lymphoid Organs, Polymerase Chain Reaction (PCR)

INTRODUCTION

Fowl adenovirus (FAdV) is a causative agent of inclusion body hepatitis (IBH), hepatitis hydropericardium syndrome (HHS), adenovirus gizzard erosion (AGE), respiratory disease and necrotizing pancreatitis (Kiss et al. 2021; Islam et al. 2023). FAdV belongs to genus of Aviadenovirus of family *Adenoviridae*. It is classified into five species (FAdV-A to FAdV-E) and has 12 serotypes (Harrach & Kajan 2011). FAdV is a non-enveloped icosahedral virion with a linear double-stranded DNA genome.

Virus can be detected by viral isolation and molecular detection using Polymerase Chain Reaction (PCR). Vertical transmission of the virus from the hens to the eggs and horizontal transmission from the infected bird to another by direct contact with faeces, respiratory discharges and fomites (Pereira et al. 2014; El-Shall et al. 2022). FAdVs are distributed globally and are recognized as economically significant pathogens in many countries. They can infect all domestic avian species across various age groups, with young broiler chickens being the most commonly affected. However, FAdVs can also be isolated from clinically healthy birds (Adel et al. 2021). Clinical disease of IBH in chickens occurs worldwide and is caused by 12 serotypes of FAdV, with mortality rates ranging from 10% to as high as 30% (Song et al. 2024).

The first case of IBH in Malaysia was reported in 2005 by Hair-Bejo. Additionally, FAdV serotype 8b was reported to be the primary agent of IBH that is highly pathogenic in SPF chickens (Sohaimi et al. 2019). Infected chickens with IBH will show clinical signs such as depression, inappetence, ruffled feathers and sudden onset death (Cizmecigil et al. 2020). The liver and kidney are primarily impacted organs in IBH. Liver appeared pale, yellow, swollen, and friable with petechial hemorrhages while the kidneys were pale and swollen (Cizmecigil et al. 2020; Norina et al. 2016). Histopathologically showed eosinophilic intranuclear inclusion body in the hepatocytes and infiltration of mononuclear inflammatory cells indicated hepatitis (Hair-Bejo 2005; Norina et al. 2016).

The IBH outbreak in chickens caused a high mortality rate and poor performance leading to serious economic losses to the affected farm. To date, the study on the effect of pathogenic FAdV serotype 8b at specific period following infection is scanty and necessitate further investigation for effective control and prevention strategies. It is important to know the impact of the disease on lymphoid organs and other tissues for better understanding of the virus tropism in chicken. The objectives of this study were to determine the pathological changes and to detect FAdV by molecular method in organs of specific-pathogen-free (SPF) chickens infected with FAdV serotype 8b.

MATERIALS AND METHODS

Virus isolate

FAdV isolate namely, UPM1901, was obtained from Johore in 2019 from 25-day-old commercial broiler chickens with a history of 1.22% mortality. Liver was swollen, necrotized and haemorrhages upon necropsy from the dead chickens. The isolate was characterized as FAdV species E serotype 8b, showing 98% nucleotide sequence identity in the L1 loop regions of the hexon gene to reference serotype 8b strains in the GenBank database (Sohaimi et al. 2022).

Preparation of virus inoculum

Liver samples were processed by subjecting them to three cycles of freezing and thawing, then pooled and macerated in a sterilized mortar and pestle to prepare a 1:2 (w/v) suspension in sterile phosphate-buffered saline (PBS, pH 7.4). The suspension was centrifuged at $381 \times g$ for 30 minutes for clarification. The supernatant was collected, filtered through a $0.45 \mu\text{m}$ membrane, and treated with Penicillin-Streptomycin-Amphotericin B solution (Gibco®, USA) at a 1:10 dilution. It was then incubated at 4°C for 1 hour prior to inoculation onto the chorioallantoic membrane (CAM) of 9-day-old SPF embryonated chicken eggs (Alemmesh et al., 2012). The eggs were incubated at 37°C until day 10 post-inoculation (pi). All embryos died, exhibiting necrotized and swollen livers within 7 to 9 days pi. Subsequently, the livers were harvested, homogenized, and processed for inoculation into SPF CEE via the CAM route as the second passage. This process was repeated for three passages to increase the volume of homogenate liver embryos. The virus inoculum from liver embryos at the third passage, with a virus titer of $10^{7.1} \text{TCID}_{50}/\text{ml}$, was used in this study (Reed & Muench 1938).

Experimental design and sampling protocol

The study protocols described were undertaken in accordance with criteria approved by Universiti Putra Malaysia (UPM), Institutional Animal Care and Use Committee (IACUC): UPM/IACUC/AUP-U019/2024. Thirty-six (36) day-old chicks were divided into 2 major group namely, FAdV infected group and control group. Sixteen (16) chicks were assigned in FAdV infected group and twenty (20) chicks in the control group. All chicks in FAdV infected group were inoculated with 1ml FAdV isolate, UPM1901, at virus titer $10^{7.1} \text{TCID}_{50}/\text{ml}$ via oral route at day old. All chicks in the control group were uninoculated and used as the control group in this study. All chickens were monitored throughout the trial with feed and water were given *ad libitum*. At 0 hour (h)

post-inoculation (pi), body weight was recorded, and blood samples were collected via wing vein from four chicks in control group prior sacrifice by cervical dislocation. Sample of liver, thymus, bursa of Fabricius, spleen, bone marrow, caecal tonsils and kidney were harvested from chicks for virus detection by PCR test and histological examination. Sampling was performed subsequently in both groups at 12 hours (h), 24 h, day 3 and 7 pi.

Clinical signs and mortality observation

All chicks were monitor for any clinical signs associated with FAdV infection throughout the trial such as changes in behavior, physical appearance and feed or water consumption. Any mortality was recorded until the end of the study (Sohaimi & Bejo 2021).

Measurement of liver and bursa of Fabricius weight

Liver and bursa of Fabricius were weight as gram (g) unit and were calculated its ratio to body weight (Sohaimi & Bejo 2021).

