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MOLECULAR AND HISTOPATHOLOGICAL STUDY ON CIRCULATING FOWL ADENOVIRUS SEROTYPES CAUSING INCLUSION BODY HEPATITIS (IBH) IN BROILER CHICKENS IN SRI LANKA

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SUMMARY: Inclusion Body Hepatitis (IBH) is an emerging acute viral disease in Sri Lanka caused by Fowl Adenoviruses (FAdVs) of the genus *Aviadenovirus*, family *Adenoviridae*. It primarily affects broilers aged 3–7 weeks, leading to liver damage and significant economic losses. Histopathological diagnosis of IBH is based on the presence of pathognomonic intranuclear basophilic inclusion bodies in degenerating hepatocytes. Molecular diagnosis of IBH involves the detection of the hexon gene of the FAdV using conventional PCR. The serotyping, based on the hexon gene L1 loop variable region, is important for developing effective vaccination strategies. This study aimed to identify circulating FAdV serotypes responsible for IBH in broilers in the Western, North Western, and Central provinces of Sri Lanka. A total of 76 dead broilers were collected/ received; Western (n = 23), North Western (n = 38), and Central (n = 15). Clinical history, vaccination data, and performance records were documented. The liver was the primary organ examined. Gross and histopathological evaluations were performed, and histopathological confirmation of IBH was based on intranuclear basophilic inclusion body detection. The gross lesions observed in all the examined livers had hepatomegaly, petechial haemorrhages and multifocal-coalescing necrotic patches. PCR was conducted on histopathologically confirmed IBH-positive samples (n = 28/76), of which 19 were PCR-positive (n = 19/28). Three PCR-positive samples from each province were selected for hexon gene sequencing using the Sanger method. Sequencing revealed serotype FAdV-8b in both the North Western and Central provinces and FAdV-11 only in the North Western province. No sequence data could be obtained from the Western province. These findings highlight the presence of different FAdV serotypes contributing to IBH in Sri Lanka and emphasise the need for region-specific surveillance and vaccination strategies.

KEYWORDS: Broiler chicken; Family *Adenoviridae*; Fowl Adenoviruses (FAdVs); Inclusion body; Inclusion Body Hepatitis (IBH)

INTRODUCTION

Inclusion Body Hepatitis (IBH) could cause a significant economic impact on the broiler industry in Sri Lanka. Therefore, a proper understanding of IBH is important for safeguarding broiler health and broiler industry. IBH is caused by Fowl Adenoviruses (FAdVs) in the Family *Adenoviridae*. Viruses belonging to this Family have non-enveloped, icosahedral capsid containing a non-segmented, linear, double-stranded DNA (dsDNA) genome, with a size ranging between 25 to 48 kbp (Benko *et al*., 2022). According to the 2024 release by the International Committee on Taxonomy of Viruses (ICTV), the family *Adenoviridae* comprises six genera, one of which is *Aviadenovirus*, known to exclusively infect birds. The Genus *Aviadenovirus* consists of 28 virus species. Among them, 12 species are responsible for causing diseases in fowls, such as in chickens. The ICTV 2024 update specifies that the 12 identified species correspond directly to 12 individual serotypes. The serotyping is based on the nucleotide sequence of the hexon gene, L1 loop variable region (Adel *et al*., 2021; Sohaimi and Hair-Bejo, 2021; Hess, 2000). Recent research has found that FAdV serotypes 2, 3, 6, 7, 8a, 8b, 9, and 11 are responsible for causing IBH (Bertran *et al*., 2021). The latest FAdV classification is given in Table 1.

Table 1: Classification of FAdV serotypes and diseases caused by the serotypes

Species	Serotype	Abbrev.	Disease
Aviadenovirus ventriculi	fowl adenovirus 1	FAdV-1	Gizzard erosions (Matczuk *et al.*, 2017)
Aviadenovirus gallinae	fowl adenovirus 2	FAdV-2	IBH (Schachner *et al.*, 2018)
Aviadenovirus gallinae	fowl adenovirus 3	FAdV-3	IBH (Bertran *et al.*, 2021)
Aviadenovirus hydropericardii	fowl adenovirus 4	FAdV-4	Hydropericardium Syndrome (Schachner *et al.*, 2018)
Aviadenovirus quintum	fowl adenovirus 5	FAdV-5	Tenosynovitis (Kaján *et al.*, 2022)
Aviadenovirus hepatitidis	fowl adenovirus 6	FAdV-6	IBH

