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Research Article

DIAGNOSIS OF THE INCLUSION BODY HEPATITIS-HYDROPERICARDIUM SYNDROME USING CONVENTIONAL TECHNIQUES

DINESH MITTAL[1], R. S. KHOKHAR[2] and N. JINDAL[2*]
[1]Department of Epidemiology and Preventive Veterinary Medicine
Guru Angad Dev Veterinary and Animal Sciences University, Ludhiana-141 004
[2]Department of Veterinary Public Health and Epidemiology, College of Veterinary Sciences
Lala Lajpat Rai University of Veterinary & Animal Sciences, Hisar-125 004

ABSTRACT

The present work was carried out to diagnose the inclusion body hepatitis-hydropericardium syndrome (IBH/HPS) in broiler chicks using conventional techniques such as histopathology and agar gel precipitation test (AGPT). Liver samples were collected from 40 commercial broiler chicken flocks suspected to be suffering from IBH/HPS. The disease caused changes in liver, kidney, heart and lymphoid organs. Enlarged, swollen and jaundiced/pale liver with pin point or diffuse white necrotic foci/hemorrhages as well as enlarged and swollen kidneys were the major necropsy findings. Histopathological changes such as small multifocal areas of coagulative necrosis and mononuclear cell infiltration were observed in liver. Basophilic intranuclear inclusion bodies were observed in liver specimens from 22 flocks. Lymphocytic depletion was observed in lymphoid organs. A line of precipitation was observed in samples from 27 flocks by AGPT. The study indicates that the disease can be diagnosed based on necropsy findings, histopathological changes in the liver and kidney and serological tests such as AGPT. However, these techniques may not provide information about the serotype of fowl adenovirus associated with IBH/HPS.

Key words: Fowl adenovirus, hydropericardium syndrome, inclusion body hepatitis, histopathology, broiler chickens

Poultry industry is one of the fastest growing sectors in India. Despite being well established and well organized, this industry is still confronted with many acute and fatal diseases. Of these, inclusion body hepatitis-hydropericardium syndrome (IBH/HPS) caused by fowl adenoviruses (FAVs) is one of the most important viral diseases causing huge economic losses to the poultry industry in India. Inclusion body hepatitis was reported for the first time from the United States as 'necrotizing hepatitis' in 7-week old chickens (Helmboldt and Frazier, 1963). Since then the disease has been reported from many countries including India. In India, IBH was first described by Grewal *et al.* (1981) in 3-wks old broiler chicks with about 15% mortality. The epidemic of HPS (popularly known as Angara disease) was first reported in broiler chicks in late 1987 from Angara Goth near Karachi, Pakistan, however, sporadic cases of this disease were recorded as early as 1985 (Cheema *et al.*, 1989). In India, the disease was named as 'Leechi disease' because the heart of the affected bird is surrounded by light yellow colored fluid which resembles to a peeled leechi fruit. The disease has subsequently been recorded from other parts of world. All 12 serotypes of group I FAVs have been incriminated in the field outbreaks of IBH (Chandra *et al.*, 1998), however, FAV serotype 4 (FAV-4) has been implicated in HPS (Mazaheri *et al.*, 1998). The present work was undertaken to diagnose the IBH/HPS in broiler chicken flocks by conventional techniques.

*Corresponding author: nareshjindal1@gmail.com

MATERIALS AND METHODS

Clinical Findings and Post Mortem Examination: The clinical findings were recorded in 40 broiler chicken flocks suspected to be suffering from IBH/HPS in different districts of Haryana during the period from January 2004 to December 2005. Necropsy findings in the dead and sacrificed birds were also recorded. The birds are generally brought to the Department of Veterinary Public Health and Epidemiology for disease investigation. Various diseases are diagnosed on the basis of clinical findings, necropsy and laboratory examination of the materials.

Histopathology: At necropsy, representative samples of liver, kidney, bursa, spleen, thymus were collected in

10% buffered formalin. The formalin fixed tissues were then processed in paraffin, sectioned at 5-6 µ and stained with Hematoxylin and Eosin (H & E) stain (Luna, 1968) for histopathological examination.

Agar Gel Precipitation Test (AGPT): This test was performed directly on homogenized liver tissue suspensions from all 40 IBH/HPS affected flocks to detect the presence of viral antigen. The test was performed following the procedure of Kumar *et al.* (1997). Briefly, the central well was filled with antiserum raised in chicken against FAV-4. The surrounding wells were filled with the homogenized liver tissue suspensions with one well having known FAV-4 as positive control. The slides were kept in a moist chamber and incubated at 37^0C for about 24-48 hours to observe precipitin bands.

RESULTS AND DISCUSSION

This study was conducted to detect IBH/HPS in commercial chicken flocks using conventional techniques. Though we tested liver specimens from flocks suspected to be suffering from IBH/HPS by molecular techniques like polymerase chain reaction, however, in this paper we have described the results of diagnosing this syndrome by conventional techniques.

