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AVIAN ADENOVIRUS ISOLATED FROM BROILER AFFECTED WITH INCLUSION BODY HEPATITIS

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ABSTRACT. Inclusion body hepatitis (IBH) has been reported in many countries in the world. The IBH characterized presence of intra-nuclear inclusion bodies in hepatocytes in chickens. On December 2015, an onset of high acute mortality in a flock of 12, 18 and 23-day-old broiler chickens in Malacca and Johore was reported to the Regional Veterinary Laboratory, Johor Bahru, Peninsular Malaysia. The birds showed lethargy, huddling, ruffled feathers, and inappetence. At necropsy, the livers were enlarged, pale yellow, friable and with multiple petechial hemorrhages, the kidney were congested and enlarged, with hydropericardium and gizzard erosion. Large eosinophilic intranuclear inclusion bodies were seen in hepatocytes. PCR revealed liver were positive of FAdV at expected band of 1219 bp and the nucleotide sequence share 95-99% identity with the fowl adenovirus species E, serotype 8b. Based on the acute high mortality, age of the broilers, gross and microscopic lesions (especially intranuclear inclusion bodies) and molecular finding, the condition was diagnosed as adenovirus inclusion body hepatitis.

Keywords: adenovirus, serotype 8b, broiler, chickens, liver, eosinophilic.

INTRODUCTION

Fowl adenovirus (FAV) is ubiquitous in chickens, with worldwide distribution. FAV is associated with naturally occurring outbreaks of inclusion body hepatitis (IBH) (Winter *et al*., 1973), hydropericardium syndrome (HPS) (Abe *et al*., 1998), respiratory disease (Dhillon and Kibenge, 1987), necrotizing pancreatitis (Ota *et al*., 1999), or gizzard erosion. Fowl adenovirus are resistant to several disinfectants, heat and pH changes (Hafez, 2011). IBH can be transmitted by both vertical and horizontal means, vertical transmission is reported as a very efficient way to spread from parent birds to progenies (Mc Ferran and Adair, 1977; McCracken and Adair, 1993) while horizontal transmission occurs by the oral-faecal route and further spread

takes place by mechanical means and by contamination with infected faeces (Hafez, 2011).

FAV can be isolated from both healthy and sick birds (Mc Ferran *et al*., 1972) due to the presence of maternal antibodies and low virulence of some strains. The most common viruses isolated belong to serotypes 4 and 8 where they are capable of producing the disease without the immunosuppressive such as infectious bursal disease (IBDV) or chicken anaemia virus (CAV). Hair-Bejo (2005) reported that IBH is characterized by sudden onset of mortality peaking after 3-4 days of infection, ending on fifth days, but with infection occasionally continuing for 2-3 weeks. Clinically, affected birds showed crouching position with ruffled feathers, huddling and inappetence. (Calnek *et al*., 1991; Hafez, 2011). Macroscopically include an enlarged pale, friable with ecchymotic hemorrhages (Howell *et al.,* 1970; Macpherson *et al*., 1974), hydropericardium syndrome (Abe *et al*., 1998) and gizzard erosion. A similar condition is observed in this paper. Gizzard erosions in chickens have been associated with diets that are deficient in vitamin B6 (Daghir and Haddad, 1981) or with the ingestion of histamine (Harry and Tucker, 1976), gizzerosine (Okazaki *et al*., 1983) and mycotoxins (Hoerr *et al*., 1982). However, several cases of gizzard erosion associated with FAV infection have been reported in chickens and quails in recent years (Abe *et al*., 2001; Goodwin, 1993; Nakamura *et al*., 2002; Tanimura *et al*., 1993).

In Malaysia, the case of IBH in chicken is under-reported, however it will cause a high economic impact on the poultry industry in Malaysia with high mortality and poor growth performances. This paper describes the case of IBH in a flock of broiler chickens in Peninsular Malaysia.

MATERIALS AND METHODS

Animals

Sudden onset of mortality affecting 30% of the flock since June 2015 in a flock of 12-day-old broiler chickens was reported to Regional Veterinary Laboratory Johore Bahru. The farm was located in southwest of Peninsular Malaysia which in the state of Malacca and Johore. In total, 35,000 broiler chickens were kept in open system houses (7,000 birds per house). The broilers were reared under open house system with slatted floor under palm trees. The birds were vaccinated against infectious bronchitis, infectious bursa disease and Newcastle disease. Birds were usually found dead but were occasionally seen in an extremely depressed condition shortly before death. Death occurred within a few hours following initial observation of signs. Outbreaks occurred most frequently at the early age of 12 days old and as late as 23 days old.