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			(Bertran *et al.*, 2021)
Aviadenovirus hepatitidis	fowl adenovirus 7	FAdV-7	IBH (Bertran *et al.*, 2021)
Aviadenovirus hepatitidis	Fowl adenovirus 8a	FAdV-8a	IBH (Schachner *et al.*, 2018)
Aviadenovirus hepatitidis	fowl adenovirus 8b	FAdV-8b	IBH (Schachner *et al.*, 2018)
Aviadenovirus gallinae	fowl adenovirus 9	FAdV-9	IBH (Bertran *et al.*, 2021)
Aviadenovirus hydropericardii	fowl adenovirus 10	FAdV-10	Hydropericardium Syndrome (Schachner *et al.*, 2019)
Aviadenovirus gallinae	fowl adenovirus 11	FAdV-11	IBH (Schachner *et al.*, 2018)

IBH is an acute viral disease that can be manifested in both broilers and layers, but broilers are the most susceptible (Matos *et al.*, 2016). The age group that IBH mainly affects is 3 – 7 weeks old broiler chickens (Bertran *et al.*, 2021; Panigrahi *et al.*, 2016). IBH can be transmitted vertically via embryonated eggs and horizontally via contaminated faeces and fomites (Panigrahi *et al.*, 2016; McFerran and Adair, 2003). There can be around 10% morbidity in IBH (Gomis *et al.*, 2006), and mortality can range from 5% up to 50% depending on the host susceptibility and virus serotype involved (Abghour *et al.*,2019; Matos *et al.*, 2016). The main replication sites are the epithelial cells of the alimentary tract and the upper respiratory tract (Saifuddin and Wilks, 1991). The completion of primary replication leads to viremia, and the virus can disseminate to other organs such as the liver, kidney, thymus, spleen, and bursa of Fabricius. According to previous studies, immunosuppressive diseases such as avian leucosis, infectious bursal disease and chicken anaemia can induce IBH infection in broilers (Chitradevi *et al.*, 2018; Meng *et al.*, 2018; Choi *et al.*, 2012). However, some studies showed that IBH is a primary disease affecting broilers (Gomis *et al.*, 2006).

The affected broilers show clinical signs such as lethargy, huddling, ruffled feathers, depression, inappetence and adopting a crouching position (Gomis *et al.*, 2006). Furthermore, the liver was identified as the principal target organ (Perera et al., 2021; Rahimi and Haghighi, 2015), exhibiting gross pathological changes characterised by friable liver, hepatomegaly, yellow discolouration of the hepatic parenchyma, necrotic foci and multifocal petechial haemorrhages (Cizmecigil *et al.*, 2020; Mariappan, 2018; Rahimi and Haghighi, 2015). Since, the clinical signs and gross lesions are non-specific, confirmation of IBH needs laboratory based diagnostic tests. There are several laboratory diagnostic methods for IBH. Most of the diagnostic methods are developed to identify FAdV and further confirmation methods might be required to diagnose IBH.

Histopathological identification of intranuclear basophilic inclusion bodies in hepatocytes is considered as the pathognomonic lesion of IBH (Mariappan *et al.*, 2018). Therefore, the examination of the liver histopathology is considered as a useful diagnosis method for IBH (Panigrahi *et al.*, 2016).

Molecular diagnosis is one of the modern, accurate and sensitive diagnostic methods. The hexon gene in the dsDNA genome of the FAdV is the common target in molecular diagnosis and commercially available primers can be used for the conventional Polymerase Chain Reaction (PCR) (Steer *et al.*, 2008). Resultant PCR products can be used for nucleotide sequencing or restriction enzyme digestion for serotyping (Meulemans, 2001) thus, serotyping serves as an important method for the definitive confirmation of IBH.

IBH was first reported in the USA in 1963 (Fadly and Winterfield, 1973), and over the past twenty years, IBH outbreaks have been reported in different regions of the world, indicating the global disease distribution, including Asia, Africa, Europe, North America, Latin America and Australia (Schachner *et al.*, 2017). Also, IBH has become more of an epidemic than a sporadic outbreak (El-Shall *et al.*, 2022).