Clinical Findings: Sudden onset of mortality without any apparent observation was the primary sign in majority of the affected flocks. The affected birds in each flock showed clinical signs such as anorexia, ruffled feathers, dullness, depression and death. Feed and water intakes were considerably reduced in the affected birds. Yellow greenish diarrhoea was observed in the affected birds. The affected birds had a typical posture with their beaks and chest resting on ground with closed eye lids and were reluctant to move. Average percent morbidity and cumulative mortality were 4.25 (range 0.67-13.33%) and 1.57 (range 0.10-8.33%), respectively due to this syndrome in 40 flocks. Since it is one time point data, overall morbidity and mortality due to this syndrome may be higher. There was sudden high mortality reaching its peak with in 3-4 days followed by an almost constant death rate for 5-7 days before declining in these flocks.

Post Mortem Findings: The post mortem examination of birds revealed gross lesions primarily in liver and kidneys in almost all the flocks affected with IBH. The changes in liver were: enlarged, swollen and jaundiced/ pale liver with pin point or diffuse white necrotic foci/ hemorrhages. The liver was mottled with rounded borders in most cases. The kidneys were invariably enlarged, congested and exhibited necrotic foci and punctiform hemorrhagic areas. In some cases, kidneys contained urate deposits in the tubules. In addition to liver and kidneys, changes were also observed in other visceral organs. Some birds also showed paleness of skin and mucous membranes. The intestinal examination revealed varying degree of congestion and hemorrhages. The only difference between IBH and HPS was characteristic hydropericardium which was seen in birds affected with HPS. The hydropericardium was characterized by accumulation of amber/ straw colored fluid in pericardial sac. Enlargement of liver and kidneys was not a consistent finding in birds affected with HPS.

More or less similar clinical observations and/or necropsy findings have been reported by various workers in commercial broiler chickens under natural and experimental conditions (Cheema *et al.*, 1989; Kumar *et al.*, 1997; Narang, 2004). The mechanism by which hydropericardium occurs is still not clearly understood. However, it has been postulated that decrease in total serum proteins and albumin along with hepatic lesions might lead to extravasation of fluid into the pericardial sac in response to decrease in colloid osmotic pressure of the plasma (Benjamin, 1978). Julian (1987) suggested that hydropericardium could be due to myocardial vascular damage and degeneration of myocardium in response to increase in hydrostatic pressure of veins and capillaries.

Histopathological Findings: Detailed histopathological studies were carried out on the tissue specimens collected from all the 40 flocks suspected to be suffering from IBH/HPS. The changes included:

Liver: The changes were: small multifocal areas of coagulative necrosis, mononuclear cell infiltration and the presence of basophilic intranuclear inclusion bodies (Fig. 1). These inclusion bodies were present in the hepatocytes and surrounded by a clear halo, or as a filling in the entire enlarged nucleus. These inclusions were suggestive of adenoviral etiology. In some specimens, centrilobular or diffuse degeneration and necrosis of hepatocytes, swelling of the cells, with partial clearing of the cytoplasm or rupture of the cell membrane, and shrunken hepatocytes with pyknotic nuclei were noticed.

In this study, inclusion bodies were detected in tissue specimens from 22 flocks thereby suggesting the

involvement of virus as a cause of IBH/HPS. Only basophilic intranuclear inclusion bodies were recorded in liver and kidneys in this study as also reported earlier (Cheema *et al.*, 1989; Philippe *et al.*, 2005; Kim *et al.*, 2008, Wilson *et al.*, 2010). However, Grewal *et al.* (1981) and Thakur and Grewal (1999) reported eosinophilic intranuclear inclusion bodies in the liver of broiler chickens affected with IBH or HPS. The basophilic intranuclear inclusion bodies contain mature virus particles and eosinophilic intranuclear inclusion bodies contain immature virus particles. In this study, the birds brought for disease investigation might be in the recovery/very late stage of disease or have died as a sequel to IBH/HPS limiting the detection of eosinophilic intranuclear inclusion bodies (Kumar *et al.*, 2003). Narang (2004) also did not record eosinophilic intranuclear inclusion bodies during recovery phase in broiler chickens experimentally infected with HPS virus.

Kidney: Basophilic inclusion bodies were also seen in the renal tubular epithelial cells (Fig. 2) in birds along with varying degree of degenerative changes. The kidneys showed marked swelling of the tubular epithelium, necrosis and extensive haemorrhages. Wilson *et al.* (2010) also observed high incidence of renal enlargement and glomerulonephropathy during the histologic evaluation of an outbreak of IBH in Mississippi broilers.

Lungs: The lungs showed congestion, oedema and infiltration by inflammatory cells. There was hemorrhagic exudate in the bronchi and alveoli and a moderate to diffuse infiltration of macrophages into the pulmonary parenchyma.

Heart: Histopathological examination of the heart sections revealed mononuclear cell infiltration, severe vascular changes, massive oedema, hemorrhages and degenerative changes. Hemorrhages under the epicardium and multifocal necrosis in the myocardium were the major findings. Heart changes were more prominent in birds affected with HPS than IBH.

