World Journal of Advanced Research and Reviews
eISSN: 2581-9615 CODEN (USA): WJARAI
Cross Ref DOI: 10.30574/wjarr
Journal homepage: https://wjarr.com/

(RESEARCH ARTICLE)

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Pathology of inclusion body hepatitis and hydro-pericardium syndrome (IBH-HPS) in broiler of Bangladesh

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World Journal of Advanced Research and Reviews, 2025, 26(02), 4253–4265

Publication history: Received on 21 April 2025; revised on 28 May 2025; accepted on 31 May 2025

Article DOI: https://doi.org/10.30574/wjarr.2025.26.2.2135

Abstract

This study aimed to investigate the presence of Inclusion Body Hepatitis and Hydropericardium Syndrome (IBH-HPS) in broiler chickens using conventional diagnostic techniques. Between January and June 2024, samples were collected from 12 privately operated broiler farms located in Faridpur and Narsingdi districts of Bangladesh. A total of 600 birds were randomly selected for clinical observation. For histopathological analysis, organ samples were collected from 30 birds—20 of which exhibited clinical signs or were found dead, while the remaining 10 appeared clinically healthy. Gross pathological findings included pale, friable, and enlarged livers with focal to diffuse necrotic lesions, along with accumulation of straw-colored fluid in the pericardial sac. Microscopic examination of the liver revealed acute degeneration of hepatocytes, necrosis, infiltration by mononuclear cells, and the presence of basophilic intranuclear inclusion bodies. Cardiac tissues demonstrated myocardial edema and necrotic changes. The overall prevalence of IBH-HPS was recorded at 9.33%, with a significantly higher prevalence observed in Narsingdi (26.67%) compared to Faridpur (3.56%).

Keywords: Broiler; Histopathology; Hydro-Pericardium; Inclusion Body Hepatitis

1. Introduction

Fowl adenovirus infections are associated with several disease syndromes in broiler chickens, among which Inclusion Body Hepatitis and Hydropericardium Syndrome (IBH-HPS) have emerged as notable threats to poultry health and production. In recent years, these conditions have gained prominence in Bangladesh, causing substantial economic damage and impacting poultry productivity globally [1]. Despite the rapid expansion of poultry farming—especially among youth and women as a source of livelihood—frequent disease outbreaks in broiler flocks continue to cause significant losses through increased mortality, stunted growth, and poor feed conversion ratios (FCR).

Over the past two decades, reports of IBH outbreaks have increased in multiple countries, including neighboring India, where broilers under five weeks of age are most susceptible [2]. Poultry farming plays a vital role in Bangladesh's agricultural economy, contributing to food security and employment. The sector is integrated into rural livelihoods and supports nearly six million people through direct and indirect employment opportunities [3]. However, the rapid growth of poultry farms has often occurred without proper feasibility assessments [4]. It was first described in 1963 in the USA [5]. Then, the disease has been reported in many countries. Horizontal and vertical transmission plays an important role in IBH [6].

In the late 1980s, a related condition resembling IBH was identified in broilers in Angara Goth, near Karachi, Pakistan. This condition was characterized by hepatic lesions and the accumulation of straw-colored fluid in the pericardial sac,

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and it was termed Hydropericardium Syndrome (HPS) or Angara disease [7]. In India, it was first detected in the northern states, where the characteristic pericardial fluid led to the nickname "leechi disease," due to its resemblance to peeled lychee fruit [8]. Subsequent outbreaks across various Indian states and in Pakistan have caused serious economic setbacks [9,10]. Over the last decade, both IBH and HPS have spread across several Asian countries, including Bangladesh, with increasing epidemic patterns rather than isolated incidents [11-13].

These diseases are associated with high mortality and significant growth retardation in broiler flocks, making them economically devastating [13-15]. Although multiple serotypes of adenoviruses have been isolated from infected birds, these viruses have also been found in clinically healthy chickens. Mortality rates in broilers under six weeks of age can range from 2% to 40%, and affected flocks may experience reduced weight gain and compromised feed efficiency [16].

In Bangladesh, IBH and HPS are increasingly being recognized as emerging threats to the broiler industry [2]. Given the reliance on poultry as a primary and affordable source of animal protein, particularly among low-income households, the impact of these diseases is severe. The high morbidity and mortality rates associated with IBH-HPS hinder efforts to meet protein demand and sustain poultry production. Chandra [17] reported that these diseases may cause sudden mortality rates of up to 80% in affected flocks, while Kataria [12] and others [18] have noted spiking mortality and uneven weight gain among young broilers.

