Journal of Animal and Veterinary Advances 18 (3): 78-83, 2019
ISSN: 1680-5593

Pathogenicity of Fowl Adenovirus Seroype 8B Isolates of Malaysia in Specific Pathogen Free Chickens

[1]M.S. Norfitriah, [1, 2]M. Hair-Bejo and [1]A. Majdi
[1]Faculty of Veterinary Medicine,
[2]Institute of Bioscience, Universiti Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia
fitriahsohaimi@gmail.com, mdhair@upm.edu.my, +60386093888

Abstract: Highly pathogenic Fowl Adenovirus (FAdV) is a causative agent of Inclusion Body Hepatitis (IBH) in poultry. It causes mortality and poor performance of the affected chickens. It was objectives of the study to determine pathogenicity of FAdV serotype 8b isolates of Malaysia in Specific Pathogen Free (SPF) chickens. The virus isolates namely, UPM11134 and UPM11142 were obtained from IBH outbreaks in broiler chicken farms and characterized as FAdV serotype 8b. The liver samples of affected chickens were prepared and inoculated into SPF embryonated chicken eggs via. Chorioallantoic Membrane (CAM) route. The liver of embryos was subsequently harvested for preparation of virus inoculum. The 36 days old SPF chicks were divided into 3 groups namely groups A-C. Each group was further divided into sacrificed and mortality groups. Chickens in groups A and B were inoculated with UPM11134 and UPM11142 FAdV isolates, respectively, via. intraperitoneal route at day old. All chicken in group C remained uninoculated and acted as the control group. The chicken were monitored for any clinical abnormalities throughout the trial. Gross lesions were recorded on necropsy and samples of liver and gizzard were collected and fixed in 10% buffered formalin for histological examination. The study showed 100 and 91% mortality of SPF chickens in groups A and B, respectively, at day 4 post inoculation (pi). Clinical signs of IBH such as depression, weakness, ruffled feathers and diarrhea were recorded within 12-24 h prior to death. On necropsy, hydropericadium with pale, friable and petechial haemorrhages of liver were recorded. Hepatitis with areas of hepatic necrosis and haemorrhages and presence of numerous basophilic Intranuclear Inclusion Bodies (INIB) in degenerated hepatocytes were recorded in all chickens in groups A and B, whilst INIB were also observed in glandular epithelium of gizzard in group A. All chicks in group C remained normal throughout the trial. It was concluded that Malaysian FAdV serotype 8b isolates are highly pathogenic in SPF chickens and acted as the primary agent of IBH.

Key words: Fowl Adenovirus (FAdV), Inclusion Body Hepatitis (IBH), Specific Pathogen Free (SPF) chicken, pathogenic, basophilic Intranuclear Inclusion Bodies (INIB), UPM11134

INTRODUCTION

Fowl Adenoviruses (FAdVs) comprised of five molecular groups species designated as letter A-E and divided further into 12 serotypes (Benko *et al*., 2005). FAdV caused several clinical diseases in poultry such as Inclusion Body Hepatitis (IBH), gizzard erosion, Hydro Pericardium Syndrome (HPS), respiratory disease and necrotizing pancreatitis (Kajan *et al*., 2013; Okuda *et al*., 2004; Balamurugam and Kataria, 2004). IBH outbreak has been reported worldwide and involved all 12 serotypes of FAdV with significant economic losses in poultry industry (Kajan *et al*., 2013; Maartens *et al*., 2014).

The FAdV serotypes 4, 8b, 9 and 11 have been identified as highly pathogenic strain of FAdV (Morshed *et al*., 2017; Gomis *et al*., 2006; Lim *et al*., 2011; Alvarado *et al*., 2007; Dahiya *et al*., 2002). Majority of the FAdV serotypes acted as secondary agent of IBH following immunosuppression in chicken due to infectious bursal disease virus or chicken anaemia virus (Ojkic *et al*., 2008; Toro *et al*., 2000). In some cases, FAdV has been isolated in both clinically healthy and sick chickens (Ojkic *et al*., 2008; El-Attrache and Villegas, 2011). It seems that pathogenicity of FAdV varies based on the virulence of a strain, intercurrent disease, the immune status of the flock or other complicating management factors (Saifuddin *et al*., 1992). Differences in virulence between serotypes highly associated with gene encoded for infectivity mainly in major capsid protein (Sohaimi *et al*., 2018). The virulence gene of FadV

