

Genetic diversity of fowl adenovirus serotype 8b isolated from cases of inclusion body hepatitis in commercial broiler chickens

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ABSTRACT

Inclusion body hepatitis (IBH) is a devastating disease of chickens caused by Fowl adenovirus (FAdV) and molecular studies of hexon gene is important for classification of FAdV isolates, epidemiology of IBH infection and development of effective control strategy. The objective of the study was to molecularly characterize FAdV isolates obtained from the field outbreaks of IBH in broiler chicken from 2017 to 2019. Liver and gizzard samples named UPM1701, UPM1801, UPM1802, UPM1901 and UPM1902 were collected and processed for FAdV detection and characterization by PCR. Swollen, necrotic and haemorrhagic liver; erosion of koilin layer of gizzard and enlargement of the proventriculus were observed. The samples were positive for FAdV with 98% to 100% identical to serotype 8b reference strains based on NCBI Blast. Thirty-one nucleotide changes that produced 10 amino acid substitutions were observed in L1 loop region of UPM1701, UPM1901 and UPM1902 isolates. All isolates clustered together with FAdV 8b reference strains and shared common ancestor with UPM1137E2 and UPM04217. Gizzard erosion in FAdV 8b infection is not common and coinciding with mutations indicate evolving novel pathogenicity. These mutations could have effect on the epidemiology of IBH and be useful in designing effective prevention and control strategy.

Keywords: Fowl adenovirus, Inclusion body hepatitis, Serotype 8b, Hexon, Substitutions.

INTRODUCTION

Fowl adenovirus (FAdV) is classified into 5 molecular species designated A to E and 12 serotypes (Steer *et al.*, 2011; Morshed *et al.*, 2017) and has been reported to be endemic in various geographical regions worldwide where they cause high levels of economic losses among poultry species (Li *et al.*, 2018; Schachner *et al.*, 2018; Jordan *et al.*, 2019). FAdV are non-enveloped, double stranded DNA virus comprised of three major structural proteins known as hexon, penton and fiber (Park *et al.*,

2016). Antigenic and most of the virulence determinants for FAdV is located in mainly hexon gene (Park *et al.*, 2016); although fiber proteins has become highly relevant due to its role in tissue tropism (Pallister *et al.*, 1996). Hexon gene contain high amino acid variability between serotype and genotype for FAdV classification (Hess 2000; Meulemans *et al.*, 2001). Hexon protein is made up of two pedestal regions P1 and P2 and 4 loop regions L1 – L4 (Sheppard *et al.*, 1995). L1 loop region being one of the projectiles is exposed, most variable and more prone to deletions and

insertions than all the loop regions; and houses major deletions that leads to least homology of the virus (Barua and Rai, 2004).

Epidemiological studies by few researchers revealed that the disease associated with FAdV especially inclusion body hepatitis (IBH), hydropericardium syndrome (HPS) and gizzard erosion (GE) have been increasing recently with major economic losses due to high mortality and poor productivity in infected farms (Cizmecigil *et al.*, 2020; Cui *et al.*, 2020). IBH is commonly caused by serotype 2, 8a, 8b and 11 (Kajan *et al.*, 2013; Marek *et al.*, 2016, Schachner *et al.*, 2016) while HPS and gizzard erosion are mainly caused by serotypes 4 and 1 respectively (Domanska-Blicharz *et al.*, 2011; Grafl *et al.*, 2015; Li *et al.*, 2018). In Malaysia, IBH was first reported in state of Perak in 2005 caused by FAdV serotype 8b with a mortality of about 10% in broiler chickens (Hair-Bejo, 2005), and later with a mortality of 30% in sate of Johore and Malacca (Norina *et al.*, 2016), the major states of poultry industry in the country. It is noteworthy that despite measures put in place by regulatory agencies against IBH in Malaysia which includes good management practices and vaccination against infectious bursal disease (IBD) and chicken anaemia virus (CAV) to control immunosuppression in poultry farms (Hair-Bejo, 2005; Juliana *et al.*, 2014; Norina *et al.*, 2016), IBH still poses a huge problem to the poultry industry. Vaccination then remains the control measure of choice in the management of IBH infections in Malaysia and worldwide (Schachner *et al.*, 2021), but unavailability of commercialized suitable vaccine against FAdV infection is a hinderance. Furthermore, characterization of current FAdV isolates from IBH outbreak remain scanty in Malaysia which is crucial for effective control strategies. It was objective of the study to molecularly characterize FAdV isolates obtained from the field outbreaks of the disease in broiler chicken farms from 2017 to 2019.

MATERIALS AND METHODS

Origin of the Isolates and Processing of Samples

Outbreaks of IBH were suspected in commercial broiler chickens aged 22 to 34-day-old from one farm in Sabah in 2017, and 3 farms in Johor in 2018 and 2019. chickens from infected farms were necrotized and gross lesion

of liver and gizzard were noted. The liver and gizzard samples were collected and homogenized according to previous published protocol (Alemnesh *et al.*, 2014) for molecular detection and characterisation of the virus. Briefly, the tissues were macerated with a sterile mortar and pestle in a 1 in 2 (w/v) suspensions in phosphate buffered saline (PBS, pH 7.4, 0.1M) prior centrifugation at 381 x g for 30 minutes. The supernatant was filtered through 0.45µm syringe filter and stored in -20°C until use.