Clinically, IBH-HPS presents with nonspecific signs such as depression, inappetence, ruffled feathers, pale comb and wattles, and sudden death. The liver may appear enlarged, discolored, and mottled with pale or hemorrhagic spots. In cases of HPS, as much as 10 mL of straw-colored fluid may be present in the pericardial sac [19]. Histopathological findings typically include hepatocellular degeneration, necrosis, mononuclear infiltration, and basophilic intranuclear inclusion bodies in the liver, while cardiac tissue may exhibit edema and necrosis.

Given these implications, it is essential to assess the current prevalence and pathology of IBH and HPS in Bangladesh. A clear understanding of these diseases is crucial for designing effective control strategies and minimizing economic losses. Therefore, the present study was designed to investigate and confirm the occurrence of IBH-HPS in broiler flocks during natural outbreaks in selected regions.

2. Materials and Methods

2.1. Study area and sample collection

A cross-sectional study was undertaken to determine the prevalence and pathological characteristics of Inclusion Body Hepatitis and Hydropericardium Syndrome (IBH & HPS) in broiler chickens across two districts in central Bangladesh—Faridpur and Narsingdi. From January to June 2024, a total of 600 broilers were assessed for clinical and pathological indicators of the diseases. The birds were sampled from 12 commercial poultry farms, including 9 in Faridpur and 3 in Narsingdi. Tissue samples were collected from birds showing clinical signs, as well as from asymptomatic birds on both affected and unaffected farms, due to the non-specific nature of IBH & HPS clinical manifestations. Emphasis was placed on identifying sick or deceased birds for detailed pathological investigation. Liver and heart tissues were selected for histopathological analysis.

2.2. Histopathological Sample Collection

Representative specimens of liver and heart were collected from naturally infected broilers. Samples were submitted to the Department of Veterinary and Animal Sciences at the University of Rajshahi for laboratory processing. Histopathological techniques were applied to confirm the presence of IBH and HPS.

2.3. Clinical Evaluation

Birds' overall health status was assessed through direct on-site examination during farm visits. Clinical signs were documented based on physical observation and supplemented by farm owner and caretaker reports regarding unusual symptoms or mortality trends.

2.4. Pathological Examination

Immediately following clinical evaluation, affected birds were transported to the Veterinary Pathology Laboratory for necropsy. Post-mortem examinations were conducted using standard protocols, and all significant gross lesions were recorded. Tissue samples exhibiting abnormalities were fixed in 10% neutral buffered formalin. The fixed tissues were processed through an ascending ethanol series for dehydration, followed by xylene clearing and paraffin infiltration at

56 °C. Paraffin blocks were then sectioned at 5 µm thickness using a rotary microtome (Model MBL 2100, Kriis, Germany). Tissue sections were stained using the standard Hematoxylin and Eosin (H&E) technique [20] for microscopic evaluation. Diagnosis was confirmed through gross pathology and histopathological lesions.

2.5. Photomicrography

Microscopic images of tissue sections were obtained using a digital photomicrography system (Model MBL 2100, Kriis, Germany) integrated with a Labomud digital camera (Labomud Inc., USA), located within the Department of Veterinary and Animal Sciences.

2.6. Statistical Analysis

Descriptive statistics were used to express results as mean ± standard deviation (SD). Statistical comparisons between infected and non-infected bird groups were made using independent samples t-tests, with significance set at $p < 0.05$. All analyses were conducted using SPSS software (version 26.0, SPSS Inc., USA). Disease prevalence was determined using the following formula:

3. Results

3.1. Prevalence of Inclusion Body Hepatitis and Hydropericardium Syndrome (IBH and HPS) in Broilers in Bangladesh

The overall occurrence of IBH & HPS among the observed broiler population was calculated to be 9.33%, as detailed in Table 1 and illustrated in Figure 1. Farms were denoted as F-1 to F-9 for Faridpur and N-1 to N-3 for Narsingdi to indicate their geographical location.

When stratified by district, Faridpur exhibited a lower prevalence rate of 3.56%, whereas Narsingdi showed a significantly higher rate of 26.67%, as presented in Table 2 and Figure 3. Among the 600 birds examined for gross pathological changes, hydropericardium was identified in 6.83% of the cases. Additional liver-related abnormalities included enlargement in 8.67%, yellowish or pale discoloration in 3.83%, and necrotic foci in 4.67% of the examined birds (Figure 2).