Corresponding Author: M. Hair-Bejo, Faculty of Veterinary Medicine, Universiti Putra Malaysia (UPM), 43400 Serdang, Selangor, Malaysia, mdhair@upm.edu.my, +60386093888

was characterized in fiber and L1 loop of hexon gene (Sohaimi *et al*., 2018; Pallister *et al*., 1996). Large variation in amino acids of fiber gene are prominent between IBH and non-IBH strains in serotype 8 (Grgic *et al*., 2014).

IBH outbreak is characterized by sudden of peaked mortality up to 30% within 3-4 days and return to normal after 5 days from onset of clinical signs (Hair-Bejo, 2005; Norina *et al*., 2016). Hepatic necrosis with presence of basophilic and eosinophilic Intranuclear Inclusion Bodies (INIB) in hepatocytes were noticed in FAdV infected liver (Norina *et al*., 2016). In Malaysia, FAdV serotype 8b under group E species was commonly isolated in broiler and layer chickens with typical case of IBH and gizzard erosion (Juliana *et al*., 2014; Norina *et al*., 2016; Norfitriah *et al*., 2018). IBH outbreaks were reported in young broiler chicks as early as 7 days old and can be older as 20 weeks old (Hair-Bejo, 2005; Norina *et al*., 2016). The disease transmitted either by vertically via. embryonated chicken eggs or horizontal modes through faecal-oral route, direct contact and fomites (Grgic *et al*., 2006; McFerran and Adair, 2003).

In the past few years, IBH outbreaks in Malaysia solely caused by serotype 8b with high mortality and poor production in the affected farms. However, the pathogenicity of Malaysian FAdV isolates is little known as the virus might take on the role as opportunistic pathogen under certain circumstances such as concurrent infections or stress factor which enhance the pathogenicity of FAdV. It was objective of the study to determine pathogenicity of FAdV serotype 8b isolates of Malaysia in Specific Pathogen Free (SPF) chickens.

MATERIALS AND METHODS

FAdV isolates: Two different FAdV isolates were used in this study namely UPM11134 and UPM11142. The UPM11134 isolate was obtained from 18 days old broiler chickens with history of 0.5-1% mortality per day started from day 7 of age. Upon necropsy, the liver was pale, enlarged and yellowish with multifocal area of necrosis and haemorrhages. The other isolate, UPM11142 originated from 24 days old broiler chickens with lesions of pale, enlarged, multifocal area of necrosis and haemorrhagic livers. Both isolates were molecularly characterized as FadV group E under serotype 8b (Juliana *et al*., 2014).

Preparation of FAdV inoculum: Liver samples from field outbreak were processed by sterile mortar and pastel in a suspension of phosphate buffer saline (PBS, pH 7.4) with ratio 1-2 dilution, according to, previous study (Alemnesh *et al*., 2012). Liver suspension was purified by centrifugation at 382×g for 30 min and the supernatant was filtered through 0.45 μm (Sartorius, Germany) membrane. The liver homogenate was treated with antibiotic antimycotic solution (Gibco, USA) at 1-10 dilution and stored in 4°C for 1 h prior inoculation into 9 days old SPF chicken embryonated eggs. All the inoculated eggs were monitored daily and liver of dead embryo was harvested at each mortality day. Liver of embryo was processed and used as inoculum for pathogenicity study.

Experimental design for pathogenicity of FAdV in SPF chickens: The 36 days old SPF chicks were divided into 3 groups namely groups A-C. Each group were further divided into sacrificed and mortality group. Chickens in groups A and B were inoculated with 0.5 mL of FAdV isolate, UPM11134 and UPM11142 via. intraperitoneal route, respectively, at day old. All chicks in group C remained uninoculated and acted as control group.

All chickens were monitored daily for any clinical signs abnormalities throughout the trial. Feed and water were given ad-libitum. The 3 chicks in group C were sacrificed by cervical dislocation at day 0 post inoculation (pi) followed by days 7 and 14 pi in all groups. On necropsy, gross lesions were recorded and samples of liver and gizzard were collected and fixed in 10% buffered formalin for histological examination. The study was conducted under approval of Institutional Animal Care and Use Committee (IACUC), Universiti Putra Malaysia with AUP No: FYP-2014/FPV.020.