Figure 1. Swollen, pale, friable and multiple petechial haemorrhages in 18-day-old broiler chicken with inclusion body hepatitis (IBH)

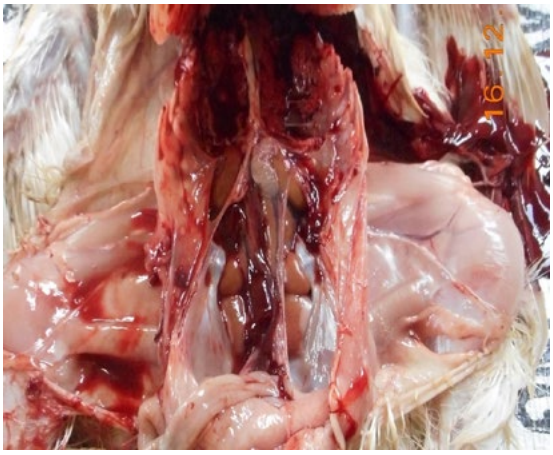


Figure 2. Kidneys appeared pale and swollen in 18-day-old broiler chicken with inclusion body hepatitis (IBH)

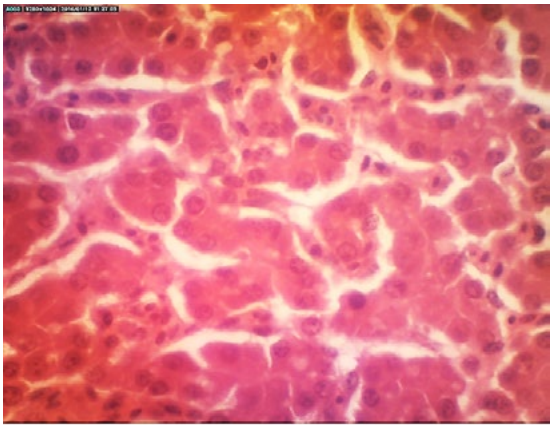


Figure 3. Eosinophilic intranuclear inclusion body in 18-day-old broiler chicken with inclusion body hepatitis (IBH), H&E, 1000×.

Laboratory Diagnosis

Twelve, 18 and 23-day-old broilers from 4 different houses in Malacca and Johore with a history of poor growth and high mortality (14%) were submitted for necropsy. On necropsy, samples of fresh liver were sent for confirmation by PCR. Liver, kidney and heart were fixed in 10% buffered formalin. They were processed according to routine procedures and stained with hematoxylin and eosin (H&E) for histopathology.

RESULTS

Details of results from the investigation are shown in Figures 1 to 5.

Necropsy findings

On necropsy, moderate enlargement of liver with pale, friable and multiple petechial haemorrhages and congestion were observed (Figure 1). The kidneys were congested and enlarged (Figure 2). Hydropericardium with yellowish colored fluid present in the sac surrounding the

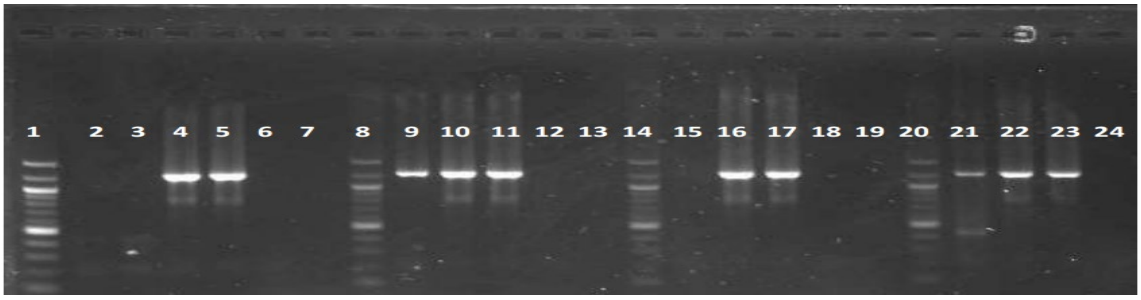
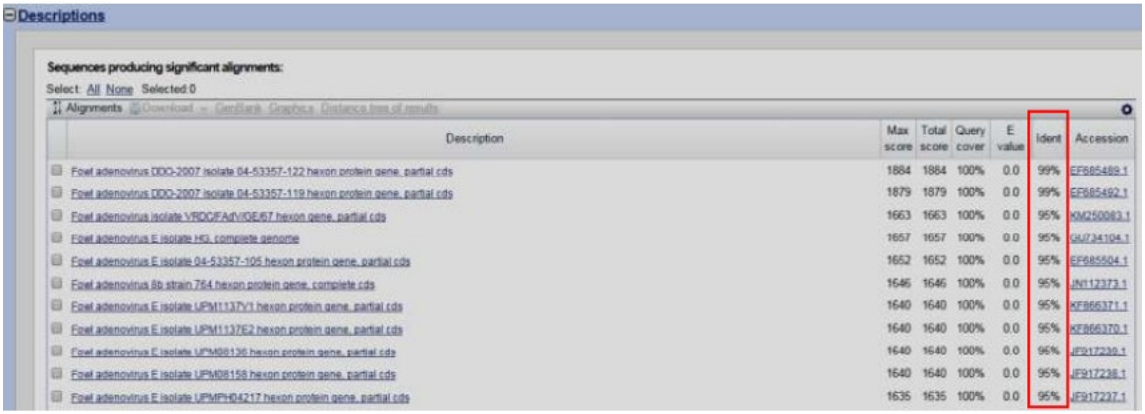


Figure 4. Detection of FAdV with PCR followed by electrophoresis on 1.5% (w/v) agarose gel, 80V for 50 min. The PCR result indicated that the samples Lanes 9, V61/15/MVKJB/4466/15 and Lane 21, V63/15/MVKJB/4473/15 are positive for FAdV. The FAdV reference strain was used as positive control with expected band of 1219 bp.