Tissue-specific prevalence analysis revealed that liver samples had a higher frequency of lesions compared to heart samples, as demonstrated in Figure 4. Age-wise distribution indicated that younger birds (1–15 days old) were more frequently affected. In contrast, the number of cases decreased markedly in birds that were 30 days of age or older, as illustrated in Figure 5. To assess whether the observed differences in IBH & HPS prevalence among the 12 broiler farms (Faridpur: F-1 to F-9; Narsingdi: N-1 to N-3) were statistically significant, a Chi-square test for independence was conducted. The test evaluated the association between the location/farm identity and the presence or absence of infection, using the frequency distribution of positive and negative cases.

The analysis yielded a Chi-square value (χ^2) that corresponded to a p-value of 4.22×10^{-35}, which is far below the conventional alpha threshold of 0.05. This result indicates an extremely statistically significant difference in disease prevalence among the farms.

The Chi-square test for prevalence differences between Faridpur and Narsingdi yielded a p-value of approximately 1.40×10^{-16}. This extremely low p-value indicates a highly significant difference in the prevalence of IBH & HPS between the two districts. Therefore, the elevated prevalence observed in Narsingdi (26.67%) compared to Faridpur (3.56%) is not due to random variation, but likely reflects true epidemiological differences, such as:

- Regional disparities in bio-security practices or farm hygiene
- Environmental conditions influencing virus survival or transmission
- Possible differences in poultry sourcing or hatchery infections
- Population density and inter-farm connectivity in Narsingdi

This statistically significant result justifies the need for district-specific control measures and targeted surveillance to mitigate the spread and impact of IBH and HPS.

Table 1 Overall prevalence of the disease

Farm numbers	No. of birds randomly checked	No. of (+) ve cases, gross & microscopic Lesions	Farm-wise prevalence (%)	Total No. of (+) ve cases	Overall prevalence (%)	*P-value*
F-1	50	0	0	56	9.33	4.22×10^{-35}
F-2	50	7	14			
F-3	50	0	0			
F-4	50	0	0			
F-5	50	4	8			
F-6	50	0	0			
F-7	50	5	10			
F-8	50	0	0			
F-9	50	0	0			
N-1	50	32	64			
N-2	50	8	16			
N-3	50	0	0			

Table 2 Area based prevalence of diseases

Source	Total examined samples	No. of positive samples	Prevalence rate (%)	*P-value*
Faridpur	450	16	3.56	1.40×10^{-16}.
Narsingdi	150	40	26.67	
Total	600	56	9.33	