The FAdV isolates were identified as UPM1701, UPM1801, UPM1802, UPM1901 and UPM1902. The UPM1701 virus was isolated from liver of 34-day-old broiler chickens in Sabah in 2017. The UPM1801 and UPM1802 isolates were obtained from 22-day-old broiler chickens in a farm in Johor from two different houses in 2018. The affected chickens showed clinical signs of depression, weakness and lateral recumbence. Mortality of 2% was recorded within 2 to 3 days of the infection. The UPM1901 isolate was also obtained from Johore in 2019 from 25-day-old broiler chickens with history of 1.22% mortality. The UPM1902 isolate was also obtained from Johore from a 26-day-old commercial broiler chickens with swollen and necrotized liver.

DNA Extraction, Conventional PCR and Agarose Gel Electrophoresis

The DNA from each sample was extracted using innuPREP Virus DNA kit (analytik jena, Germany) following manufacturer's recommended protocol. Measurement of DNA concentration was performed at wavelength 260/280nm by biophotometer (Eppendorf). Amplification of DNA nucleic acid was conducted by PCR using published universal hexon gene primer, HexA1/HexB1 (Meulemans *et al.*, 2001; Marek *et al.*, 2010; Norfitriah *et al.*, 2018) in a total of 50µl PCR reaction using PCR Mastermix (Bioline, UK). The PCR products were separated in a 1% agarose gel electrophoresis using RedSafe™ Nuclei Acid Staining solution (iNtRON, Korea) and 1kb DNA Marker (GeneDirex, USA). Electrophoresis was conducted at 70 volts for 45 minutes prior visualization of DNA fragment band under U.V. transillumination.

DNA Purification, Sequencing and Nucleotide Sequence Analysis

All positive PCR product were purified by

fragment DNA purification kit (iNtRON, Korea) according to manufacture procedure. The purified PCR products were submitted for sequencing (1st BASE, Singapore). Nucleotide sequences were retrieved, assembled and analyzed using Bioedit Version 7.2.5 software (Sohaimi *et al.*, 2019). Online web based namely NCBI BLAST GenBank was used for sequence verification. Nucleotide sequence comparison between FAdV reference strains, UPM1137E2 and UPM04217 and the recent FAdV isolates were performed by multiple sequence alignment (MSA) using ClustalW program. The percentage of identity between isolates was carried out by sequence identity matrix in BioEdit package.

Phylogenetic Tree Analysis

Twenty-one published FAdV hexon gene sequences were obtained from GenBank database with accession numbers as follows: Z67970 (CELO), AF508952 (340), AF339917 (J2-A), EU979377 (C2B), AF508946 (SR48), AF339915 (P7-A), AF508948 (SR49), AF508949 (75), NC_000899 (A-2A), KT862812 (380), DQ323984 (1047), EU979378 (UF71), AF508954 (CR119), AF508955 (YR36), AF339922 (B-3A), AF508957 (58), EU979374 (TR59), JN112373 (764), KU517714 (UPM04217), JF917238 (UPM08158) and KF866370 (UPM1137E2). Phylogenetic tree was generated in MEGA version 5 software using Neighbour-joining method after MSA between recent FAdV isolates and 21 reference strains (Juliana *et al.*, 2014).

RESULTS

Gross Lesions

The liver of UPM1701 was swollen, congested with mild multifocal areas of necrosis and petechial haemorrhages. The UPM1801 and UPM1802 livers were also swollen and yellowish with mild area of petechial haemorrhages. The liver of UPM1901 was pale and swollen with areas of necrosis and haemorrhages. Erosion at the koilin layer of gizzard and enlargement of the proventriculus were also recorded (Figure 1). The UPM1902 showed swollen and necrotized liver.

Molecular Detection by PCR

The UPM1701, UPM1801, UPM1802, UPM1901 and UPM1902 obtained from liver tissues were positive for FAdV with PCR

fragment size of 897 base pairs (bp) (Figure 2). Similarly, for gizzard sample from UPM1901 (Figure 2).

Nucleotide and Amino Acid Sequences Analysis

Nucleotide (nt) sequences of partial hexon gene with length 596 bases for recent isolates were confirmed as Fowl adenovirus in NCBI Blast which corresponds to the L1 loop region of hexon. The percentage identity value is highest for UPM1801 and UPM1802 isolates with 100% identity (E value = 0.0) to reference strain, UPM04217 from serotype 8b species E. There are 98% to 99% identity between UPM1701, UPM1901 and UPM1902 to reference strains under serotype 8b in GenBank databases.

Multiple sequence alignment of 596bp nucleotide sequences in L1 loop region region on hexon gene between recent FAdV isolates and Malaysian's FAdV reference strains, UPM1137E2 and UPM04217 (Figure 3). These nucleotides translated to 198 amino acids (Figure 4). There were 31 nucleotide base substitutions among the sequences of isolates UPM1701, UPM1901 and UPM1902 compared to the earlier reference Malaysian isolates in GenBank. Out of these changes 18 were synonymous while 13 were non-synonymous which yielded 10 amino acid substitutions. These includes **I⁴⁰V**, **D⁴³E**, **G⁴⁴D**, **T⁴⁸S**, **A⁶¹Q**, **A⁷⁰G**, **G⁸⁷T**, **L¹²⁷I**, **G¹⁷⁸R** and **E¹⁷⁹A**. These changes were not observed among the UPM1801 and UPM1802 isolates and could singly or in combination play a role in pathogenicity of the isolates.