Gross and histopathological examinations

All chicks were observed for any abnormalities associated with FAdV upon necropsy. Any gross lesions were recorded throughout the trial. Sample of liver, thymus, bursa of Fabricius, spleen, bone marrow, caecal tonsils and kidney were harvested and fixed in 10% buffer formalin for histological examination. All samples were stained with Haematoxylin and Eosin (H&E) according to method by Gurina & Simms (2023). All slides were viewed, evaluated and described based on the severity of the lesions.

Statistical data analysis

Mean data for body weight, liver weight, liver to body weight ratio and bursa of Fabricius to body weight ratio were compared between infected and control groups using independent T test based on sampling day using SPSS software version 27. Significant differences were measured at alpha $P < 0.05$ value (Sohaimi & Bejo 2021).

Molecular detection (DNA extraction, conventional PCR and gel electrophoresis)

All the tissue samples from chickens were extracted using DNA extraction kit (Kylt® RNA/DNA

purification, Germany) according to the manufacturer's protocol. The eluted DNA was measured for concentration and DNA purity by using a biophotometer (Eppendorf, Germany). Extracted DNA sample was used as template for amplifying hexon gene using MyTaq™ HS Mix (Bioline, UK). Published primer was used namely Hexon A (forward) and Hexon B (reverse) with expected PCR size product at 897 base pairs (bp) (Sohaimi et al. 2022). Conventional PCR was performed according to the protocol published by Sohaimi et al. (2022). Subsequently, all PCR products were separated in a 1.5% agarose gel electrophoresis using RedSafe™ Nuclei Acid Staining solution (iNtRON, Korea) and 1kb DNA Marker (GeneDirex, USA). Electrophoresis was conducted at 110 volts for 25 minutes prior visualization of DNA fragment band under U.V. transillumination.

RESULTS AND DISCUSSION

Clinical signs and mortality

It was demonstrated that pathogenic FAdV serotype 8b isolate UPM1901 into day old SPF chicks via oral route induced IBH similarly as seen in the natural outbreak throughout the trial. The virus isolate caused several clinical signs associated with FAdV infection such as depression, diarrhoea, ruffled feathers and inappetence in chickens prior to sudden death at day 3pi onwards. 48% mortality rate was recorded in infected chickens during the experiment. Three chickens (9%) died at day 3pi, followed by seven chickens (23%) died at day 4pi and five chickens (16%) died at day 5pi (Figure 1). All chickens in the control group were clinically healthy and normal throughout the trial. This finding was consistent with previous works shown in SPF chickens due to FAdV serotype 8b infection using intramuscular route at 1 day old (Sabarudin et al. 2021).

Mean body weight of chickens

For control group, mean body weight at 0h pi was 47 ± 2.04 g and increasing to 60.33 ± 1.20 g at day 7 pi. Similarly, body weight for the infected group was increased from 46.8 ± 1.39 g at day 3 pi to 57.4 ± 2.0 g at day 7 pi. However, the mean body weight of the infected group was significantly lower ($P < 0.05$) than the control group at day 3 and 7 pi (Figure 2) due to inappetence and reduced feed consumption which similarly showed in the field IBH disease in chickens (Tsiouris et al. 2022).

Liver weight and liver-to-body weight ratio

Mean liver weight of the control group was 2.8 ± 0.5 g, 2.2 ± 0.2 g, 2.9 ± 0.3 g, 3.1 ± 0.2 g and 3.8 ± 0.3 at 0 h, 12

h, 24 h, day 3 and 7 pi, respectively. For FAdV infected group, mean liver weight was 3.6 ± 0.1 g, 3.1 ± 0.3 g, 4.1 ± 0.2 g and 5 ± 0.3 g at 12 h, 24 h, day 3 and 7 pi, respectively. There is high liver weight significantly ($P<0.05$) at 12 h, day 3 and 7 pi in the infected group than control group (Figure 3). Analysis on liver-to-body weight ratio revealed infected group has high ratio significantly ($P<0.05$) than control group at 12h, days 3 and 7pi (Figure 4). It indicated that the increased liver weight and liver-to-body weight ratio observed in infected chickens as early as 12 h pi, and consistently at day 3 and 7 pi, reflect liver damage caused by FAdV replication, characterized by liver enlargement and swelling due to the virus's primary tropism. This finding consistent with previous report after inoculated via subcutaneous route (Sun et al. 2024).

Mean bursa of Fabricius weight

Mean bursa weight for control group was 0.01 ± 0 g at three consecutive periods, 0 h, 12 h and 24 h pi. At day 3 and 7 pi, it was 0.02 ± 0 g and 0.05 ± 0 g, respectively. In infected group, the means was 0.01 ± 0 g at 12 h and 24 h pi followed by 0.02 ± 0 g at day 3 pi and 0.03 ± 0 g at day 7 pi. There is no significant difference ($P>0.05$) in mean bursa weight between groups at 12 h, 24 h and day 3 pi, however, the mean was low significantly ($P<0.05$) in the infected group (0.03 ± 0 g) compared to the control group (0.05 ± 0 g) (Figure 5). Low bursa of Fabricius weight suggested due to atrophy and compatible with previous work after infected with FAdV serotype 4 isolates in the immunosuppressed birds (Naeem et al. 1995; (Rashid et al. 2024).

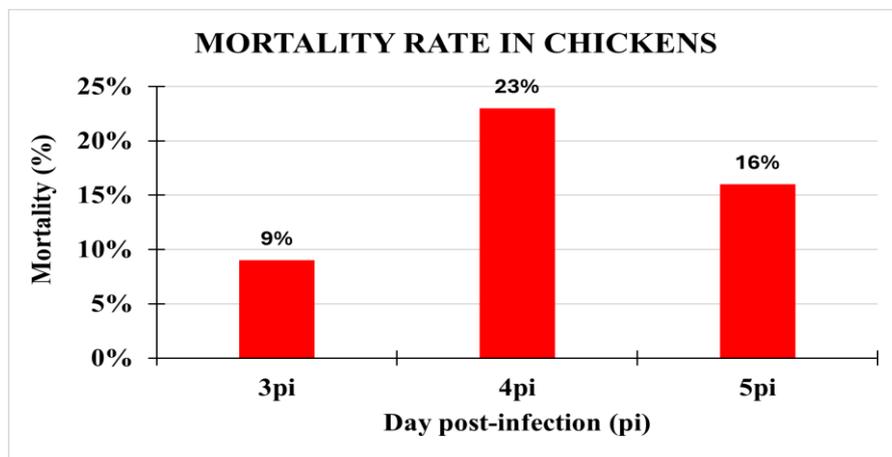


Figure 1. Mortality rate in specific pathogen free (SPF) chickens following infection with FAdV serotype 8b isolate UPM1901 via oral route at day-1-old