RESULTS AND DISCUSSION

Clinical signs: The chickens in groups A and B showed clinical signs of inactive, weakness, prostration, depression, diarrhea with white dropping in cloaca, ruffled feathers within 12-24 h prior to death (Fig. 1a, b). Cumulative mortality of 55, 91 and 100% at days 2-4 pi were recorded in group A. In group B, cumulative mortality of 45, 82 and 91% were recorded at days 2-4 pi (Fig. 2a, b). All chickens in group C were remained normal.

Gross lesions: Subcutaneous jaundice with swollen, friable and petechial haemorrhages of liver in chickens from groups A and B were recorded at days 2-4 pi (Fig. 3a, b). Hydropericardium with accumulation of straw colour fluid in pericardial sac along with pale and marked enlargement of spleen in both groups were also recorded. At day 7 pi, the liver was pale in sacrificed chicken in group B. No gross lesion was recorded in group C throughout the trial.

Fig. 1: Clinical signs of IBH following FAdV isolate serotype 8b inoculation in group A (UPM11134) and B (UPM11142): a) Depression, ruffled feather and prostration after 24 h post-inoculation (pi) and b) Diarrhea in infected chicks with presence of white pasty dropping in cloaca region (arrow)

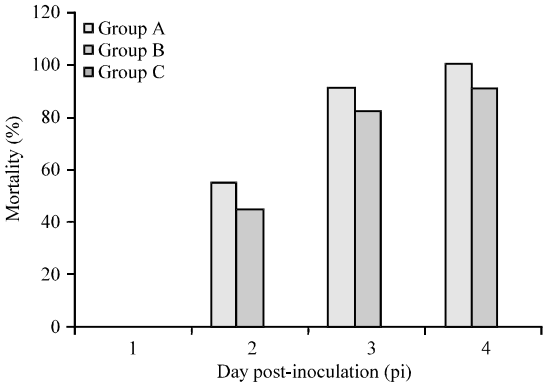

Fig. 2: Cumulative mortality of SPF chickens in groups A and B following inoculation with FAdV isolates, UPM11134 and UPM11142, respectively. At day 4 pi, 100 and 91% mortality were recorded in groups A and B, respectively. No mortality was recorded in group C (Control) throughout the trial

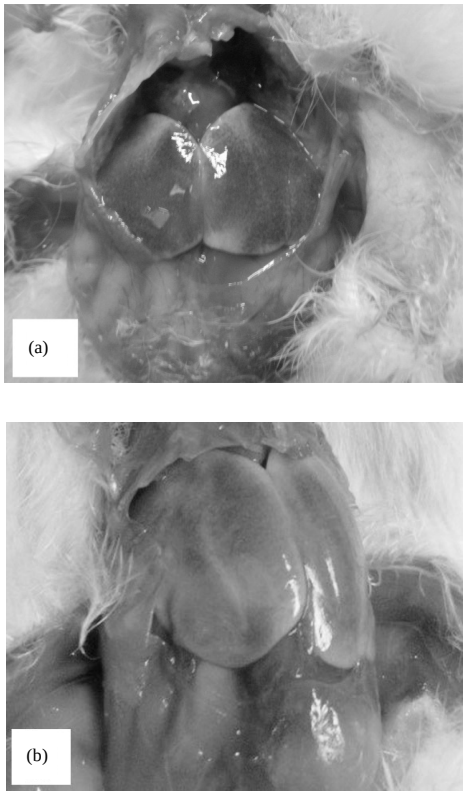

Fig. 3: Gross lesions of liver in chickens infected with FAdV isolates, UPM11134 (Group A) and UPM11142 (Group B): a) Yellowish discoloration liver, enlarged, friable and petechial haemorrhages in dead chick at day 3 pi in group A and b): Yellowish discoloration of the entire liver with swollen, friable and petechial haemorrhages in chick died at day 4 pi in group B

Histological lesions: Mild haemorrhagic hepatitis with a few basophilic Intranuclear Inclusion Bodies (INIB) were detected at day 2 pi in groups A and B (Fig. 4a, b). Few basophilic INIB were also detected in glandular epithelium of gizzard at day 2 pi typically in group A (Fig. 4c). These lesions became moderate at day 3 pi and subsequently, severe at day 4 pi. Numerous basophilic INIB were observed in the hepatocytes at day 4 pi (Fig. 5a, b). At day 7 pi, basophilic INIB were also detected in hepatocytes in group B with severe haemorrhagic hepatitis and necrosis of hepatocytes. No histological lesion was recorded in group C throughout the trial.