3.2. Graphical representation of various prevalence rates

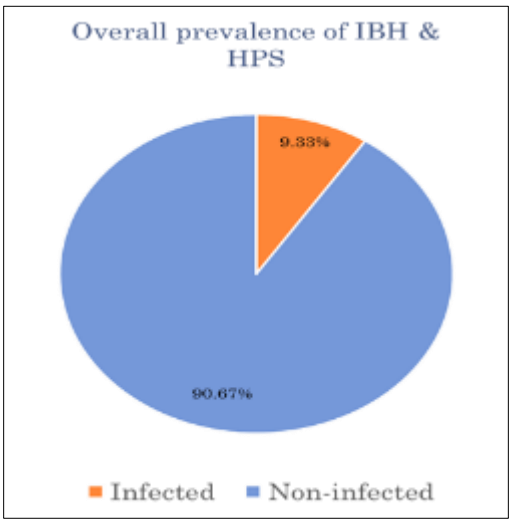

Figure 1 Overall prevalence of IBH & HPS

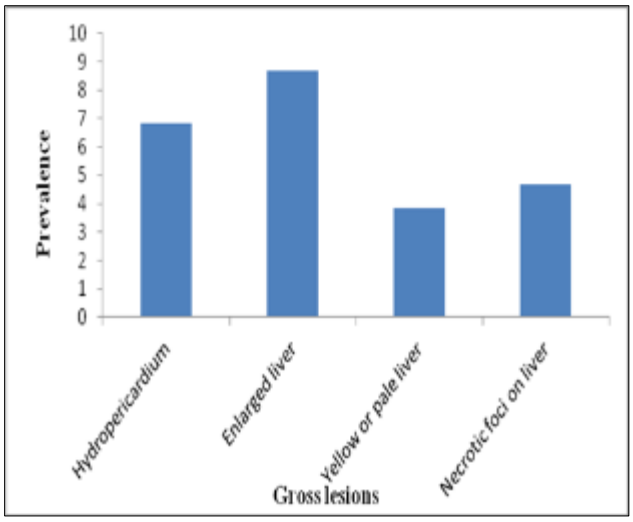

Figure 2 Distribution of gross lesion

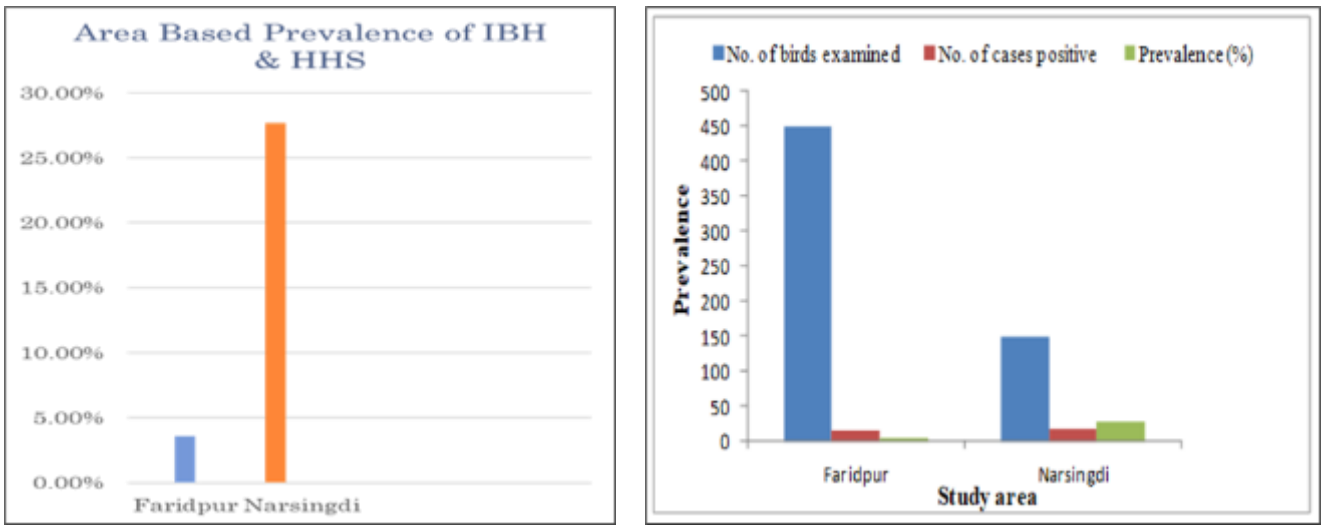

Figure 3 Area based prevalence rate of IBH & HPS in broiler

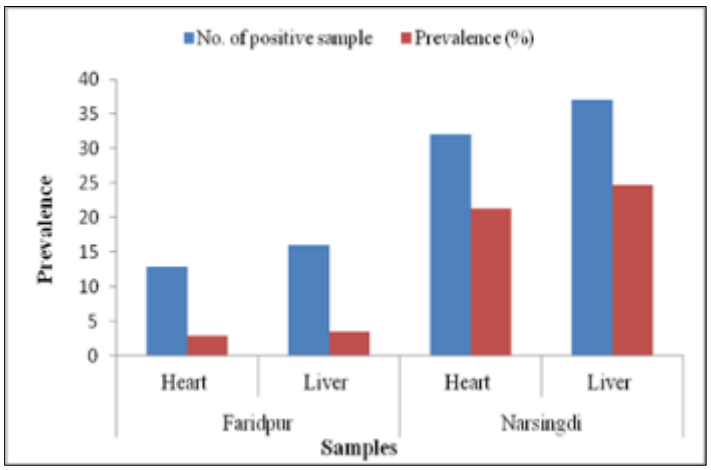

Figure 4 Sample based prevalence

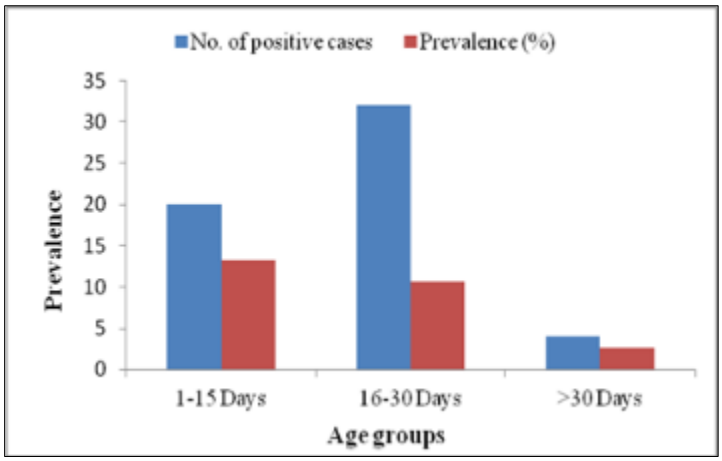

Figure 5 Age based prevalence of diseases

3.3. Pathological study

3.3.1. Gross lesions

Gross pathological assessments were primarily concentrated on the liver and heart, as these organs consistently exhibited characteristic lesions associated with Inclusion Body Hepatitis (IBH) and Hydropericardium Syndrome (HPS). Affected birds displayed variable organ involvement—some with liver lesions only, some with heart involvement, and others with concurrent damage in both organs.