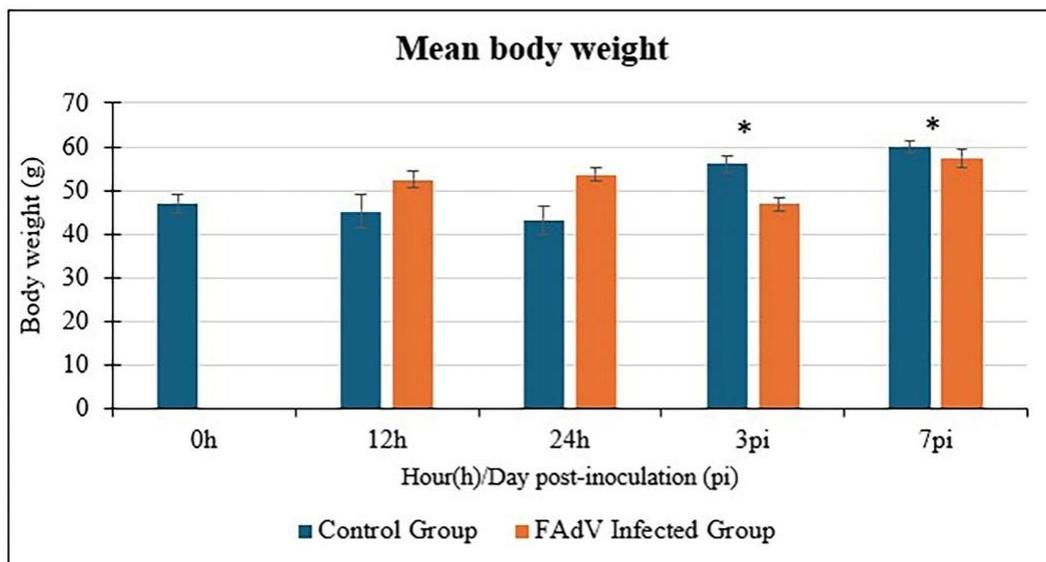


Figure 2. Mean body weight in chickens between control and infected group following inoculation with FAdV-8b isolate UPM1901 at day old. The body weight of the infected group was significantly lower ($P<0.05$) than the control group on days 3 and 7 pi. Asterisk * indicates significant difference between group at alpha value $P<0.05$

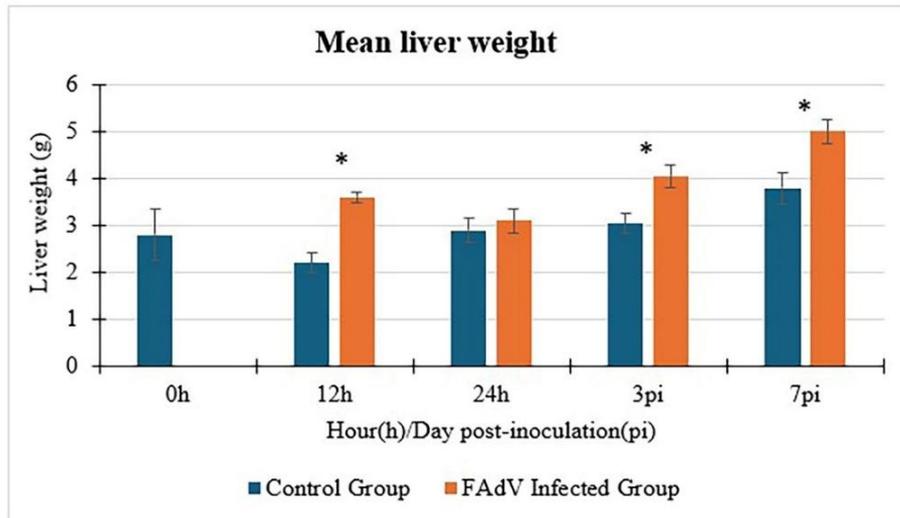


Figure 3. Mean liver weight of chicken between control and infected group with FAdV-8b isolate UPM1901. *= indicates significant difference between group at alpha value $P < 0.05$

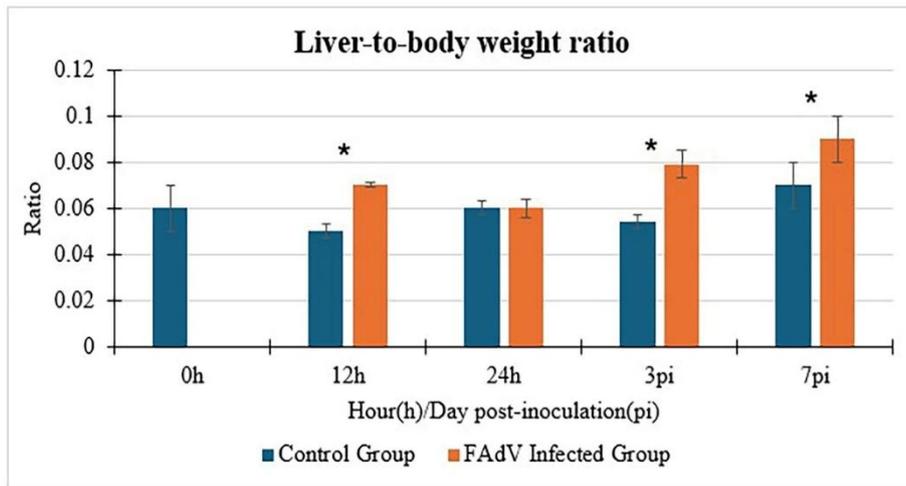


Figure 4. Liver-to-body weight ratio in chicken between control chickens and infected group with FAdV-8b isolate UPM1901. *= indicates significant difference between group at alpha value $P < 0.05$