The first reported IBH cases in Sri Lanka were published in 2021 by Perera *et al*. According to their findings, suspected IBH outbreaks were reported between July 2019 and March 2020 in 13 commercial broiler farms and one layer farm in Kurunegala, Puttalam, and Gampaha districts in Sri Lanka. The liver was observed as the most affected organ. Hepatocellular degeneration and necrosis with predominant intranuclear basophilic inclusion bodies were reported as histopathological data. The condition was diagnosed as IBH using histopathological findings (Perera *et al.*, 2021). Except for the above-mentioned article, there was no published literature about the epidemiology of IBH in Sri Lanka.

There is a high demand for poultry meat worldwide, especially broiler meat. According to the Food and Agriculture Organization (FAO), broiler meat contributes to 90% of global poultry meat production (FAO, 2025). In Sri Lanka during the year 2020, 64% of the Gross Domestic Production (GDP) in the livestock sector was from broiler meat (DAPH, 2021). Considering this high global demand for broiler meat, disease conditions such as IBH could affect the broiler industry in many ways. Several studies conducted in North America found that IBH was responsible for heavy mortalities, weight loss and high carcass condemnation at

slaughterhouses (Abghour *et al*., 2019; Gomis *et al*., 2006).

Given the economic impact of IBH, the implementation of effective prevention and control strategies is of critical importance. Consistent disease surveillance, strict biosecurity measures, regular monitoring of breeder flocks and implementation of vaccination programmes are considered the most effective prevention and control methods for IBH (Bertran et *al*., 2021). Live or inactivated vaccines are commonly used in many countries (Monis *et al*., 2005), and serotypes FAdV-4 and FAdV-8 are used in commercial vaccines (Saifuddin and Wilks, 1990). Vaccination of broiler breeders is done to develop immunity against IBH in progeny (broilers) via the transfer of maternal antibodies (Gomis *et al*., 2006). Understanding the circulating FAdV serotypes is essential for the development and implementation of an effective vaccination program against IBH (Schachner *et al*., 2018). The autogenous inactivated vaccine against the prevalent serotype of FAdV is recommended in IBH-endemic areas (Alvarado *et al*., 2007).

Since there is no published data on circulating FAdV serotypes responsible for IBH in Sri Lanka, the effectiveness of the currently used vaccines is uncertain. Therefore, it is necessary to detect the prevalent FAdV serotypes in Sri Lanka.

This research was aimed to study the circulating FAdV serotypes responsible for causing IBH in broiler chickens, which show suspected clinical signs of the disease in three poultry-dense provinces of Sri Lanka.

MATERIALS AND METHODS

Sample collection

Samples were collected from commercial broiler farms located in the Western, North Western and Central provinces of Sri Lanka. These three provinces have the highest number of broiler farms in Sri Lanka (Statistical Bulletin 2022, DAPH) and thus, were selected for the study. The study was conducted over a period of one year and nine months from 2022 June to 2024 March. Dead broilers with suspected clinical signs of IBH were collected or received for the research and 1-3 broilers from one farm/cage were randomly selected for sampling. Suspected clinical signs were lethargy, huddling, ruffled feathers, depression, inappetence and adopting a crouching position which was consistence with the signs of IBH in previous studies (Gomis *et al*., 2006). Number of farms/cages from each province and the total number of dead broilers included in this study are given in Table 2.

Table 2: Number of samples collected for the study

Province	Farms / Cages	No. of dead broilers
Western	13	23
North Western	18	38
Central	6	15

When collecting or receiving dead broilers, information regarding flock history, clinical signs, performance metrics, and vaccination records was systematically collected. The age of the dead broilers, initial stocking density, cumulative mortality and other management data were also collected.

Postmortem exanimation and gross pathology

All collected/received broiler carcasses with suspected IBH clinical signs were subjected to necropsy examinations and liver samples were collected for further histopathological and molecular analysis. Gross pathological features of the liver were recorded for each carcass.

Histopathology

Liver samples were collected and placed in appropriately labelled containers with 10% neutral buffered formalin for fixation. Histopathological examination was performed on all collected liver samples (Total = 76). The routine tissue processing procedure was employed. Processed tissue samples were embedded in paraffin wax blocks, and cut into 2-5 µm sections. Hematoxylin and Eosin (H&E) stain was used for staining tissue sections (Dolka *et al*., 2012) and histopathological features associated with IBH infection were investigated using light microscopy and the observations were recorded. The presences of pathognomonic intranuclear basophilic inclusion bodies in hepatocytes with hepatocellular degeneration and necrosis were considered as IBH positive cases (Mariappan *et al*., 2018; Rahimi and Haghighi, 2015; Dolka *et al*., 2012; Hair-Bejo, 2005).