The liver in most cases appeared pale, yellowish, friable, and enlarged, often accompanied by focal to diffuse necrotic areas (Figures 6 & 7). In several birds, the hepatic tissue also presented pinpoint or ecchymotic hemorrhages. Occasionally, an icteric liver was observed, either with or without hemorrhagic patches (Figures 7 & 8). Such degenerative and necrotic hepatic alterations may impair liver function, potentially leading to reduced plasma oncotic pressure and the subsequent accumulation of transudate in the pericardial sac.

In birds exhibiting both IBH and HPS, up to 10 mL of straw-colored serous fluid was found within the pericardial cavity, imparting a distinctive "Lychee Heart" appearance to the organ (Figures 7 & 8). This fluid accumulation is considered a hallmark of HPS and strongly supports the co-infection of both syndromes.

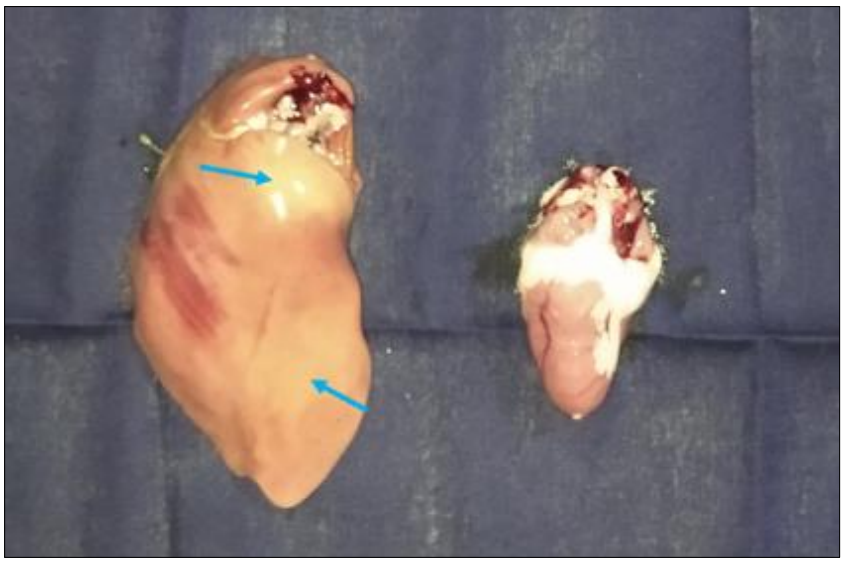

Figure 6 Heart (right) and liver (left) of a 25 days old broiler, liver showing pale, yellowish discoloration, friable and enlarged with the presence of focal or diffuses areas of necrosis while heart was normal

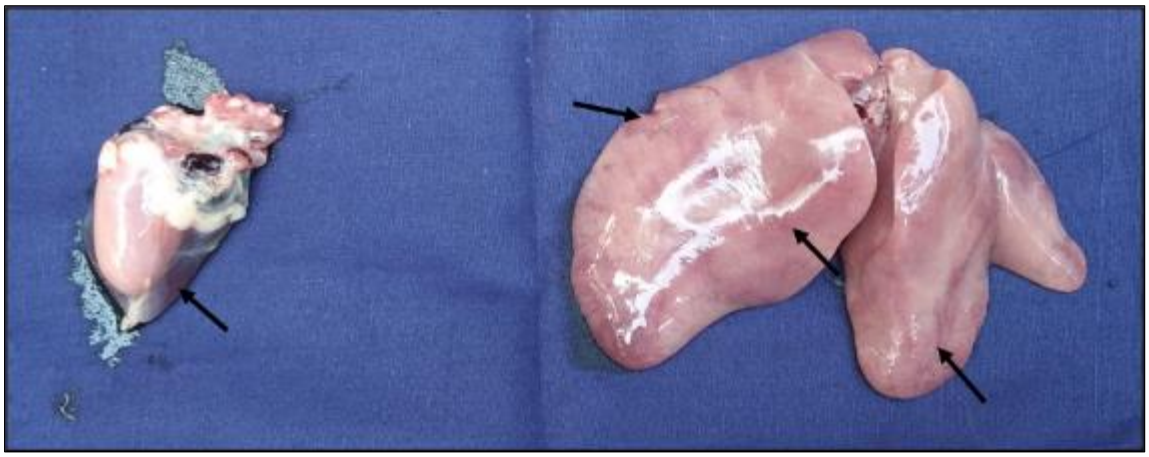

Figure 7 Heart (left) and liver (right) of a 24-day old broiler; liver is enlarged and showing pale discoloration (Necrosis), and heart showing hydropericardium