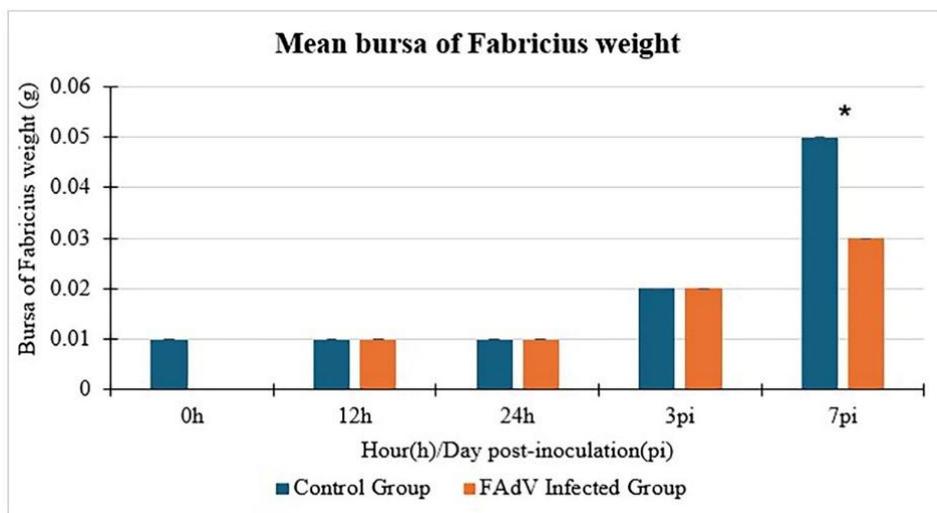


Figure 5. Mean bursa of Fabricius weight in chicken between control and FAdV infected group inoculated with FAdV-8b isolate UPM1901. *= indicates significant difference between group at alpha value $P < 0.05$

Gross lesions

Liver was normal in control chickens with brownish coloration, glistening surface and smooth edges at 0 h, 12 h, 24 h, day 3 and 7 pi. For FAdV infected group, swollen, pale and haemorrhages liver with sharp edges was observed from day 3 and 7 pi (Figure 6). The isolate induced lesions to other organs in infected chickens with mild petechial haemorrhages of the caecal tonsils as well as swollen and haemorrhagic spleen and kidney at day 3 and 7 pi. Based on the gross lesion findings, the changes were pronounced during acute infection phase after day 3 pi following virus distribution throughout gastrointestinal tract (GIT) from oral route. These findings indicates that the disease impact of IBH in chicken which also similarly found in the natural outbreak regardless FAdV serotype in worldwide (Sun et al. 2024; Qiao et al. 2024; Zhang et al. 2023). Additionally, the severity of disease manifestations is influenced by both the route of inoculation and the viral serotypes. Compared to chickens infected via the intramuscular route, as reported in previous studies, the lesions observed in this study are more extensive, showing greater congestion and necrosis in organs such

as the pancreas and proventriculus (Lou et al. 2024). In contrast to FAdV serotype 4 strains, the virus caused hydropericardium with excessive accumulation of straw-colored fluid in the pericardial sac surrounding the flabby heart (Rashid et al. 2024; Zhao et al. 2015). It has become a major cause of death in meat-producing chickens due to heart failure, with mortality rates reaching up to 80% as reported in India and China (Chen et al. 2019; Asthana et al. 2013).

Histopathological lesions

Based on the current study, serotype 8b virus isolate induced histopathological lesions in liver, kidney and lymphoid organs particularly at day 3 pi onwards. Histopathological findings revealed normal liver architecture in control chicken (Figure 7A), while numerous basophilic intranuclear inclusion bodies (INIB) in the nucleus of the hepatocytes were detected in infected groups at day 3 and day 7 pi (Figure 7B and 7C) (Table 1). Liver is the primary tropism for FAdV with evidence of numerous basophilic INIB in the hepatocytes as shown at day 3 and 7 pi under microscopic observation.

