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Prevalence and Molecular Diagnosis of Hydropericardium Hepatitis Syndrome in the Poultry Population of Mizoram, India

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ABSTRACT

Background: Hydropericardium hepatitis syndrome (HHS) is an important emerging disease having economic importance and affecting particularly broiler chickens. Although the disease has been reported as a major problem in almost all the regions of India, not much is known from the North Eastern states of India. The present study was aimed to study prevalence, clinico-pathology and molecular diagnosis of HHS in poultry population of Mizoram.

Methods: A poultry population of total 2430 from 15 different farms located in different districts of Mizoram were studied for outbreaks of HHS. Clinical manifestation of the disease in affected birds was recorded and pathological studies were carried out in total 190 dead birds. The outbreaks were further confirmed by detection of hexon gene of FAdV in tissue lesions by PCR.

Result: Outbreak of HHS was recorded in 5 (33.3%) different broiler farms with sudden onset of mortality (18 to 25.2%) in broiler birds of 4 to 6 weeks of age. Pathological studies revealed characteristic lesions comprising hydro pericardium, hepatitis and basophilic intranuclear inclusion bodies in hepatocytes. Detection of hexon gene of FAdV in tissue lesions by PCR assay has further confirmed the outbreaks. This study reports for the first time, high prevalence of HHS in poultry population of Mizoram.

Key words: Hydropericardium hepatitis syndrome, Mizoram, Pathology, PCR, Poultry.

INTRODUCTION

Hydropericardium hepatitis syndrome (HHS) is an economically important emerging disease particularly of broiler birds. *Fowl aviadenovirus* (FAdV 4) is the etiological agent for HHS which is classified under the genus *Aviadenovirus* and family *Adenoviridae* (Virus taxonomy, 2018). The virus is a linear double stranded DNA virus. The genome is approximately 43 to 46 kb, non-enveloped and icosahedral in shape. Broiler birds of 3-6 weeks of age are mostly affected by HHS.

The disease is characterized by rapid onset of sudden mortality in broiler chicken ranging from typically 10-30% (Kataria *et al.*, 2013). Litchi appearance of the heart due to hydropericardium and hepatitis are the most characteristic lesions of the disease. Hydropericardium hepatitis syndrome was first observed in 1987 at Angara Goth, Pakistan and therefore it was named as Angara disease. Later, the disease was reported in other countries including India where the disease was first detected in Jammu followed by Punjab and Delhi in 1994 (Gowda and Satyanarayana, 1994). Since then, several outbreaks of the disease have been reported from different states and regions of India like Madhya Pradesh, Uttarakhand, Uttar Pradesh, Karnataka, Tamil Nadu, Kerala, *etc* (Kumar *et al.*, 1997; Asrani *et al.*, 1997; Asthana *et al.*, 2013). Based on clinicopathological findings, the outbreak of HHS in the northeast region was first reported in 2002 among the poultry population of Mizoram (Rajkhowa, 2002). Subsequently incidences of the disease were reported during 2013-2014 in Mizoram (Bhutia and Singh, 2017) and Assam in 2017 (Dutta *et al.,* 2017). Although there have been reports on outbreaks of the disease from poultry population of Mizoram on the basis of clinicopathological finding, it has never been confirmed by molecular detection of FAdV in the tissue lesions of affected birds. Therefore, the present study was undertaken to gather information on the prevalence, pathology and molecular diagnosis of Fowl aviadenovirus (FAdV) in the poultry population of Mizoram.

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MATERIALS AND METHODS

Sample collection

A poultry population of total 2430 numbers from 15 different farms located in different districts of Mizoram were visited regularly to study outbreak of HHS during the period from March 2018 to March 2019. Suspected flocks were clinically examined, clinical sign and symptoms were recorded. The relevant epidemiological information like location of farm, strength of affected flock, age of the affected flock, morbidity and mortality rates, history and type of vaccination and previous outbreaks of diseases were recorded. A total of 190 dead birds from 15 different farms were collected for

postmortem examination and gross lesions were recorded. Representative tissue samples from liver, kidneys, heart, lungs, spleen, thymus and bursa of Fabricius were collected and fixed in 10% buffered neutral formalin for histopathological studies. Representative tissue samples from liver was also collected and stored at -80°C for molecular diagnosis. Fixed tissue samples in formalin were processed for routine histopathological examination and stained with Hematoxylin and Eosin (H&E) following standard procedures (Bancroft and Gamble, 2008).