Figure 8 IBH & HPS affected 22 days old broiler having Hydropericardium or "Litchi heart appearance". The liver showing enlarged with the presence of hemorrhage

3.3.2. Microscopic lesions

Necrosis of hepatic tissues (Figure 9), mononuclear cell infiltration (Figure 11), acute hepatocytic degeneration, hemorrhage, basophilic intranuclear inclusion bodies were present in the hepatocytes and surrounded by a clear halo or sometimes filling the entire nucleus (Figure 10 &12). In heart, there was myocardial edema and degeneration of the cardiac muscle fibre (Figure 13) and myocardial necrosis (Figure 14).

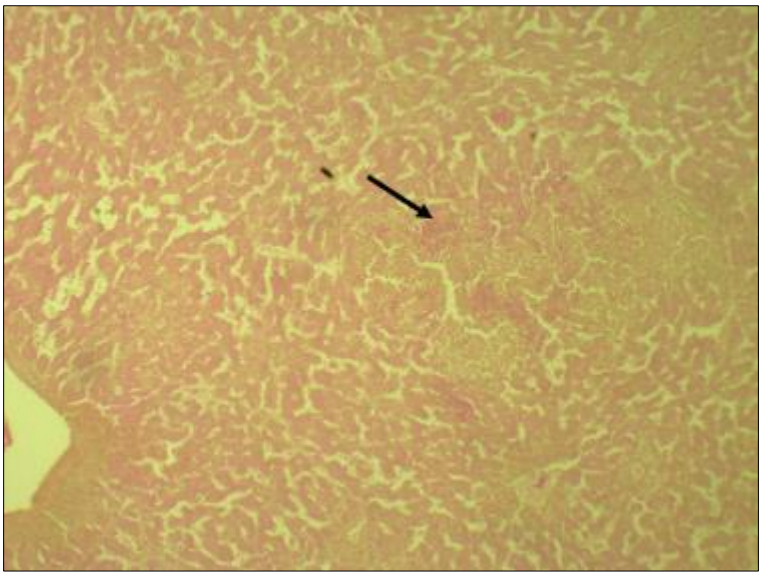

Figure 9 Liver of 18 days old broiler showing necrosis of hepatic tissues (H & E, 10X)

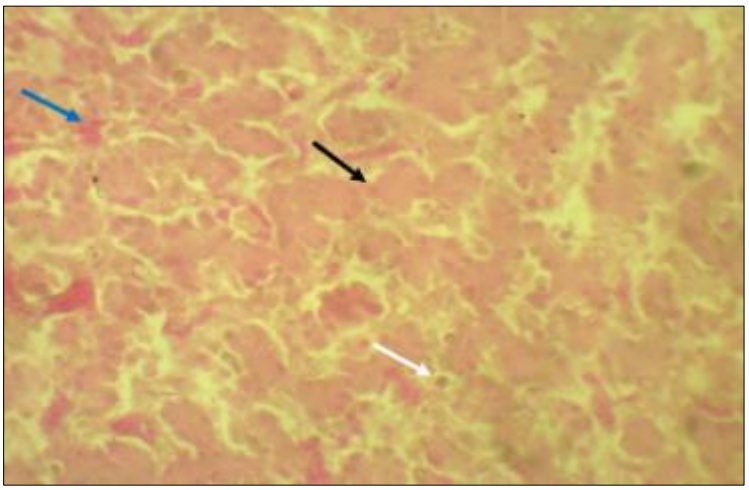

Figure 10 Liver of 18 days old broiler showing basophilic intranuclear inclusion bodies (white arrows), hemorrhage (blue arrow) and acute hepatocytic degeneration (black arrows), (H & E, 40X)