It was demonstrated that both FAdV isolates serotype 8b, UPM11132 and UPM11142 are highly pathogenic in 1-day-old SPF chickens with high mortality and severe lesions in the liver. Mortality rate of 100

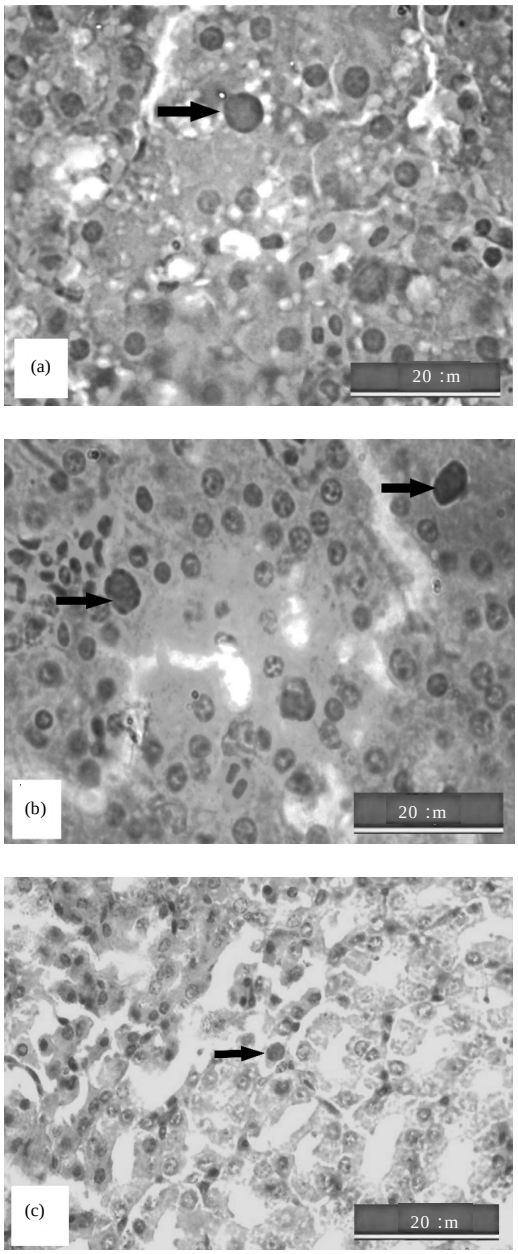

Fig. 4: Histopathological changes in liver and gizzard in groups A and B after inoculated with FAdV isolate, UPM11134 and UPM11142, respectively at day 2 pi: a) Basophilic Intranuclear Inclusion Bodies (INIB) in hepatocytes (arrow) in group A; b): Numerous basophilic INIB in hepatocytes (arrow) with mild haemorrhages in group B and c): Basophilic INIB in glandular epithelium of gizzard (arrow) in group A. HE. Scale bar = 20.0 μm

and 91% were recorded in SPF chicks within 4 days post-inoculation (pi) following inoculation with FAdV