Sequence identity matrix revealed 100% identities among UPM1701, UPM1901 and UPM1902 isolates on entire nucleotide sequence length (Table 1). Comparison those three isolates with UPM1801 and UPM1802 isolates showed 94.8% identity. The percentage identity between current isolates and Malaysian reference strains, UPM1137E2 and UPM04217 revealed highest identity to both UPM1801 and UPM1802 with 99.3% and 99.6% identity, respectively. For UPM1701, UPM1901 and UPM1902 isolates, the identity was 94.7% and 95.1% identity compared to UPM1137E2 and UPM04217, respectively.

Phylogenetic Tree Analysis

Phylogenetic tree of 26 aligned nucleotide bases on partial hexon gene revealed FAdV

Table 1. Sequence identity matrix between recent FAdV isolates and reference strains, UPM04217 and UPM1137E2 on partial nucleotide sequences in hexon gene

ID ¹	UPM1137E2	UPM04217	UPM1701	UPM1801	UPM1802	UPM1901	UPM1902
UPM1137E2	ID ¹	99.6	94.7	99.3	99.3	94.7	94.7
UPM04217	99.6	ID ¹	95.1	99.6	99.6	95.1	95.1
UPM1701	94.7	95.1	ID ¹	94.8	94.8	100	100
UPM1801	99.3	99.6	94.8	ID ¹	100	94.8	94.8
UPM1802	97.8	98.1	95.9	0.984	ID ¹	95.9	95.9
UPM1901	94.7	95.1	100	94.8	94.8	ID ¹	100
UPM1902	94.7	95.1	100	94.8	94.8	100	ID ¹

ID¹ : FAdV isolate

classification into 5 species designated as letter A to E as differentiated by colour indicator (Figure 5). Recent FAdV isolates, UPM1701, UPM1801, UPM1802, UPM1901 and UPM1902 were classified under species E serotype 8b and shared common ancestor with UPM04217, UPM1137E2 and 764 strains.

DISCUSSION

The FAdV serotype 8b has been reported to be the most prominent cause of IBH in broiler chickens worldwide leading to significant economic losses in poultry industry due to high mortality, low productivity and performance (Grafl *et al.*, 2012; Lim *et al.*, 2012; Kajan *et al.*, 2013; Morshed *et al.*, 2017). The gross lesions recorded in the present IBH cases which include swollen and yellowish liver with areas of haemorrhages and necrosis are consistent with IBH cases reported previously (Kumar *et al.*, 2013; Maartens *et al.*, 2014). But the presence of gizzard erosion in of this case (UPM1901) which was more commonly recorded in serotype 1 of

the virus, but not for the serotype 8b is noteworthy and could indicate increase in pathogenic effects. Severe gizzard erosion was previously reported in a case of IBH outbreaks (UPM1137) involving 25 to 27 weeks old layer chickens in the country in 2011 in which intranuclear inclusion bodies and FAdV serotype 8b were detected both in the liver and gizzard (Norfitriah *et al.*, 2018). The pathogenicity of FAdVs are highly related to virulence determinant gene which is located in L1 loop of hexon gene (Marek *et al.*, 2010; Sohaimi *et al.*, 2019). Liver is the major predilection site for FAdV replication where it causes destruction of tissue based on necropsy findings, but gizzard could also be affected (Steer *et al.*, 2015).

All the 5 FAdV isolates in the present study were closely related to UPM1137E2 reference strain obtained from previous concurrent IBH infection with gizzard erosion (Sohaimi *et al.*, 2018). The hexon gene primers used in this study were previously used successfully to detect 12 serotypes of FAdV as reported in previous finding (Meulemans *et al.*, 2001) and yielded

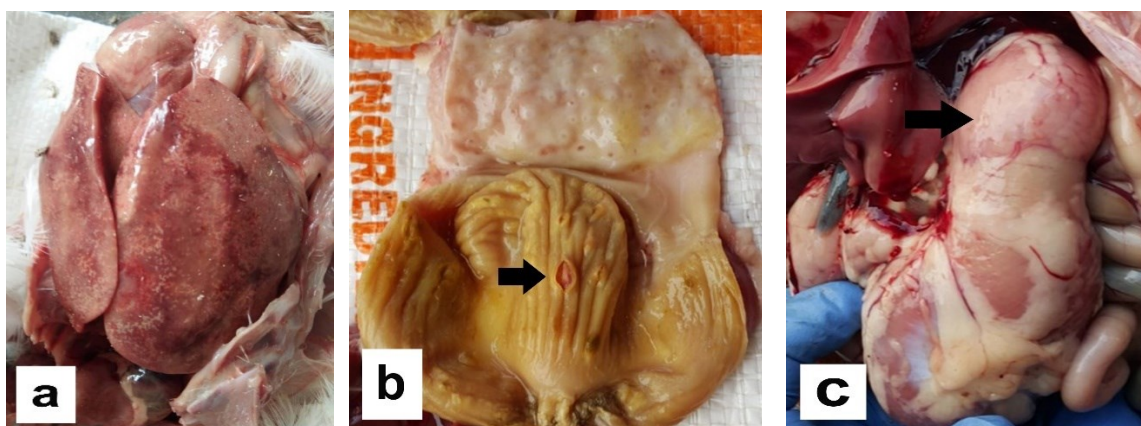


Figure 1. Gross lesions of liver, gizzard and proventriculus of the commercial broiler chickens (UPM1901) infected with FAdV (a): Pale and swollen liver with areas of necrosis and haemorrhages. (b): Erosion at koilin layer of gizzard (arrow). (c): Enlargement and flabby proventriculus (arrow).