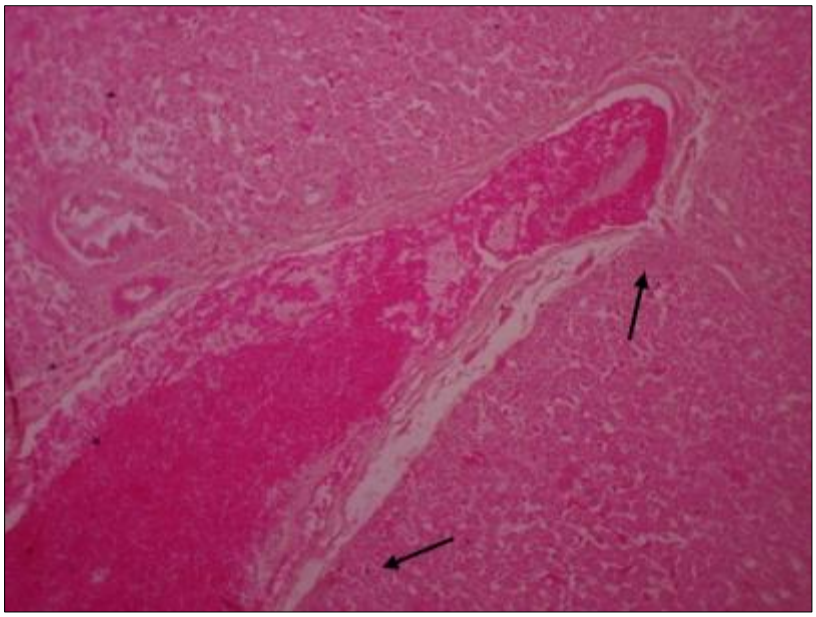

Figure 11 Liver of a 16 days old broiler showing mononuclear cell infiltration, (H & E, 10X)

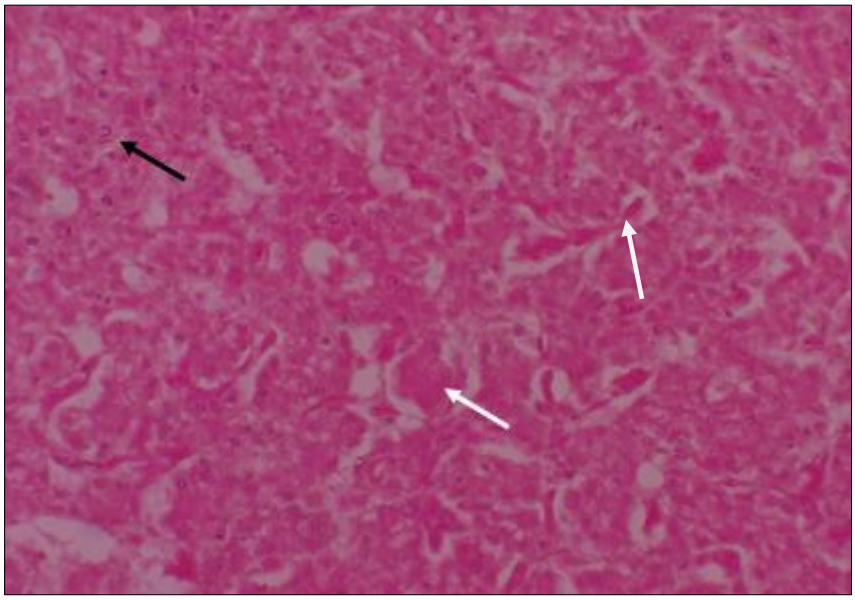

Figure 12 Liver of 23 days old broiler showing basophilic intranuclear inclusion bodies (black arrows) and acute hepatocytic degeneration (white arrows), (H & E, 40X)

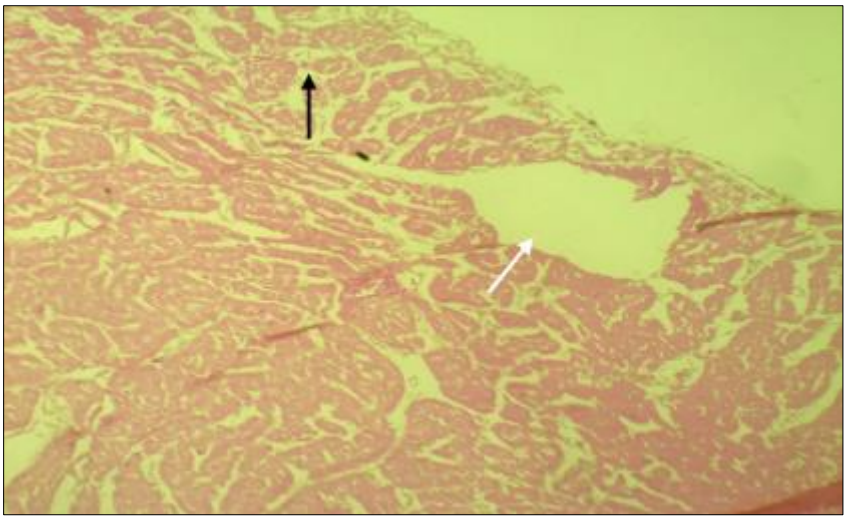

Figure 13 Heart of a 25 days old broiler showing myocardial edema (white arrow) and degeneration of the cardiac muscle fibre (black arrow), (H & E, 40X)