Molecular analysis

Following the postmortem examination, liver samples were collected into labelled Eppendorf tubes and stored in -20 °C for further confirmation using a conventional PCR. Due to resource constraints, PCR was performed only on samples histopathologically confirmed as IBH-positive. Total DNA extraction was performed using QIAGEN DNeasy® mini kit, according to the manufacturer's protocol. Conventional PCR was performed with the extracted total DNA. The hexon gene of the FAdV was the target of the molecular

diagnosis. The following primer pair was used in this study and the anticipated amplicon size was 897 bp.

•FAdV - Hexon A - CCRTTCAGRCAGACGGT

•FAdV - Hexon B - TAGTGATGMCGSGACATCAT

The PCR protocol consisted of an initial denaturation at 95 °C for 15 minutes, followed by 35 amplification cycles of denaturation at 94 °C for 1 minute, annealing at 60 °C for 1 minute, and extension at 72 °C for 1 minute. A final extension was at 72 °C for 10 minutes (Gowthaman *et al.*, 2012). Commercially available, inactivated IBH vaccine was used as the positive control and nuclease free water was used as the negative control.

The gel electrophoresis was done using 1% agarose gel and the gel image was captured using Geneflash Syngene gel imaging system (Cambridge, UK).

Nucleotide sequencing and serotyping of FAdV

Selected PCR-positive liver samples representing Western, North Western and Central provinces were subjected to gene sequencing targeting the hexon gene of the FAdV using the Sanger sequencing technique. Gene sequencing was done for 3 liver samples from each province.

A small aliquot of PCR positive liver tissue samples (stored in -20 °C) were spotted directly onto a QIAcard® FTA™ Classic cards (QIAGEN, Hilden, Germany), ensuring complete saturation of the sample area. The cards were air-dried at room temperature for 1–3 hours in a PCR hood, previously cleaned and UV-irradiated to minimize the risk of cross-contamination. After drying, the FTA™ cards were individually placed in sterile zip-lock bags with silica desiccant packs and stored at room temperature. Samples were transported to the molecular laboratory under ambient conditions for further analysis. Molecular analysis and gene sequencing was performed by *Laboratory Diagnostics Germany GmbH, Abschnede 64, Cuxhaven, Germany (Vaxxinova Autogenous Vaccines, Germany) and BioChek B.V., Fokkerstraat 14, 2811ER, Reeuwijk, The Netherlands.*

The resulting nucleotide sequences were compared to known sequences in the NCBI GenBank database using BLASTn (Basic Local Alignment Search Tool for nucleotides) to identify the closest matching Fowl Adenovirus strains. Sequences with the highest percentage identity and lowest E-values were used for serotype identification.

RESULTS

Performance data

The summary of the collected data from broiler farms in each province is included in Table 3.

Table 3: The age range, approximate initial input of the broilers in the farm, range of cumulative mortality and vaccination history of the dead broilers used for the study

Province	Age range (days)	Input (broilers)	Mortality (%)	Vaccination*
Western	26 – 47	35,000	3 – 9	ND, IBD, IB
North Western	26 – 38	30,000	6 – 8	ND, IBD, IB
Central	21 – 40	30,000	16 -35	ND, IBD, IB

*****ND: Newcastle disease, IBD: Infectious bursal disease, IB: Infectious bronchitis

Most of the farms in these provinces were large scale commercial broiler farms with intensive management systems. The clinical signs shown by the IBH suspected broilers were lethargy, huddling, ruffled feathers, depression, pale wattle and anorexia (Figure 1).