596bp nucleotide sequence corresponding to L1 loop region of hexon gene. Analysis of the L1 loop region of hexon gene of the present isolates revealed 98% to 100% identities to FAdV species E serotype 8b reference strain despite originating from different farms and year of collection. Hexon gene, mainly L1 loop region is commonly used for FAdV serotype characterization (Raue *et al.*, 1998; Ojkic *et al.*, 2008). It indicates that FAdV serotype 8b could be the sole causative agent of IBH outbreaks in Malaysia and is highly pathogenic in chickens as previously reported (Hair-Bejo, 2005; Norina *et al.*, 2016; Norfitriah *et al.*, 2019).

The phylogenetic tree of 5 hexon nucleotide sequences obtained from the present 5 IBH cases and 21 reference strains in GenBank derived from 12 serotypes confirmed that the recent isolates belong to FAdV serotype 8b share common ancestry with reference strains from Malaysia (UPM1137E2 and UPM04217) and high pathogenic strain 764 from United Kingdom (Dar *et al.*, 2012). This has shown that it is probably FAdV 8b from same origin that has been circulating in Malaysia, and it seems the isolate is evolving into higher pathogenicity across outbreaks. This was shown in the occurrence of gizzard erosion in this IBH case associated with FAdV 8b. This calls for stringent

preventive measures in Malaysia and IBH endemic regions because the spread of FAdV 8b could follow the Indian experience where the first outbreak of FAdV occurred in 1994 in some parts of Jammu and Kashmir, Punjab and Delhi (Gowda and Satyanarayana, 1994), later spread to Uttar Pradesh same year (Kumar *et al.*, 1997) and then nationwide (Bhowmik, 1996; Nighot *et al.*, 1996; Asrani *et al.*, 1997).

There was a total of 31 nucleotide substitutions which translated to 10 amino acid mutations among isolates UPM1701 (Sabah), UPM1901 and UPM1902 (Johore) when compared to UPM04217 and UPM08158 isolated in 2004 and 2008 respectively (Juliana *et al.*, 2014), and UPM1137E2 isolated in 2011 (Sohaimi *et al.*, 2018). But these changes were not observed in UPM1801 and UPM1802 isolates which have closer similarity to the earlier isolates. This has demonstrated the occurrence of the FAdV serotype 8b divergence or mutation since the first outbreak of the disease reported in 2005 (Hair-Bejo, 2005). Divergence in FAdV 8b genome occurring in different outbreaks has not been reported in Malaysia before and this is coming with novel lesion (gizzard erosion) that is not associated with serotype 8b infections which is worrisome. Viruses are always involved with changes in

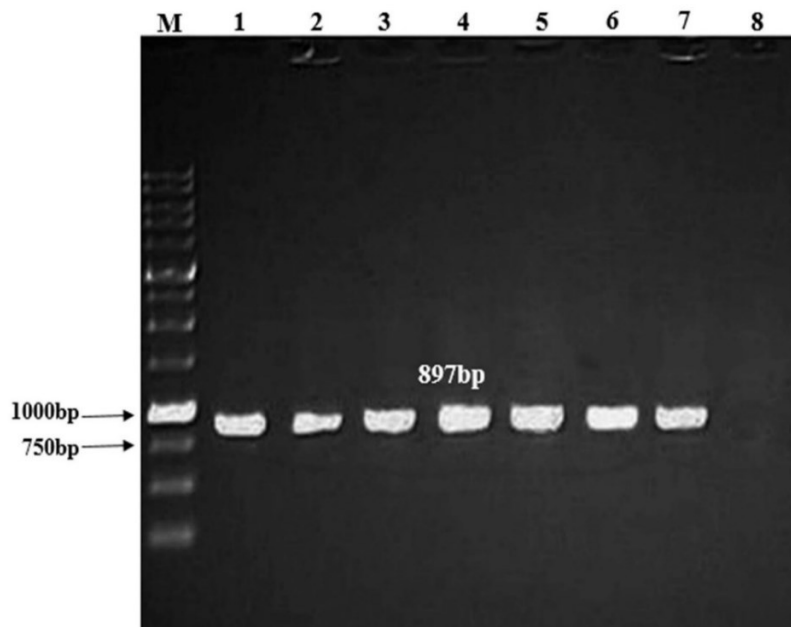


Figure 2. Electrophoresis of polymerase chain reaction products with fragment size of 897 base pairs (bp) in 1% agarose gel using HexA1/HexB1 primer for detection of Fowl adenovirus. Lane M: 1kb DNA marker, Lane 1: Positive control (UPM1137E2), Lane 2: UPM1701, Lane 3: UPM1801, Lane 4: UPM1802, Lane 5: UPM1901 (Liver), Lane 6: UPM1901 (Gizzard), Lane 7: UPM1902, Lane 8: Negative control.