Polymerase chain reaction

Total DNA was extracted from tissue lesions, using conventional method (Sambrook *et al.,* 1989). The tissue sample was minced and 500 µl of TENS solution along with 2.5 µl of Proteinase K was added. The digestion was carried out in a water bath at 56°C for 3 hours and 500 µl of phenol: chloroform: isoamyl alcohol (25:24:1) was added to the digested tissue sample, mixed and spun after which the aqueous phase was separated. This was then treated again with phenol, chloroform, isoamyl alcohol and the process was repeated. Next, 100% Isopropanol was added, mixed and kept for precipitation of DNA. The sample was then spun at 12000 rpm and resultant DNA pellet was washed with 70% ethanol, spun again and pellet was then dried in speed vacuum. The DNA extracted was stored by re-suspending in 20µl of TE buffer and stored at -20°C till further use. PCR was performed on extracted DNA by using the primer set H3 5′- AACGT CAACCCCTTCAACCACC-3′, H4 5′-TTGCC TGTGGCGAAAGGCG-3′ to amplify a relatively conserved region of hexon gene of FAdV (Raue and Hess, 1998). Amplification of PCR products was visualized in agarose gel electrophoresis using 1.5% agarose gel containing ethidium bromide against a 1 kb DNA ladder (Fermentas-O'Gene Ruler 1 kb DNA ladder plus, SM0313). The gel was visualized in a UV trans-illuminator (Bio-Rad Laboratories Inc, USA).

RESULTS AND DISCUSSION

The poultry population in Mizoram, India, has increased five-fold in the last decade. With the increase in the density of population, the occurrence of disease outbreaks like IBD, NDV, MDV, coccidiosis have also been recorded frequently (Rajkhowa *et al.,* 2012, Bhutia and Singh, 2016; Rajkhowa *et al.,* 2018). However, the information regarding prevalence, epidemiology, pathology and molecular diagnosis of HHS in poultry population of Mizoram are lacking till date.

The present study has confirmed outbreak of HHS in 5 out of 15 poultry farms covering a chicken population of 2430. All the outbreaks were recorded during the summer months from March to June in broiler birds of 4 to 6 weeks of age. Similar findings were previously described by Sawale *et al.* (2012), Palanivelu *et al.* (2014), Das *et al.* (2015) and Bhutia and Singh (2017). Clinically disease appeared suddenly with mortality of 18 to 25.2% in the birds. General signs of illness like dullness, depression, lethargy and huddling were observed in affected birds. Similar observations were earlier recorded by Palanivelu *et al.* (2014), Bhutia and Singh (2017) and Dutta *et al.* (2017).

At necropsy, the most consistent gross lesion observed in all the cases was hydropericardium characterized by the presence of 5-10 ml of clear to straw coloured fluid in the pericardial sac of the heart (Fig 1) and hepatitis. The heart showed congestion and focal petechial haemorrhage at the base of the epicardium. The liver was enlarged, congested and friable with rounding of the edges in the FAdV affected birds. There were focal areas of necrosis and focal to diffused petechial haemorrhages on the surface (Fig 2). Such lesions were earlier described as the characteristic lesions of HHS (Meenakshi-Bal *et al.,* 2005; Sawale *et al.,* 2012; Thakor *et al.,* 2012; Palanivelu *et al.*, 2014; Das *et al.*, 2015; Dutta *et al.,* 2017). Enlarged, congested and friable kidneys were observed in all of the cases. In some cases there were focal necrotic areas and petechial haemorrhages on the surface of the kidneys (Fig 3). Palanivelu *et al.* (2014) and Das *et al.* (2015) also reported similar findings. The lungs showed moderate to severe congestion and edema in almost all the cases. The bursa of Fabricius was edematous and showed mild to severe degree of congestion. The spleen showed moderate to severe congestion and in some cases it was enlarged and friable. Congestion of thymus was observed



Fig 1: Accumulation of straw colored fluid in the pericardial sac of the heart of 4-week-old FAdV affected bird.

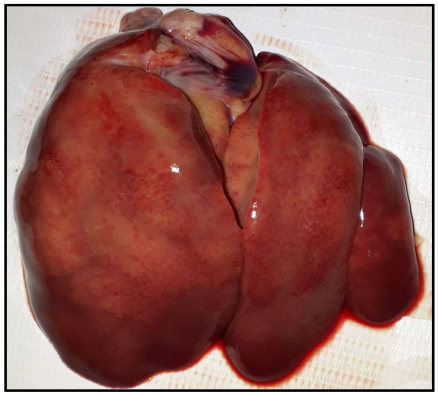


Fig 2: Enlargement of the liver with focal area of necrosis and haemorrhage of 5-week-old FAdV affected bird.