Descriptions

Sequences producing significant alignments:

Select: All None Selected:0

Alignments Download GenBank Graphics Distance tree of results

Description	Max score	Total score	Query cover	E value	Ident	Accession
Fowl adenovirus DDO-2007 isolate 04-53357-122 hexon protein gene, partial cds	1884	1884	100%	0.0	99%	EF685489.1
Fowl adenovirus DDO-2007 isolate 04-53357-119 hexon protein gene, partial cds	1879	1879	100%	0.0	99%	EF685492.1
Fowl adenovirus isolate VRDC/FAdV/GE/67 hexon gene, partial cds	1663	1663	100%	0.0	95%	KM250083.1
Fowl adenovirus E isolate HG, complete genome	1657	1657	100%	0.0	95%	GU734104.1
Fowl adenovirus E isolate 04-53357-105 hexon protein gene, partial cds	1652	1652	100%	0.0	95%	EF685504.1
Fowl adenovirus 8b strain 764 hexon protein gene, complete cds	1646	1646	100%	0.0	95%	JN112373.1
Fowl adenovirus E isolate UPM1137V1 hexon protein gene, partial cds	1640	1640	100%	0.0	95%	KF866371.1
Fowl adenovirus E isolate UPM1137E2 hexon protein gene, partial cds	1640	1640	100%	0.0	95%	KF866370.1
Fowl adenovirus E isolate UPM08136 hexon protein gene, partial cds	1640	1640	100%	0.0	95%	JF917239.1
Fowl adenovirus E isolate UPM08158 hexon protein gene, partial cds	1640	1640	100%	0.0	95%	JF917238.1
Fowl adenovirus E isolate UPMPH04217 hexon protein gene, partial cds	1635	1635	100%	0.0	95%	JF917237.1

Figure 5. The nucleotide identity matching revealed that the isolates share 95-99% identity with the fowl adenovirus species E, serotype 8b.

heart, and gizzard erosion were observed too.

On histological examination of the liver with numerous eosinophilic and basophilic intranuclear inclusion bodies were observed in the hepatocytes (Figure 3). Focal hepatitis with infiltration of mononuclear inflammatory cells was noted. Kidneys showed severe hyperemia, tubular epithelial cells degeneration, intra-tubular cellular cast formation, and mild interstitial nephritis. Mild focal myocarditis, with degenerated muscle fibers was seen in the heart sections

Laboratory results

PCR result revealed that two samples of liver were positive of FAdV at expected band of 1219 bp (Figure 4). Nucleotide sequence identity of the positive samples were determined using Basic Local Alignment Search Tool (BLAST, NCBI http://blast.ncbi.nlm.nih.gov/Blast.cgi.).

Results revealed that the isolates share 95-99% identity with the fowl adenovirus species E, serotype 8b (Figure 5).

After trimming of the raw sequencing data, the 700 bp nucleotide of hexon protein gene sequence of FadV isolates was analysed with BLAST.

DISCUSSION

In this study, high acute mortality started with 12-day-old broiler chickens. The mortality peaked on the 5th day and gradually declined to normal at the age of 21 days. As the age of the host increases, the degree of multiplication of the viruses within the host is restricted (Clemmer, 1972) and the mortality they produce is reduced (Cook, 1974). IBH can affect all ages of chicken and all found to be susceptible during the first 2 to 3 weeks of life.

Inclusion bodies are generally associated with a viral aetiology, either eosinophilic or basophilic (Itakura *et al*., 1974; Grimes *et al.*, 1977). Hair-Bejo (2005) reported that the basophilic intranuclear inclusion bodies in IBH contain numerous adenoviruses when examined under transmission electron microscopy (TEM), whilst the eosinophilic inclusion bodies contain only fibrillary granular material and filaments. From the findings, it can be concluded that presence of the eosinophilic inclusion bodies in hepatocytes indicates an early stage in the formation of virus or a late stage after the virus has left the nucleus (Riddell, 1987).

In this study, the clinical signs and lesions of the disease here were similar to those of hydropericardium syndrome (HPS, Angara disease) which been reported by Ahmad *et al*., (1989). The main pathological findings of HPS are the accumulation of a clear, straw colored fluid in the pericardial sac (Jaffery, 1988). Based on the acute high mortality, age of the broilers, gross and microscopic lesions (especially intranuclear inclusion bodies) and molecular finding, the condition was diagnosed as adenovirus inclusion body hepatitis.

Further studies have to be done to assess the prevalence of serotypes of fowl adenoviruses and occurrence of IBH in poultry flocks in Malaysia. Demonstration of pathognomonic intra-nuclear inclusion bodies in hepatocytes indicates the involvement of a DNA containing virus. Serology can be used to monitor progression but obviously does not indicate active infection. Virus isolation and molecular methods can also be used for detection and typing of field isolates.

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