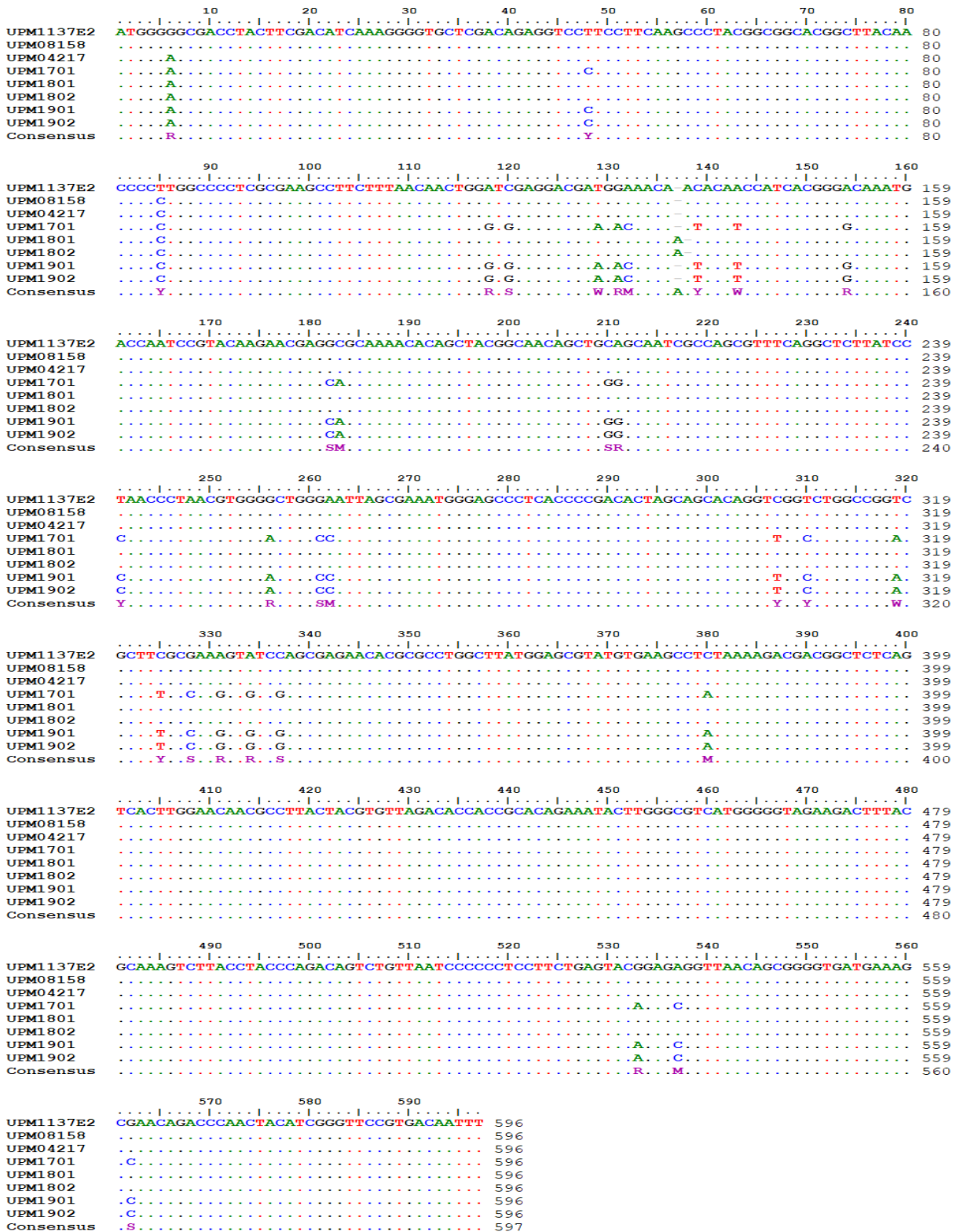


Figure 3. Multiple sequence alignment by ClustalW of 596bp nucleotide sequences of L1 loop in hexon gene between recent FAdV isolates, UPM1701, UPM1801, UPM1802, UPM1901 and UPM1902 and Malaysian's FAdV reference strains (UPM1137E2 UPM08158 and UPM04217).