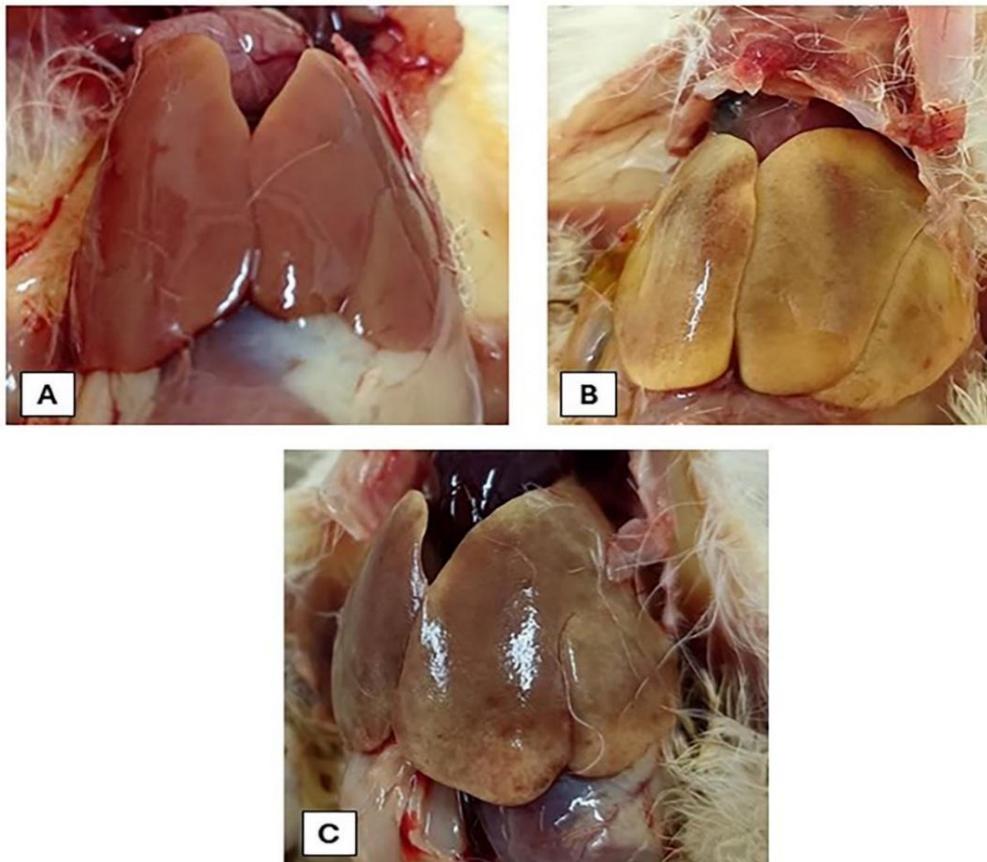


Figure 6. Necropsy findings in the liver of chicken for control and FAdV infected group following inoculation with FAdV-8b isolate UPM1901 at day 7 pi. (A): Normal liver appeared glistening surface, smooth edges and brownish coloration in control chicken, (B): Swollen, pale and petechial haemorrhages with sharp edges liver in the infected chicken. (C): Pale, necrotised liver with sharp edges and petechial haemorrhages in dead chicken at day 3 pi

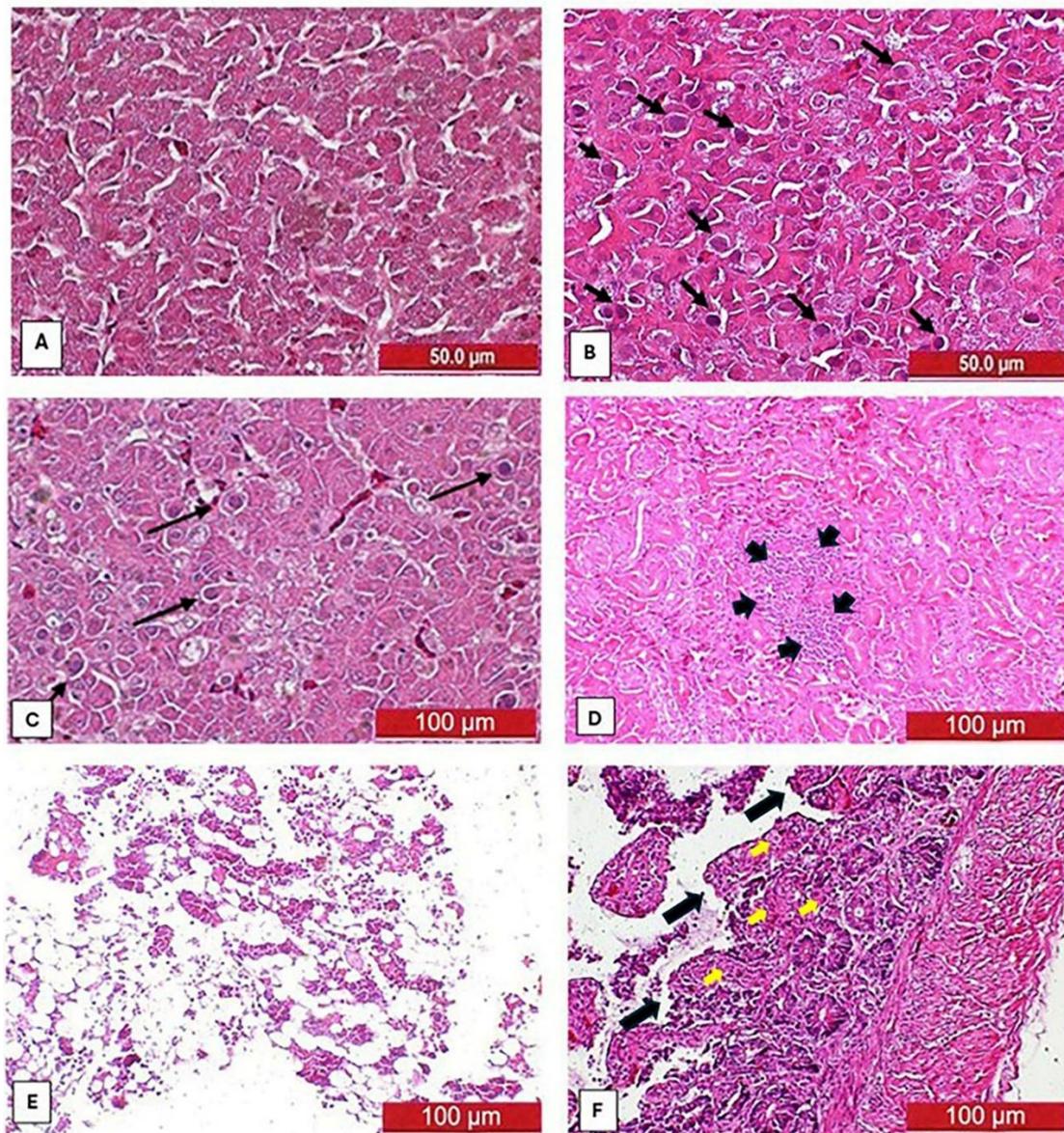


Figure 7: Histopathological findings of the collected samples from infected chicken with FAdV-8b isolate UPM1901. (A): Normal liver architecture in the control chicken, (B): liver of infected chicks at day pi with presence of numerous basophilic intranuclear inclusion bodies (INIB) (arrow), (H&E, 200X), (C): liver from dead chickens at day 5 pi with numerous basophilic INIB (arrow), (H&E, 400X), (D): infiltration of inflammatory cells in kidney tubule (arrows) at day 3 pi (H & E, 400X), (E): low bone marrow cellularity at 12 h pi (H & E, 400X), (F): necrosis (yellow arrows) and degeneration (black arrows) of villi in caeca tonsil at day 3 pi (H & E, 400X).

It showed that this INIB primarily a big, spherical, irregular shape and basophilic with clear and pale halo which contain virus particles in those intranuclear bodies. These findings are in agreement with previous paper studies, INIB in the liver is a hallmark of FAdV infection and has been reported in both IBH and HHS worldwide (Nakamura et al., 2011; Yuan et al. 2021); El-Shall et al. 2022). In addition, the formation of INIB was believed that the inflammatory cells, Kupffer cells phagocytose circulating viruses and eventually multiply in the hepatic cells lead to liver injury (Saifuddin & Wilks 1992).