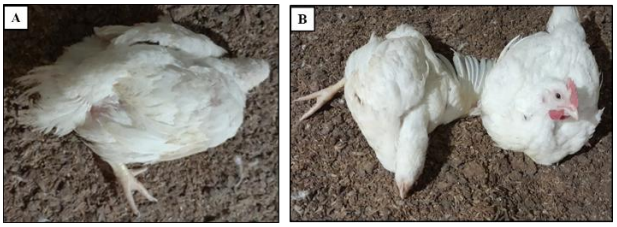

Figure 1: Clinical signs of IBH.
(A) Ruffled feathers, depression and lethargy (B) Pale wattle and depression

Gross pathology

Hepatomegaly, friable pale yellow liver with multifocal - coalescing necrotic patches and petechial haemorrhages, were observed as gross lesions in all collected liver samples of the dead broilers suspected of having IBH clinical signs (Figure 2).

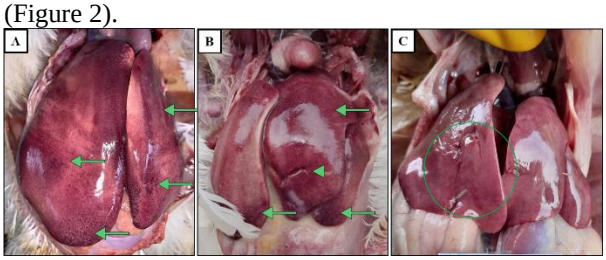

Figure 2: Gross lesions of IBH
A: hepatomegaly, pale yellow discolouration of the liver parenchyma and petechial haemorrhages (arrows); B: petechial haemorrhages (arrows) and liver rupture (arrowhead); C: necrotic patches and ruptured areas on the liver (circled area).

Histopathology

Histopathological examination revealed IBH-associated lesions in 5 liver samples from the Western Province (n = 5/23), 16 samples from the North Western Province (n = 16/38) and
7 samples from the Central Province (n = 7/15), yielding a total of 28 IBH-positive livers out of 76 examined (n = 28/76). Those histopathological findings included, pathognomonic large intranuclear basophilic inclusion bodies consistent with previous studies (Perera *et al*., 2021; Mariappan *et al*., 2018) together with varying degrees of hepatocellular degeneration and necrosis (swollen cells, reduced cytoplasmic eosinophilia with vacuolation, unclear cell margins as well as nuclear changes: pyknosis, karyorrhexis and karyolysis) in hepatocytes. Few hepatocytes in these histopathologically confirmed IBH positive livers had intranuclear eosinophilic inclusion bodies.

The remaining liver samples,18 from the Western Province (n = 18/23), 22 from the North Western Province (n = 22/38), and 8 from the Central Province (n = 8/15) exhibited hepatocellular degeneration and necrosis but lacked intranuclear basophilic inclusion bodies. Accordingly, these samples were classified as IBH-negative cases, totalling 48 out of 76 examined livers (n = 48/76). Light microscopic images of histopathologically confirmed IBH positive livers and IBH negative livers are included in Figure 3.

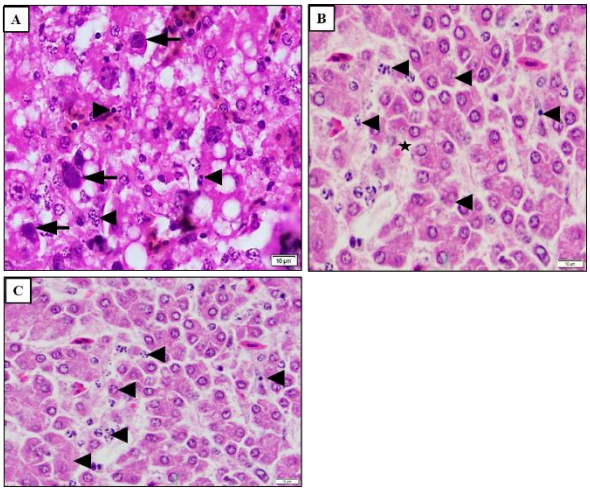

Figure 3: Histopathology of IBH, A: Intranuclear basophilic inclusion bodies in hepatocytes (arrows) and degeneration of hepatocytes and nuclear changes due to necrosis (arrowheads) in IBH positive liver, B: Intranuclear eosinophilic inclusion body in hepatocyte (asterisk) and degeneration of hepatocytes and nuclear changes due to necrosis (arrowheads) in a IBH positive liver, C: Degeneration of hepatocytes and nuclear changes due to necrosis (arrowheads) without intranuclear basophilic inclusion bodies in hepatocytes in a IBH negative liver.

Molecular analysis

The PCR positive livers showed the anticipated bands at the level of 897 bp together with the positive control. The negative control did not show any PCR band.