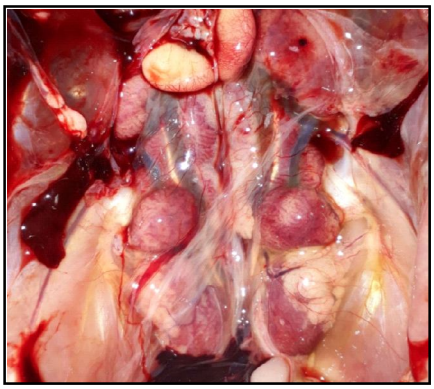


Fig 3: Enlargement and congestion of the kidney with necrotic areas and petechial haemorrhages of 5-week-old FAdV affected bird.

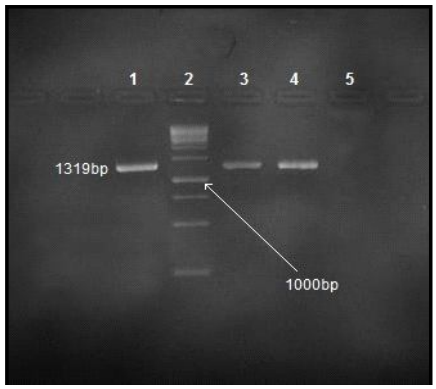


Fig 4: Agarose gel electrophoresis stained with Ethidium bromide showing the 1319 bp hexon gene fragment in tissue sample (Lane 1 = +ve control, Lane 2 = Marker, Lane 3 = +ve sample, Lane 4 = +ve sample, 5= -ve control).

in some cases. These findings are in accordance with reports from Kumar *et al.* (1997), Meenakshi-Bal *et al.* (2005), Sawale *et al.,* 2012, Palanivelu *et al.* (2014), Das *et al.* (2015) and Dutta *et al.,* 2017. Moderate to severe ascites was observed in dead birds of 3 farms along with the hydropericardium and hepatitis as earlier described by Das *et al.* (2015).

Histopathological examination of the liver revealed mononuclear infiltration and moderate to severe congestion in portal area. Sinusoidal spaces were congested and dilated along with disorientation of the hepatic chords and vacuolar degeneration (Fig 5). Hepatocytes revealed cellular swelling and degeneration. Many of the swollen hepatocytes contained basophilic intranuclear inclusion bodies completely replacing the nucleus of the degenerating hepatocyte which is considered as pathognomonic lesion (Fig 6). These findings were found to be similar with the earlier reports of Sawale *et al.* (2012), Thakor *et al.* (2012), Palanivelu *et al.* (2014) and Dutta *et al*. (2017). Kidneys of affected birds showed infiltration of mononuclear cells in the interstitium, hypercellularity in glomerular tuft, congestion in the interstitium, marked degeneration, swelling, necrosis and desquamation of the renal tubular epithelium (Fig 7). Similar findings were earlier described by Sawale *et al.* (2012) and Palanivelu *et al.* (2014). The lungs revealed severe congestion of the parabronchiolar wall and mononuclear cell infiltration towards the lumen leading to compression of respiratory atria and air capillaries and dilatation of the inter-parabronchiolar septae due to oedema. The findings were similar to earlier reports by Venkatesha *et al.* (2005) and Dutta *et al*. (2017). Heart revealed congestion, lack of cross striations indicating degeneration and disintegration of the cardiac muscle fibres due to degeneration. Sharma *et al.* (2014) and Dutta *et al*. (2017) reported similar findings. Bursa showed congested and haemorrhagic areas and dilatation of the interfollicular septa due to oedema and lymphoid depletion in the germinal centre of the follicle (Fig 8). Similar observations were reported by Thakor *et al.* (2012) and Palanivelu *et al.* (2014). Microscopic examination of spleen showed severe congestion and haemorrhages in splenic parenchyma and depletion of the

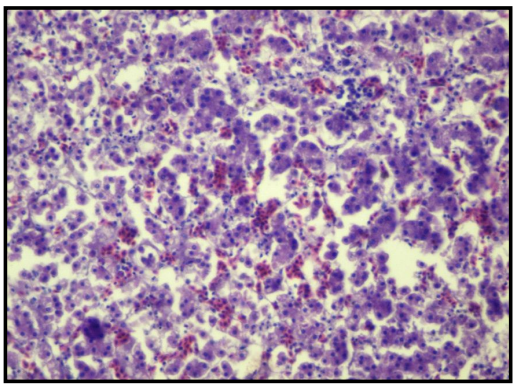


Fig 5: Section of liver showing congestion and dilatation of the sinusoidal spaces, vacuolar degeneration. H&E 400X.