Examination to other organs revealed swollen and haemorrhagic kidney with presence of infiltration of inflammatory cells in the at day 3 and 7 pi (Figure 7D). There was a depletion of lymphocytes in both spleen and bursa of Fabricius on day 3 pi. Thinning of the cortex was also found in the thymus at day 3 pi and reduced bone marrow cellularity at 12 h pi (Figure 7E). In caecal tonsil, lesion was only found in the dead chicks at day 3 pi, with degeneration and necrosis of the villi (Figure 7F). The lesions were noticed in kidney and lymphoid organs from day 3pi as disease continued progressed due to virus distribution in circulation. These findings were

consistent with previous works as reported by Cizmecigil et al. (2020) and Islam et al. (2023). According to Saifuddin & Wilks (1992), the disease outbreak caused severe lesions in the lymphoid organs without involvement of other immunosuppressive viruses such as infectious bursal disease virus (IBDV) or chicken

anaemia virus (CAV). Infected SPF chicks in this study showed lymphocytic depletion in spleen, bursa of Fabricius and thymus in histopathological changes which suggested that the immune system was compromised by the FAdV infection which led to weakness and depression chicks.

Table 1. Summary of histopathological findings of the collected samples from infected SPF chicken with FAdV-8b isolate UPM1901

Day post-inoculation (pi)	Sample	Histopathological findings
12 hour (h) pi	Bone Marrow	Reduced bone marrow cellularity
24h pi	Bone Marrow	Reduced bone marrow cellularity
Day 3pi	Liver	Presence of numerous basophilic intranuclear inclusion bodies (INIB) in the nucleus of the hepatocytes
	Kidney	Swollen and haemorrhages kidney with presence of infiltration of inflammatory cells
	Spleen	Depletion of lymphocytes
	Bursa of Fabricius	Depletion of lymphocytes
	Thymus	Thinning of the cortex
	Caecal tonsil	Degeneration and necrosis of the villi in the dead chicks
Day 7pi	Liver	Presence of numerous basophilic intranuclear inclusion bodies (INIB) in the nucleus of the hepatocytes
	Kidney	Swollen and haemorrhages with the presence of infiltration of inflammatory cells

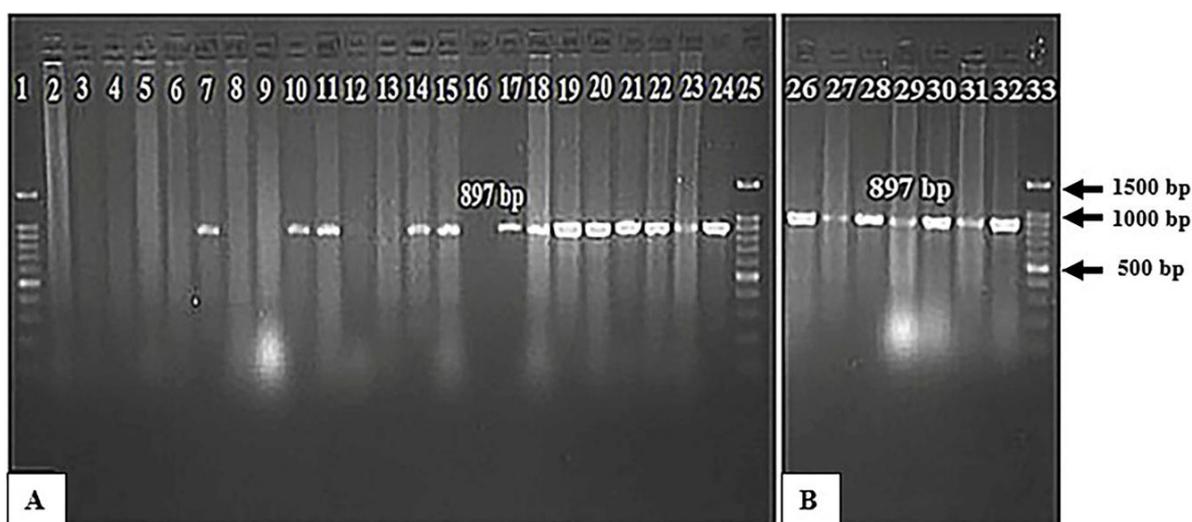


Figure 8. Agarose gel electrophoresis on amplification of PCR product with fragment size 897 base pairs (bp) using hexon gene primer of fowl adenovirus. (A). Lane 1 & 25: 100bp DNA ladder, Lane 16: Positive control (UPM1137), Lane 17: Negative control, Lane 2 – 8: Organs samples at 12 hour(h) post-inoculation (pi), Lane 2: Thymus, Lane 3: Spleen, Lane 4: Liver, Lane 5: Kidney, Lane 6: Bursa of Fabricius, Lane 7: Bone Marrow, Lane 8: Cecal Tonsil, Lane 9-15: organ samples at 24h pi, Lane 9: Thymus, Lane 10: Spleen, Lane 11: Liver, Lane 12: Kidney, Lane 13: Bursa of Fabricius, Lane 14: Bone Marrow, Lane 15: Cecal Tonsil, Lane 18-24: Organ samples of chicken at day 3pi, Lane 18: Thymus, Lane 19: Spleen, Lane 20: Liver, Lane 21: Kidney, Lane 22: Bursa of Fabricius, Lane 23: Bone Marrow; Lane 24: Cecal Tonsil. (B). Lane 26-32: Organ samples of chicken at day 7pi were positive for FAdV. Lane 33: 100bp DNA ladder