Gel image of PCR products are in the Figure 4 and the summary of PCR results for the histopathologically positive IBH livers are shown in Table 4.

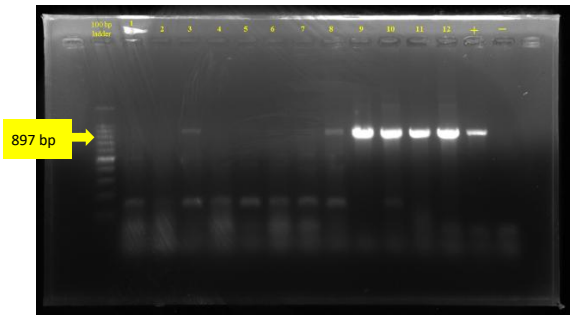

Figure 4: PCR gel image. Lane 1 to lane 12 contains liver samples which were histopathologically positive for IBH. The positive control, samples 3, 8, 9, 10, 11 and 12 are PCR positive for FAdV and have bands at the 897 bp level. The samples 3 and 8 are moderately positive whereas sample 9, 10, 11 and 12 are highly positive. There is no band at the 897 bp level for negative control.

Table 4: Summary of PCR results for histopathologically confirmed IBH positive livers

Province	Total Histopathology	Histopathology positive	PCR positive	PCR negative
Western	23	5	3	2
North Western	38	16	10	6
Central	15	7	6	1
Total	76	28	19	9

Nucleotide sequencing and serotyping of FAdV

Sequencing results were obtained for 6 PCR positive livers including all 3 samples from North Western and Central provinces. Unfortunately, we could not obtain sequence results for 3 liver samples submitted from Western province. This might be due to degradation of DNA material or inadequate amount of DNA in the sample to perform the analysis.

The resultant serotypes from the comparison between obtained nucleotide sequences with the NCBI GenBank database sequences are presented in Table 5.

Table 5: Serotypes circulating in selected provinces

Province	Serotypes
Western	-
North Western	FAdV-8b and FAdV-11
Central	FAdV-8b

The sequence homology between the nucleotide sequences obtained in this study and known sequences in the NCBI GenBank database that were used for serotype identification, is presented in Table 6**.**

Table 6: Nucleotide sequence homology of study isolates with GenBank reference serotypes

Province	Serotype	Sequence homology %	Accession number	Reference
North Western	FAdV-8b	98.52%	JN112373.1	Dar *et al.*, 2012
	FAdV-8b	98.84%	LC604663.1	Yamaguchi *et al*., 2022
	FAdV-11	99.06%	KY229174.1	De la Torre *et al*., 2018
Central	FAdV-8b	99.07%	JN112373.1	Dar *et al.*, 2012
	FAdV-8b	99.25%	LC604663.1	Yamaguchi *et al*., 2022
	FAdV-8b	99.15%	LC604663.1	Yamaguchi *et al*., 2022

DISCUSSION

According to Rahimi and Haghighi (2015), broilers infected with IBH exhibit clinical signs such as lethargy, huddling in a crouching position, ruffled feathers, depression, and anorexia. All IBH-suspected dead broilers collected for this study exhibited these clinical signs. Additionally, the gross pathological examination of the livers from the deceased broilers revealed lesions consistent with IBH, as described by Schachner et al. (2018). However, it is important to note that mycotoxicosis and fatty liver haemorrhagic syndrome may present with similar gross lesions, such as friable liver tissue with petechial haemorrhages in the hepatic parenchyma (Nagar *et al*., 2024; El Nabarawy *et al*., 2020). A study conducted in Canada in 2015 also indicated that immunosuppression caused by a variant Infectious Bursal Disease (IBD) virus could lead to hepatitis and necrotic patches in the liver (Amini *et al*., 2015). Therefore, until these conditions are excluded through histopathological examination or PCR, field veterinarians should consider them as differential diagnoses for IBH.