Lymphoid Organs: Macrophages containing erythrocytes and prominent yellow pigment in the red pulp were also recorded in the spleen. Other changes observed were lymphocytolysis and cyst formation in the bursa of Fabricius, thymus and spleen leading to depletion of the lymphocytes in the medullae of the follicles in the bursa of Fabricius, thymus and spleen. The lymphocytic depletion in IBH/HPS affected birds as observed in this study has a significance from field point of view as the deleterious effects on these organs

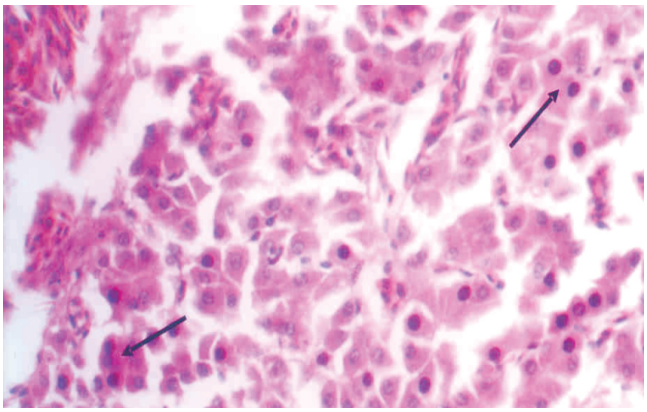

Fig 1. Basophillic intranuclear inclusion bodies (arrow) in hepatocytes of a bird affected with IBH. (H. & E. x 132)

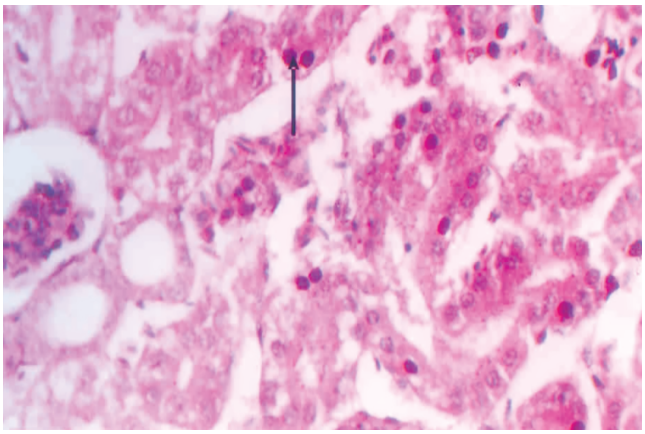

Fig 2. Basophillic intranuclear inclusion bodies (arrow) in tubular cells of kidney of a bird affected with IBH. (H. & E. x 132)

might reduce the resistance of the birds, thereby, making them susceptible to secondary complications. Concurrent infection with other diseases may further complicate the situation by increasing the morbidity and mortality or by decreasing the weight gain in commercial broiler chickens.

The histopathological findings in different organs of birds observed in this study are in conformity with the previous reports (Cheema *et al.*, 1989; Kumar *et al.*, 1997; Narang, 2004; Philippe *et al.*, 2005).

Agar Gel Precipitation Test: The AGPT was performed on liver homogenized suspension from all the 40 affected flocks using antiserum raised in chicken against FAV-4. A line of precipitation was observed in liver suspensions from positive control as well as from 27 flocks suspected to be affected with IBH/HPS. No precipitation line was observed in negative control as well as in liver suspensions from 13 flocks which may

be due to failure of AGPT to detect primary response to adenovirus infection. The AGPT is a widely used serologic test for the detection of FAV antibodies because it is fast and economical. However, it has disadvantages like lack of sensitivity and detection of group antigen (McFerran and Adair, 2003). This has been confirmed by experimental studies which have demonstrated that birds undergoing a primary infection as a result of natural exposure may not respond with precipitin antibodies. The detection of infection might be delayed in fowl since precipitins may develop only after a second exposure to FAV (McFerran and Smyth, 2000). There is an evidence that adenovirus infection can remain latent and undetected for at least one generation in a specific pathogen free flock (Fadly *et al.*, 1980). The apparent sensitivity of the AGPT test in the field is more when birds are being infected with two or more strains. This test has also been employed for diagnosis of FAV infections by many workers and reported more or less similar results (Kumar *et al.*, 1997; Kumar *et al.*, 2003; Philippe *et al.*, 2005).

This study revealed that the syndrome caused pathological changes in liver, kidney, heart and to certain extent in lymphoid organs and the disease can be diagnosed by necropsy findings, histopathological changes in the liver and kidneys and by serological tests such as AGPT. However, the possibility of false negatives can not be ruled out considering the sensitivity/ specificity of AGPT. These 40 samples were also tested for the presence of FAVs by polymerase chain reaction (PCR). All 27 samples that were positive for FAVs by AGPT, were also positive by PCR. In addition, six more samples were positive by PCR, thereby making total 33 samples positive by PCR (Mittal, 2007). The diagnostic laboratories lacking the facilities can rely on conventional techniques for the diagnosis of IBH/ HPS. However, molecular methods would not only help in accurate diagnosis but would also help identify the serotypes of FAVs associated with IBH/HPS.

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