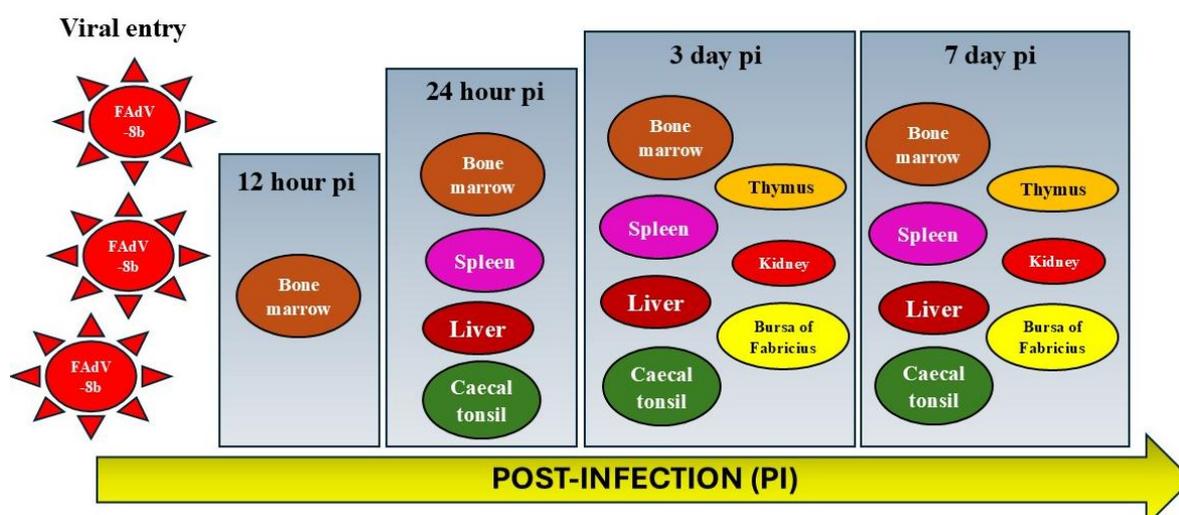


Figure 9. Schematic illustration of viral distribution kinetics across the organs over time in SPF chickens after infected by fowl adenovirus serotype 8b isolate UPM1901

Virus detection in organs

At 12h pi, only bone marrow was positive to FAdV with 897 bp as shown in Figure 8A. Subsequently, at 24 h pi, samples of spleen, liver, bone marrow and caeca tonsils were positive to FAdV. Nevertheless, at day 3 and day 7 pi, all the collected organs which are bone marrow, spleen, liver, kidney, thymus, caeca tonsils and bursa of Fabricius was positive to FAdV infection (Figure 8B). This study used PCR as the molecular detection by amplifying the hexon genes, which enables broad-range detection of the FAdV nucleic acid regardless the serotype (Adel et al. 2021). Based on the current findings, FAdV was first detected in bone marrow as early as 12h pi, followed by other organs in spleen, liver and caecal tonsils at 24 h pi. It indicates that FAdV was replicated primarily in lymphoid organs prior distributed to other organs as shown at day 3 and 7 pi, which all organs include thymus, bursa of Fabricius and kidney were positive to FAdV (Figure 9). Furthermore, the PCR found in this study showed that the targeted organ which is liver in infected chickens was positive to FAdV started from 24 h pi and persistent until day 7 pi. These findings were consistent with liver weight, gross and histological lesions predominantly in the liver organ which suggested that the incubation period was 2 days for this study. It also found that the viral nucleic acid was detected in all infected chickens, although few studies states that FAdVs may isolated from both healthy and sick chicks (Schachner et al. 2018; Niczyporuk 2016).

CONCLUSION

It was concluded that pathogenic FAdV serotype 8b induced pathological changes in the liver and lymphoid organs in SPF chickens following oral or natural route

with evidence of viral nucleic acid as early as 12 h pi in the bone marrow and existed in various organs up to day 7 pi. Therefore, effective control strategies such vaccination programs against FAdV infection are needed to overcome disease and immunosuppressive effects in the poultry flock.

ACKNOWLEDGEMENT

The author would like to thank Universiti Putra Malaysia for research funding with vote number under GP-IPM/2020/9690900.

REFERENCES

- Adel A, Mohamed AAE, Samir M, Hagag NM, Erfan A, Said M, Arafa AES, Hassan WMM, El Zowalaty ME, Shahien MA. 2021. Epidemiological and molecular analysis of circulating fowl adenoviruses and emerging of serotypes 1, 3, and 8b in Egypt. *Heliyon*. 7:e08366. DOI:10.1016/j.heliyon.2021.e08366.
- Asthana M, Chandra R, Kumar R. 2013. Hydropericardium syndrome: current state and future developments. *Arch Virol*. 158:921–931. DOI:10.1007/s00705-012-1570-x.
- Chen Z, Shi S, Qi B, Lin S, Chen C, Zhu C, Huang Y. 2019. Hydropericardium syndrome caused by fowl adenovirus serotype 4 in replacement pullets. *J Vet Med Sci*. 81:245–251. DOI:10.1292/jvms.18-0168.
- Cizmecigil UY, Umar S, Yilmaz A, Bayraktar E, Turan N, Tali B, Aydin O, Tali HE, Yaramanoglu M, Yilmaz SG, et al. 2020. Characterisation of fowl adenovirus (FAdV-8b) strain concerning the geographic analysis and pathological lesions associated with inclusion body hepatitis in broiler flocks in Turkey. *J Vet Res*. 64:231–237. DOI:10.2478/jvetres-2020-0026.