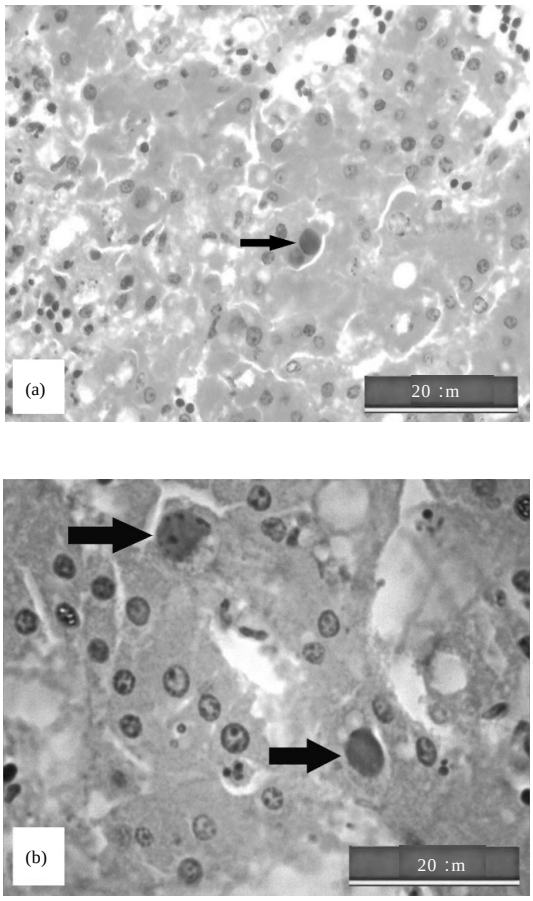

Fig. 5: Histopathological changes in liver in groups A and B after inoculated with FAdV isolate, UPM11134 and UPM11142, respectively at day 4 pi: a): Large basophilic INIB (arrow) in degenerated hepatocytes with marked vacuolation in group A and b): Numerous large basophilic INIB in degenerated hepatocytes and marked vacuolation in group B. HE. Scale bar = 20.0 μm

isolates UPM11134 and UPM11142, respectively. High mortality was recorded as early as day 2 pi in both groups A and B. It was demonstrated that the isolates are highly pathogenic in SPF chicks. These findings are consistent with several previous studies (Li *et al*., 2018; El-Attrache and Villegas, 2011; Alvarado *et al*., 2007; Lim *et al*., 2011). Infection of FAdV-8 isolates via. parenteral route resulting high mortality in day old SPF chicks compared to oral route (El-Attrache and Villegas, 2011; Okuda *et al*., 2004). In contrast, neither mortality nor gross and histological lesions were observed in commercial broiler chickens due to neutralization of viral antigen by maternal derived antibodies (El-Attrache and Villegas,

2011). This suggests that FAdV varying in pathogenicity due to different route of inoculation, type of chicken and perhaps different virus strains (Erny *et al*., 1991).

Acute clinical signs of IBH which includes depression, ruffled feathers and prostration were observed within 12-24 h prior to death (Balamurugam and Kataria, 2004; Alavarado *et al*., 2007; Zadrvec *et al*., 2011). The gross and histological lesions were confined mainly in the liver with moderate to severe necrosis, haemorrhages and inflammation of the organ and present of INIB within the hepatocytes (El-Attrache and Villegas, 2011; Okuda *et al*., 2004). Liver is the tissue tropism of the virus following rapid systemic absorption through intraperitoneal route. The FAdV isolates used in the present study were also caused hydropericardium which is commonly reported in chickens infected with FAdV serotype 4 (Balamurugam and Kataria, 2004).

Histopathological changes revealed large basophilic INIB in liver was detected as early as day 2 pi in both groups A and B. Those changes were consistent until day 4 pi in group A and day 7 pi for group B with severe hepatic necrosis. Interestingly, basophilic INIB were also detected in glandular epithelium of gizzard in group A (UPM11134 isolate). In most IBH cases with gizzard erosion, FAdV serotype 1 were commonly isolated from the infected chicken (Kajan *et al*., 2013). However, both liver and gizzard are favorable for FAdV UPM11134 serotype 8b isolate which also shown similar characteristic with other serotype 8 isolate in Japan (Okuda *et al*., 2004). Those differences in histopathological finding of gizzard between isolates perhaps highly associated with gene encoded for virulent and viral tropism in chickens (Marek *et al*., 2010).