Histopathology and molecular analysis using PCR can be considered as effective diagnostic methods for IBH according to Panigrahi *et al*., (2016) and Steer *et al*., (2008). Detection of pathognomonic intranuclear basophilic inclusion bodies in hepatocytes is the histopathological diagnoses for IBH (Mariappan *et al*., 2018). In the study, observing this lesion in livers was considered as histopathologically positive for IBH. Some of these livers contained intranuclear eosinophilic inclusion bodies together with basophilic inclusion bodies. In the present study, livers which did not show intranuclear basophilic inclusion bodies in hepatocytes were considered as histopathologically negative for IBH. However, during the histopathological examination, all the subjected livers (n=76) had varying degrees of hepatocellular degeneration and necrosis with or without intranuclear basophilic inclusion bodies in hepatocytes. The only criterion to assess the IBH status was the presence of intranuclear basophilic inclusion bodies in hepatocytes. This might lead to false negative results because histopathologically negative livers could contain intranuclear basophilic inclusion bodies in hepatocytes which were not observed in the prepared tissue section. Therefore, tissue sections should be selected from multiple areas of the liver to avoid this error.

Molecular diagnosis using PCR is the accurate and sensitive method for diagnosing IBH together with histopathology, gross lesions and clinical signs. Conventional PCR was used in this study to detect the hexon gene in the FAdV genome. PCR was carried out only on liver samples that tested positive for IBH through histopathological examination. Since PCR has a specific detection limit, the amount of target DNA must be above a certain threshold to produce a reliable result. This threshold can vary depending on the PCR protocol used (Hajia, 2018). Therefore, PCR protocols should be properly optimised before being used for diagnosis to reduce the chance of false negatives. Accurate quantification of nucleic acids using tools such as the Qubit® Fluorometer or NanoDrop® spectrophotometer is important during this process (Nakayama *et al*., 2016).

The PCR protocol used in this study was optimised prior to molecular diagnosis. However, due to resource limitations, viral DNA quantification in the liver samples was not performed. This may explain the PCR-negative results in some histopathologically positive samples, as the viral DNA concentration in those livers may have been below the detection threshold of the PCR assay. For future studies, it is recommended to determine the minimum detectable viral DNA concentration for the protocol described by Gowthaman et al. (2012), to improve the accuracy of IBH molecular diagnosis in Sri Lanka.

According to a study by Hair-Bejo, (2005), the transmission electron microscopy of the intranuclear inclusion bodies in IBH infected hepatocytes revealed that, basophilic inclusion bodies contain adenoviral particles whereas eosinophilic inclusion bodies consist of granular protein material indicating a cellular degeneration process/ early stage of virus assembly in the hepatocyte/ remaining protein material after virus replication. Additionally, histopathology results of the study revealed that some hepatocytes contained intranuclear eosinophilic inclusion bodies which might be an accumulation of protein material rather than viral DNA particle as described by Hair-Bejo, 2005. This could be another reason for PCR negativity of FAdV in histopathologically positive liver samples,

because the PCR used in this study only detect viral DNA.

Nucleotide sequence analysis from the present study identified FAdV serotypes 8b and 11 as the circulating strains associated with IBH in the North Western and Central provinces of Sri Lanka. The detection of these serotypes is consistent with global trends, highlighting their widespread distribution and significance in poultry health.

FAdV-11 has been reported as the predominant serotype causing IBH in India (Chitradevi *et al*., 2021), and has also been detected in both breeder and broiler flocks in Pakistan (Sharif *et al*., 2020) and Iran (Mirzazadeh *et al*., 2020). Similarly, both FAdV-8a and FAdV-8b have been identified in China (Chen *et al*., 2019), and FAdV-8b has been isolated in Japan (Mase *et al*., 2020). Outside of Asia, FAdV-2 has been reported as the causative agent of IBH in southwestern Ontario, Canada (Philippe *et al*., 2005). In South America, FAdV-8a, 8b, and 11 were detected in IBH cases in Brazil (De la Torre *et al*., 2018). In Australia, FAdV-8b and FAdV-11 were similarly associated with IBH outbreaks (Steer *et al*., 2011).

CONCLUSION

Veterinarians working in the Western, North-Western and Central provinces recently identified disease outbreaks in broiler farms, raising concerns about the possible presence of IBH. Our investigation confirmed the circulation of IBH in these regions, emphasising the importance of including it in the differential diagnosis.

The findings of this study contribute valuable molecular data on circulating FAdV serotypes associated with IBH in broiler chickens in Sri Lanka.

They also emphasise the need for region-specific surveillance and tailored vaccination strategies to effectively manage IBH in broiler breeders, ensuring the protection of the poultry industry in Sri Lanka.

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