- El-Shall NA, El-Hamid HSA, Elkady MF, Ellakany HF, Elbestawy AR, Gado AR, Geneedy AM, Hasan ME, Jaremko M, Selim S, et al. 2022. Epidemiology, pathology, prevention, and control strategies of inclusion body hepatitis and hepatitis-hydropericardium syndrome in poultry: A comprehensive review. *Front Vet Sci.* 9. DOI:0.3389/fvets.2022.963199/full.
- Gurina TS, Simms L. 2023. *Histology, Staining. Treasure Island (FL): StatPearls Publishing.*
- Hair-Bejo M. 2005. Inclusion body hepatitis in a flock of commercial broiler chickens. *J Vet Malaysia.* 7:23–26.
- Harrach B, Kajan G. 2011. Aviadnavirus. In: Tidona C, Darai G, editors. *The springer index of viruses.* 2nd ed. Springer; p. 13–28.
- Islam MN, Rahman MM, Rahman MK, Alam J. 2023. First evidence of fowl adenovirus induced inclusion body hepatitis in chicken in Bangladesh. *Batra L, editor. Can J Infect Dis Med Microbiol.* 2023:1–11. DOI:10.1155/2023/7253433.
- Kiss I, Homonnay ZG, Mató T, Bányai K, Palya V. 2021. Research Note: An overview on distribution of fowl adenoviruses. *Poult Sci.* 100:101052. DOI:10.1016/j.psj.2021.101052.
- Lou M, Shi H, Cao X, Li J, Zhang R, Pan Q, Yin Y, Wang J. 2024. Growth retardation and immunosuppression in SPF chickens infected by fowl adenovirus serotype-8b isolated in China. *Vet México OA.* 11. DOI:10.22201/fmvz.24486760e.2024.1265.
- Naeem K, Niazi T, Malik SA, Cheema AH. 1995. Immunosuppressive Potential and Pathogenicity of an Avian Adenovirus Isolate Involved in Hydropericardium Syndrome in Broilers. *Avian Dis.* 39:723. DOI:10.2307/1592408.
- Niczyporuk JS. 2016. Phylogenetic and geographic analysis of fowl adenovirus field strains isolated from poultry in Poland. *Arch Virol.* 161:33–42. DOI:10.1007/s00705-015-2635-4.
- Norina L, Norsharina A, Nurnadiah A, Redzuan I, Nor-Ismaaliza A. 2016. Avian adenovirus isolated from broiler affected with inclusion body hepatitis. *Malaysian J Vet Res.* 7:121–126.
- Pereira C, Marin S, Santos B, Resende JS, Resende M, Gomes A, Martins NRS. 2014. Occurrence of aviadenovirus in chickens from the poultry industry of Minas Gerais. *Arq Bras Med Veterinária e Zootec.* 66:801–808. DOI:10.1590/1678-41625899.
- Qiao Q, Xu M, Wang X, Tian J, Zhang Y, Song C, Liu J, Li Yan, Li X, Yang P, et al. 2024. Genomic characterization and pathogenicity of a novel fowl adenovirus serotype 11 isolated from chickens with inclusion body hepatitis in China. *Poult Sci.* 103:103642. DOI:10.1016/j.psj.2024.103642.
- Rashid F, Xie Zhixun, Wei Y, Xie Zhiqin, Xie L, Li M, Luo S. 2024. Biological features of fowl adenovirus serotype-4. *Front Cell Infect Microbiol.* 14. DOI:10.3389/fcimb.2024.1370414/full.
- Reed LJ, Muench H. 1938. A simple method of estimating fifty per cent endpoints. *Am J Epidemiol.* 27:493–497. DOI:10.1093/oxfordjournals.aje.a118408.
- Sabarudin NS, Tan SW, Phang YF, Omar AR. 2021. Molecular characterization of Malaysian fowl adenovirus (FAdV) serotype 8b species E and pathogenicity of the virus in specific-pathogen-free chicken. *J Vet Sci.* 22. DOI:10.4142/jvs.2021.22.e42.
- Saifuddin M, Wilks CR. 1992. Effects of fowl adenovirus infection on the immune system of chickens. *J Comp Pathol.* 107:285–294. DOI:10.1016/0021-9975(92)90004-E.
- Schachner A, Matos M, Grafl B, Hess M. 2018. Fowl adenovirus-induced diseases and strategies for their control – a review on the current global situation. *Avian Pathol.* 47:111–126. DOI:10.1080/03079457.2017.1385724.
- Sohaimi NM, Bejo MH. 2021. Efficacy of live attenuated fowl adenovirus serotype 8b isolate of Malaysia in specific pathogen-free chickens. *Malaysian Appl Biol.* 50:135–143. DOI:10.55230/mabjournal.v50i3.1998.
- Sohaimi NM, Clifford UC, Hair-Bejo M. 2022. Genetic diversity of fowl adenovirus serotype 8b isolated from cases of inclusion body hepatitis in commercial broiler chickens. *J Indones Trop Anim Agric.* 47:97–106. DOI: 10.14710/jitaa.47.2.97-106.
- Sohaimi NM, Hair-Bejo M, Majdi A. 2019. Pathogenicity of Fowl Adenovirus Serotype 8B Isolates of Malaysia in Specific Pathogen Free Chickens. *J Anim Vet Adv.* 18:78–83.
- Song Y, Liu L, Sun W, Gao W, Song X, Wang Y, Wei Q, Huang Z, Li X. 2024. Identification, pathogenicity and molecular characterization of a novel fowl adenovirus 8b strain. *Poult Sci.* 103:103725. DOI:10.1016/j.psj.2024.103725.
- Sun Q, Li Y, Huang Y, Li S, Fu Q, Liu S. 2024. FAdV-4 can cause more noticeable clinical symptoms compared to FAdV-8b after infecting specific pathogen free chickens. *Poult Sci.* 103:104006. DOI:10.1016/j.psj.2024.104006.
- Tsiouris V, Mantzios T, Kiskinis K, Guérin J-L, Croville G, Brellou GD, Apostolopoulou EP, Petridou EJ, Georgopoulou I. 2022. First Detection and Identification of FAdV-8b as the Causative Agent of an Outbreak of Inclusion Body Hepatitis in a Commercial Broiler Farm in Greece. *Vet Sci.* 9:160. DOI:10.3390/vetsci9040160.
- Yuan F, Hou L, Wei L, Quan R, Wang J, Liu H, Liu J. 2021. Fowl Adenovirus Serotype 4 Induces Hepatic Steatosis via Activation of Liver X Receptor- α . *Banks L, editor. J Virol.* 95. DOI:10.1128/JVI.01938-20.
- Zhang X, Liu L, Wang F, Li H, Fan J, Xie J, Jiao Y, Han Z, Ma D. 2023. Pathogenicity and innate immune responses induced by fowl adenovirus serotype 8b in specific pathogen-free chicken. *Poult Sci.* 102:102846. DOI:10.1016/j.psj.2023.102846.
- Zhao J, Zhong Q, Zhao Y, Hu Y, Zhang G. 2015. Pathogenicity and complete genome characterization of Fowl

adenoviruses isolated from chickens associated with inclusion body hepatitis and hydropericardium syndrome in China. Devlin J, editor. PLoS One. 10:e0133073. DOI:10.1371/journal.pone.0133073.