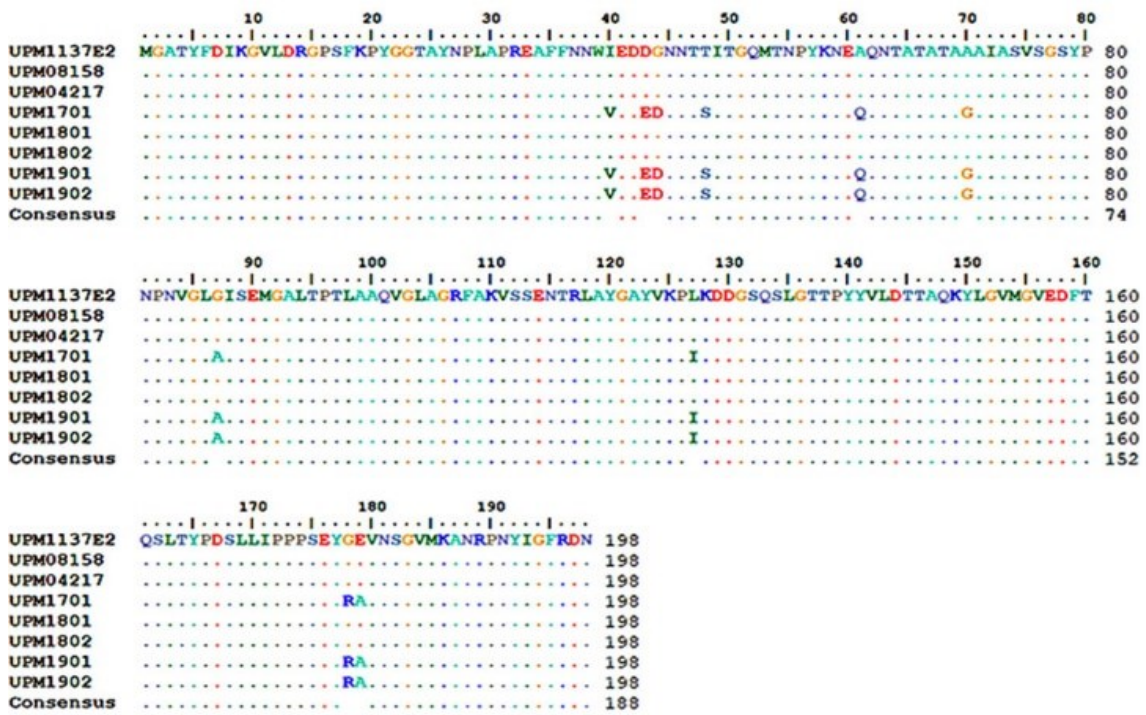


Figure 4. Multiple sequence alignment by ClustalW of 198 amino acids residue of L1 loop in hexon gene between recent FAdV isolates, UPM1701, UPM1801, UPM1802, UPM1901 and UPM1902 and Malaysian's FAdV reference strains (UPM1137E2, UPM08158 and UPM04217).

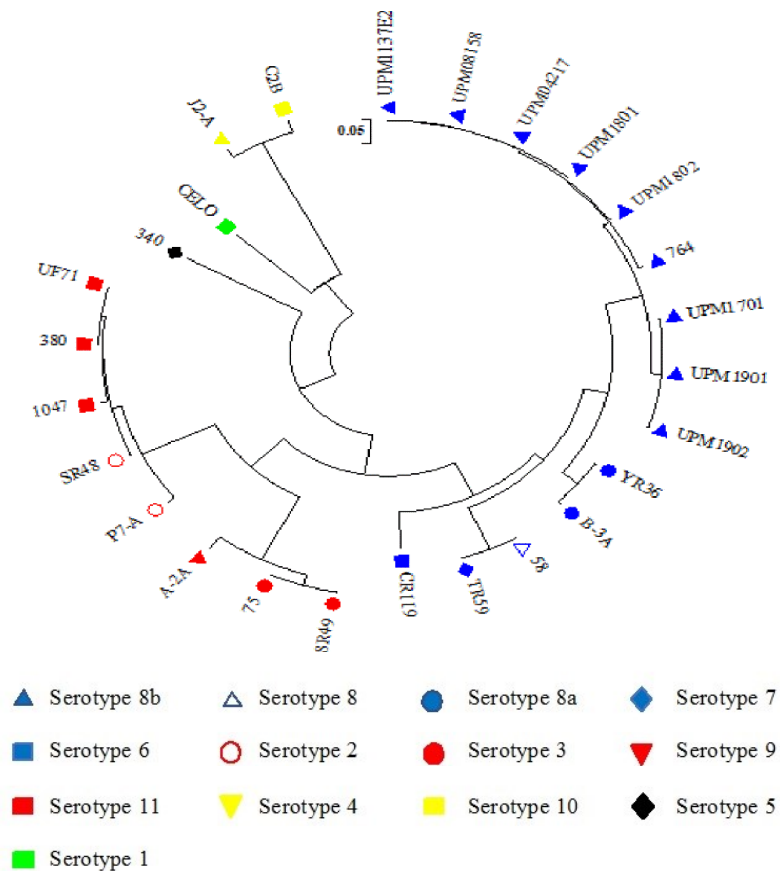


Figure 5. Phylogenetic tree of 596 nucleotide bases on partial hexon gene of fowl adenovirus (FAdV) isolates with 21 reference strains retrieved from GenBank database. The recent isolates, UPM1701, UPM1801, UPM1802, UPM1901 and UPM1902 were classified under species E and belong to serotype 8b FAdV. Color indicator : 1) Blue = Group E, 2) Red = Group D, 3) Yellow = Group C, 4) Black = Group B, 5) Green = Group A

their genome due to genetic selection that could lead to mutations; and mutations could be detrimental, beneficial or of no effect (Fleischmann, 1996). It could produce viruses with increased or reduced pathogenicity, changed host range or tissue tropism (Fleischmann, 1996). Since FAdV infection are spreading to various areas, development of vaccines is crucial and understanding the genetic diversities is very important in designing appropriate vaccines for prevention and control of IBH. Although adequate biosecurity measures could be of help in limiting the spread of IBH since horizontal transfer is an established transmission mechanism (McFerran and Adair, 2003), vaccination using inactivated or attenuated FadV as established elsewhere (Steer *et al.*, 2009; Kim *et al.*, 2014; Schachner *et al.*, 2018), could be the best method of control and eradication. However, following the discovery of molecular changes in recent isolates, regular monitoring of successive outbreaks is highly recommended in endemic areas of the world to safeguard against vaccine failure where vaccines exist and guide in development of appropriate vaccines where it is currently lacking.

CONCLUSION

The recent isolates of FadV were serotype 8b and were the cause of the IBH outbreaks in the poultry farms in Malaysia. These outbreaks occurred with gizzard erosion in one case which is uncommon with FadV serotype 8b. There were also nucleotide changes and amino acid mutations in the isolates from different regions of the country which could be important in the pathogenicity of the virus and could be helpful in the design of vaccine for prevention and control of IBH disease.

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.