Based on molecular analysis conducted by Juliana (2014), high nucleotide (nt) and amino acid (aa) differences in L1 loop region between Malaysian FAdV isolates and non-IBH strain namely TR59 from serotype 8a were noted. It shown that 106 nt and 29 aa differences were detected, although, classified within the similar group species. In addition, both isolates UPM11134 and UPM11142 are highly identical with IBH strains namely, 764 and other Australian strains from field outbreak with only 11-13 nt and 1-2 aa different which in contrast to TR59 strain (Juliana, 2014; Steer *et al*., 2011; Ojkic *et al*., 2008). It seems that L1 loop region play important role in determination of FAdV virulence with significant impact for FAdV infectivity in chicken as shown in previous study (Sohaimi *et al*., 2018). Moreover, those molecular changes in virulent strains is essential to cause IBH in chickens as demonstrated by both Malaysian FAdV isolates (Morshed *et al*., 2017; Maartens *et al*., 2014).

CONCLUSION

It was concluded that both the UPM1132 and UPM1142 FAdV isolates of serotype 8b in the present study are the primary agent of IBH. The isolates are highly pathogenic in SPF chickens.

ACKNOWLEDGEMENT

This study was supported by the Ministry of Higher Education and the Ministry of Science and Technology, Malaysia, through research grants 6369101 and 6364002, respectively.

REFERENCES

Alemnesh, W., M. Hair-Bejo, I. Aini and A.R. Omar, 2012. Pathogenicity of fowl adenovirus in specific pathogen free chicken embryos. J. Comp. Pathol., 146: 223-229.

Alvarado, I.R., P. Villegas, J. El-Attrache, E. Jensen and G. Rosales *et al*., 2007. Genetic characterization, pathogenicity and protection studies with an avian adenovirus isolate associated with inclusion body hepatitis. Avian Dis., 51: 27-32.

Balamurugan, V. and J.M. Kataria, 2004. The hydropericardium syndrome in poultry: A current scenario. Vet. Res. Commun., 28: 127-148.

Benko, M., B. Harrach, G. Both, W.C. Russell and B.M. Adair *et al*., 2005. Family Adenoviridae. In: Virus Taxonomy: Eighth Report of the International Committee on Taxonomy of Viruses, Fauquet, C.M., M.A. Mayo, J. Maniloff, U. Desselberger and L.A. Ball (Eds.). Elsevier, San Diego, California, USA., pp: 213-218.

Dahiya, S., R.N. Srivastava, M. Hess and B.R. Gulati, 2002. Fowl adenovirus serotype 4 associated with outbreaks of infectious hydropericardium in Haryana, India. Avian dis., 46: 230-233.

El-Attrache, J. and P. Villegas, 2011. Genomic identification and characterization of avian adenoviruses associated with inclusion body hepatitis. Avian Dis., 45: 80-787.

Erny, K.M., D.A. Barr and K.J. Fahey, 1991. Molecular characterization of highly virulent fowl adenoviruses associated with outbreaks of inclusion body hepatitis. Avian Pathol., 20: 597-606.

Gomis, S., R. Goodhope, D. Ojkic and P. Willson, 2006. Inclusion body hepatitis as a primary disease in broilers in Saskatchewan, Canada. Avian Dis., 50: 550-555.

Grgic, H., C. Philippe, D. Ojkic and E. Nagy, 2006. Study of vertical transmission of fowl adenoviruses. Can. J. Vet. Res., 70: 230-233.

Grgic, H., P.J. Krell and E. Nagy, 2014. Comparison of fiber gene sequences of Inclusion Body Hepatitis (IBH) and non-IBH strains of serotype 8 and 11 fowl adenoviruses. Virus Genes, 48: 74-80.

Hair-Bejo, M., 2005. Inclusion body hepatitis in a flock of commercial broiler chickens. J. Vet. Malaysia, 17: 23-26.

Juliana, M.A., 2014. Molecular characterization and genomic organization of fowl adenovirus isolates of Malaysia. Master Thesis, Universiti Putra Malaysia, Kembangan, Selangor, Malaysia.

Juliana, M.A., I. Nurulfiza, M. Hair-Bejo, A.R. Omar and I. Aini, 2014. Molecular characterization of fowl adenoviruses isolated from inclusion body hepatitis outbreaks in commercial broiler chickens in Malaysia. Pertanika J. Trop. Agric. Sci., 37: 483-497.

Kajan, G.L., S. Kecskemeti, B. Harrach and M. Benko, 2013. Molecular typing of fowl adenoviruses, isolated in Hungary recently, reveals high diversity. Vet. Microbiol., 167: 357-363.