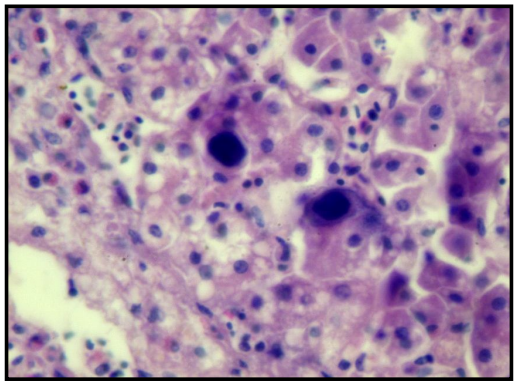


Fig 6: Section showing swelling and degeneration of the hepatocytes and presence of basophilic intranuclear inclusion bodies in the degenerating hepatocyte. H&E 1000X.

lymphocytes in the germinal centre of the follicle. This was similar to findings reported by Meenakshi-Bal *et al*. (2005), Thakor *et al.* (2012) and Dutta *et al*. (2017).

Following clinicopathological examination, all the outbreaks of HHS were further confirmed by detection of hexon gene of FAdV in liver tissue lesions by PCR. Out of 15 farms included in the study, 5 (33.3%) farms were tested

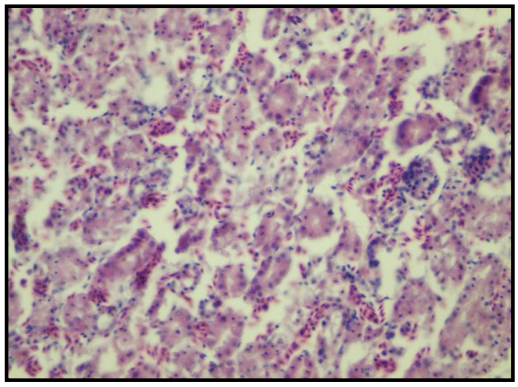


Fig 7: Section of kidney showing congestion, infiltration of mononuclear cells in the interstitium, degeneration and necrosis of the renal tubular epithelium. H&E 400X.

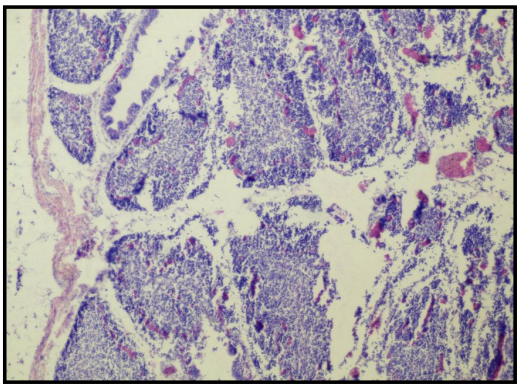


Fig 8: Section of bursa of Fabricius showing depletion of lymphoid tissue in follicles and oedema in the interfollicular septa. H&E 100X.

positive for FAdV by PCR detection of a 1319bp region of hexon gene (Fig 4). PCR technique targeting the hexon gene of FAdV to confirm diagnosis of HHS was earlier described by Raue and Hess (1998) and Shamim *et al.* (2009).

CONCLUSION

This study has recorded high prevalence of FAdV infection in poultry population of Mizoram, which has resulted huge economic losses to the poultry farmers through morbidity (20 to 36%) and mortality (18 to 25.2%). The disease was particularly recorded in broiler birds of 4 to 6 weeks of age during the period of March 2018 to March 2019. Typical gross lesions comprising of hydropericardium and hepatitis were observed consistently in affected birds. Basophilic intranuclear inclusion bodies in the degenerated swollen hepatocytes confirmed the pathological diagnosis of the FAdV infection in the affected birds. Finally the diagnosis was further confirmed by PCR detection of hexon gene of FAdV in the representative tissue lesions from liver. This study is confirming for the first time outbreak of HHS in poultry population of Mizoram by using PCR assay to detect FAdV. Occurrence of FAdV infection with high morbidity and mortality in poultry population which is confirmed by this study should be considered as a major health concern for the poultry population of Mizoram.

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