Asrani, R.K., B.K. Gupta, S.K. Sharma, S.P. Singh and R.C. Katoch. 1997. Hydropericardium - hepatopathy syndrome in Asian poultry. *Vet. Rec.* 141: 271-273.

Barua, S. And A. Rai. 2004. Phylogenetic analysis of fowl adenovirus 4 hexon gene. *J.*

Immunol.Immunopathol. 6(2): 64-68.

Bhowmik, M.K. 1996. Leechi disease (hydropericardium syndrome) in broiler chicken in West Bengal. *Proceedings, XXth World's Poultry Congress, New Delhi, India, P.317.*

Cizmecigil, U.Y., S. Umar, A. Yilmaz, E. Bayraktar, N. Turan, B. Tali, O. Aydin, H.E. Tali, M. Yaramanoglu, S.G. Yilmaz, A. Kolukisa, J.R. Sadeyen, M. Iqbal and H. Yilmaz. 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(2):231-237.

Cui, J., Y. Xu, Z. Zhou, Q. Xu, J. Wang, Y. Xiao, Z. Li and D. Bi. 2020. Pathogenicity and molecular typing of Fowl Adenovirus-associated associated with hepatitis/hydropericardium syndrome in Central China (2015-2018). *Front. Vet. Sci.* 7.

Dar, A., S. Gomis, I. Shirley, G. Mutwiri, R. Brownlie, A. Potter, V. Gerds and S.K. Tikoo. 2012. Pathotypic and molecular characterization of a fowl adenovirus associated with inclusion body hepatitis in Saskatchewan chickens. *Avian Dis.* 56(1): 73-81.

Domanska-Blicharz, K., G. Tomczyk, K. Smietanka, W. Kozaczynski and Z. Minta. 2011. Molecular characterization of fowl adenoviruses isolated from chickens with gizzard erosions. *Poult. Sci.* 90(5): 983-989.

Fleischmann WR. 1996. *Viral Genetics.* In: *Medical Microbiology.* (S. Baron, eds) 4th edition, Galveston (Tx), 43.

Gowda, R.N.S. and M.L. Satyanarayana. 1994. Hydropericardium syndrome in poultry. *Ind. J. Vet. Pathol.* 18: 159-161.

Grafl, B., F. Aigner, D. Liebhart, A. Marek, I. Prokofieva, J. Bachmeier and M. Hess. 2012. Vertical transmission and clinical signs in broiler breeders and broilers experiencing adenoviral gizzard erosion. *Avian Pathol.* 41(6): 599-604.

Hair-Bejo, M. 2005. Inclusion body hepatitis in commercial broiler chickens. *J. Vet. Malays.* 17: 23-26.

Hess, M. 2000. Detection and differentiation of avian adenoviruses: A review. *Avian Pathol.* 29: 195-206.

Jordan, A.B., L. Blake, J. Bisnath, C. Ramgattie, C.V. Carrington and C.A.L. Oura. 2019.

- Identification of four serotypes of fowl adenovirus in clinically affected commercial poultry co-infected with chicken infectious anaemia virus in Trinidad and Tobago. *Transbound. Emerg. Dis.* 66: 1341–1348.
- Juliana, M.A., M.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 (4): 483-497.
- Junnu, S., P. Lertwatcharasarakul, S. Jala, S. Phattanakulan, A. Monkong, S. Kulprasertsri, C. Thivalai, W. Chakritbudsabong, K. Chaichoun and T. Songserm. 2015. An inactivated vaccine for prevention and control of inclusion body hepatitis in broiler breeders. *Thai. J. Vet. Med.* 45(1): 55-62.
- Kajan, G.L., S. Kecskeméti, B. Harrach and M. Benkő. 2013. Molecular typing of fowl adenoviruses, isolated in Hungary recently, reveals high diversity. *Vet. Microbiol.* 167: 357-363.
- Kim, M.S., T.H. Lim, D.H. Lee, H.N. Youn, S.S. Yuk, B.Y. Kim, S.W. Choi, C.H. Jung, J.H. Han and C.S. Song. 2014. An inactivated oil-emulsion fowl adenovirus serotype 4 vaccine provides broad cross-protection against various serotypes of fowl adenovirus. *Vaccine.* 32(28): 3564-3568.
- Kumar, R., R. Chandra, S.K. Shukla, D.K. Agrawal and M. Kumar. 1997. Hydropericardium syndrome in India: a preliminary study on causative agent and control of disease by inactivated autogenous vaccine. *Trop. Anim. Health Prod.* 29: 158-164.
- Kumar, V., R. Kumar, R. Chandra, P. Bhatt and K. Dhama. 2013. Outbreaks of inclusion body hepatitis (IBH) in chickens; pathological studies and isolation of fowl adenovirus. *Adv. Anim. Vet. Sci.* 1(3S): 21–24.
- Li, L., J. Wang, P. Chen, S. Zhang, J. Sun and W. Yuan. 2018. Pathogenicity and molecular characterization of a fowl adenovirus 4 isolated from chicken associated with IBH and HPS in China. *BMC Vet. Res.* 14: 400.
- Lim, T.H., B.Y. Kim, M.S. Kim, J.H. Jang, D.H. Lee, Y.K. Kwon, J.B. Lee, S.Y. Park, I.S. Choi and C.S. Song. 2012. Outbreak of gizzard erosion associated with fowl adenovirus infection in Korea. *Poult. Sci.* 91 (5): 1113-1117.
- 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. S. Afr. Vet. Assoc.* 85: 1-5.
- Marek, A., G.L. Kaján, C. Kosiol, M. Benkő, A. Schachner and M. Hess. 2016. Genetic diversity of species Fowl aviadenovirus D and Fowl aviadenovirus E. *J. Gen. Virol.* 97 (9): 2323-2332.
- 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(6): 937-941.
- McFerran, J.B. and B.M. Adair. 2003. Group I Adenovirus Infections. In: *Diseases of Poultry*, 12th ed. (Y.M. Saif, A.M. Fadly, J.R. Glisson, L.R. McDougald, L.K. Nolan and D.E. Swayne, eds). Blackwell Publishing, Iowa, USA. P.214-27.
- Meulemans, G., M. Boschmans, T.P. van den Berg and M. Decaesstecker. 2001. Polymerase chain reaction combined with restriction enzyme analysis for detection and differentiation of fowl adenoviruses. *Avian Pathol.* 30: 655-660.
- 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(2): 205-210.
- Night, P.K., D.B. Deshmukh, G.R. Ghalasasi and B.D. Sarvashe. 1996. Inclusion body hepatitis, hydropericardium syndrome: sequential histopathology. *Proceedings, XXth World's Poultry Congress, New Delhi, India.* P.321.
- Norfitriah, M.S., M. Hair-Bejo and A. Majdi. 2019. Pathogenicity of fowl adenovirus serotype 8b isolates of Malaysia in specific pathogen free chicken. *J. Anim. Vet. Adv.* 18(3): 78-83.
- 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. *Int. J. Agric. Sci. Vet. Med.* 6(1): 73-84.