Li, L., J. Wang, P. Chen, S. Zhang and J. Sun *et al*., 2018. Pathogenicity and molecular characterization of a fowl adenovirus 4 isolated from chicken associated with IBH and HPS in China. BMC. Vet. Res., 14: 1-8.

Lim, T.H., H.J. Lee, D.H. Lee, Y.N. Lee and J.K. Park *et al*., 2011. Identification and virulence characterization of fowl adenoviruses in Korea. Avian Dis., 55: 554-560.

Maartens, L.H., H.W. Joubert, H. Aitchison and E.H. Venter, 2014. Inclusion body hepatitis associated with an outbreak of fowl adenovirus type 2 and type 8b in broiler flocks in South Africa. J. South Afr. Vet. Assoc., 85: 1-5.

Marek, A., E. Schulz, C. Hess and M. Hess, 2010. Comparison of the fibers of fowl adenovirus A serotype 1 isolates from chickens with gizzard erosions in Europe and apathogenic reference strains. J. Vet. Diagn. Invest., 22: 937-941.

McFerran, J.B. and B.M. Adair, 2003. Group I Adenovirus Infection. In: Disease of Poultry, Saif, Y.M. (Ed.). Iowa State University, Ames, Iowa, USA., ISBN:9780813804231, pp: 214-227.

Morshed, R., H. Hosseini, A.G. Langeroudi, M.H.B. Fard and S. Charkhkar, 2017. Fowl adenoviruses D and E cause inclusion body hepatitis outbreaks in broiler and broiler breeder pullet flocks. Avian Dis., 61: 205-210.

Norfitriah, M.S., M. Hair-Bejo, A.R. Omar, I. Aini and M.I. Nurulfiza, 2018. Molecular detection and pathogenicity of fowl adenovirus isolated from disease outbreak in commercial layer chickens. Intl. J. Agric. Sci. Vet. Med., 6: 73-84.

Norina, L., A. Norsharina, A.H. Nurnadiah, I. Redzuan and A. Ardy *et al*., 2016. Avian adenovirus isolated from broiler affected with inclusion body hepatitis. Malaysian J. Vet. Res., 7: 121-126.

Ojkic, D., E. Martin, J. Swinton, J.P. Vaillancourt and M. Boulianne *et al*., 2008. Genotyping of Canadian isolates of fowl adenoviruses. Avian Pathol., 37: 95-100.

Okuda, Y., M. Ono, I. Shibata and S. Sato, 2004. Pathogenicity of serotype 8 fowl adenovirus isolated from gizzard erosions of slaughtered broiler chickens. J. Vet. Med. Sci., 66: 1561-1566.

Pallister, J., P.J. Wright and M. Sheppard, 1996. A single gene encoding the fiber is responsible for variations in virulence in the fowl adenoviruses. J. Virol., 70: 5115-5122.

Saifuddin, M.D., C.R. Wilks and A. Murray, 1992. Characterisation of avian adenoviruses associated with inclusion body hepatitis. N. Z. Vet. J., 40: 52-55.

Sohaimi, N.M., M.H. Bejo, A.R. Omar, A. Ideris and N.M. Isa, 2018. Hexon and fiber gene changes in an attenuated fowl adenovirus isolate from Malaysia in embryonated chicken eggs and its infectivity in chickens. J. Vet. Sci., 19: 759-770.

Steer, P.A., D. O'rourke, S.A. Ghorashi and A.H. Noormohammadi, 2011. Application of high-resolution melting curve analysis for typing of fowl adenoviruses in field cases of inclusion body hepatitis. Aust. Vet. J., 89: 184-192.

Toro, H., C. Gonzalez, L. Cerda, M. Hess, E. Reyes and C. Geisse, 2000. Chicken anemia virus and fowl adenoviruses: Association to induce the inclusion body hepatitis/hydropericardium syndrome. Avian Dis., 44: 51-58.

Zadrvec, M., S. Brigita, U. Krapez, G.L. Kajan and J. Racnik *et al*., 2011. Inclusion body hepatitis associated with fowl adenovirus type 8b in broiler flock in Slovenia: A case report. Slov. Vet. Res., 48: 107-113.