- Norina, L., A. Norsharina, A.H. Nurnadiah, I. Redzuan, A. Ardy and A. Nor-Ismaliza. 2016. Avian adenovirus isolated broiler affected with inclusion body hepatitis. *Malays. J. Vet. Res.* 7(2): 121-126.
- Ojkić, D., E. Martin, J. Swinton, J.P. Vaillancourt, M. Boulianne and S. Gomis. 2008. Genotyping of Canadian isolates of fowl adenoviruses. *Avian Pathol.* 37(1): 95-100.
- Okuda, Y., M. Ono, I. Shibata, S. Sato and H. Akashi. 2006. Comparison of the polymerase chain reaction-restriction fragment length polymorphism pattern of the fiber gene and pathogenicity of serotype -1 fowl adenovirus isolates from gizzard erosions and from feces of clinically healthy chickens in Japan. *J. Vet. Diagn. Invest.* 18 (2): 162-167.
- 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(8): 5115-5122.
- Raue, R. and M. Hess. 1998. Hexon based PCRs combined with restriction enzyme analysis for rapid detection and differentiation of fowl adenoviruses and egg drop syndrome virus. *J. Virol. Methods.* 73(2): 211-217.
- Sheppard, M., R.J. McCoy and W. Werner. 1995. Genomic mapping and sequence analysis of the fowl adenovirus serotype 10 hexon gene. *J. Gen. Virol.* 76(10): 2595-2600.
- Schachner, A., G. Beatrice and M. Hess. 2021. Spotlight on avian pathology: fowl adenovirus (FAdV) in chickens and beyond – an unresolved host-pathogen interplay. *Avian Pathol.* 50(1): 2-5.
- Schachner, A., A. Marek, B. Grafl and M. Hess. 2016. Detailed molecular analyses of the hexon loop-1 and fibers of fowl aviadenoviruses reveal new insights into the antigenic relationship and confirm that specific genotypes are involved in field outbreaks of inclusion body hepatitis. *Vet. Microbiol.* 186: 13-20.
- Schachner, A., M. Matos, B. Grafl and M. Hess. 2018. Fowl adenovirus-induced diseases and strategies for their control – a review on the current global situation. *Avian Pathol.* 47(2): 111-126.
- Singh, A., M.S. Oberoi, S.K. Jand. et al. 1996. Epidemiology of inclusion body hepatitis in poultry in northern India from 1990 to 1994. *Rev. Sci. Tech. Off. Int. Epiz.* 15: 1053-1060.
- 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(6): 759-770.
- Sohaimi, N.M., M.H. Bejo, A.R. Omar, A. Ideris and N.M. Isa. 2019. Molecular characterisation of fowl adenovirus isolate of Malaysia attenuated in chicken embryo liver cells and its pathogenicity and immunogenicity in chickens. *PLoS One.* 14 (12): e0225863.
- Steer, P.A., N.C. Kirkpatrick, D. O'Rourke and A.H. Noormohammadi. 2009. Classification of fowl adenovirus serotypes by use of high-resolution melting-curve analysis of the hexon gene region. *J. Clin. Microbiol.* 47: 311-321.
- 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. *Aus. Vet. J.* 89: 184-192.
- Steer, P.A., J.R. Sandy, D. O'Rourke, P.C. Scott, G.F. Browning and A.H. Noormohammadi. 2015. Chronological analysis of gross and histological lesions induced by field strains of fowl adenovirus serotypes 1, 8b and 11 in one-day-old chickens. *Avian Pathol.* 44(2): 106-113.
- Ugwu, C.C., M. Hair-Bejo, M.I. Nurulfiza, A.R. Omar and A. Ideris. 2020. Propagation and Molecular Characterization of Fowl Adenovirus Serotype 8b Isolates in Chicken Embryo Liver Cells Adapted on Cytodex™ 1 Microcarrier Using Stirred Tank Bioreactor. *Processes.* 8: 1065.