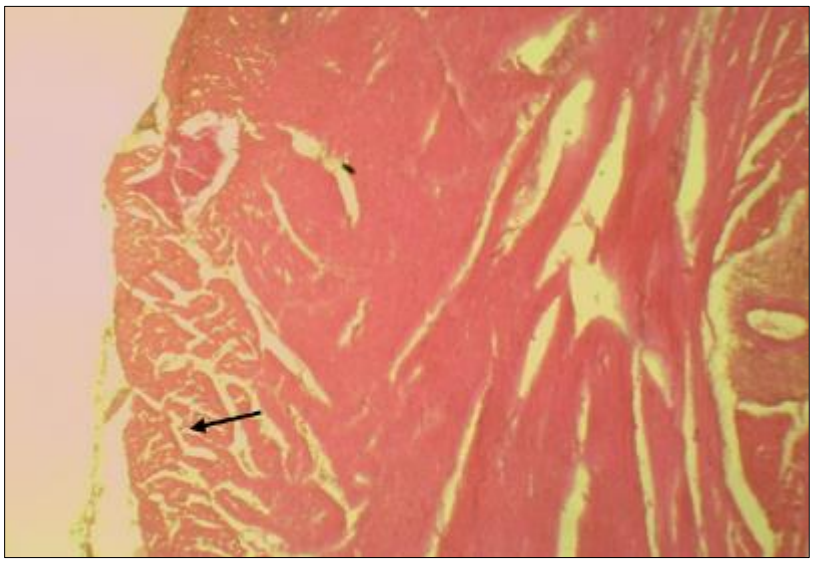

Figure 14 Heart of an 18 days old broiler showing myocardial necrosis (H & E, 40X)

4. Discussion

The current investigation revealed an overall prevalence of 9.33% for Inclusion Body Hepatitis and Hydropericardium Syndrome (IBH & HPS) in broiler chickens. This finding closely aligns with a study conducted in Poland, which reported a prevalence of 10.41% [21]. Slightly elevated rates were noted in other studies, including 11% in Odisha, India [22], and 12.57% in the Sonapur area of Kamrup District, Assam [19]. Conversely, lower prevalence figures such as 6.69% in Haryana [23] and significantly higher rates like 33.3% in Mizoram [24] have also been documented. Ahmad [25] recorded even more severe morbidity (32.73%) and mortality (94.44%) in Giriraja chickens affected by IBH/HPS. These variations are likely influenced by multiple epidemiological determinants, including geographic differences, seasonal effects, age and genetic background of the birds, and the immunological status of flocks.

Age appeared to play a crucial role in disease susceptibility. In this study, the highest prevalence (13.33%) was observed among birds aged 1–15 days, followed by 10.67% in the 16–30 day group, and 2.67% in birds older than 30 days. These findings support earlier studies: Sawale [18] noted outbreaks in broilers aged 15–37 days, Kataria [12] reported infection between 3–6 weeks of age, and Shiyamala [26] documented cases between 2–6 weeks. Other reports similarly highlight the vulnerability of young chicks, particularly around the third week of life [22]. Rahimi [27] emphasized that

younger hosts exhibit higher viral replication and mortality rates, which decline as birds age. A study in Haryana [23] also found that most outbreaks occurred between 21–30 days, followed by 31–40 days, 1–10 days, and beyond 50 days.

In the current study, many birds demonstrated classic signs of hydropericardium, characterized by the accumulation of straw-colored transudate in the pericardial sac, consistent with previous reports [7, 28, 29]. The liver frequently appeared enlarged and mottled, showing a reticular pattern, diffuse hepatitis, and the presence of intranuclear basophilic inclusion bodies within hepatocytes, along with mononuclear infiltration around portal triads [18]. Additional changes, such as fatty degeneration and vacuolation of hepatocytes, were also occasionally observed. Chandra [17] reported similar findings, including focal necrosis and mononuclear cell infiltration, while other studies noted centrilobular degeneration, cytoplasmic clearing, and cell membrane rupture. Dutta [30] described the liver as pale, friable, and enlarged, with multifocal coagulative necrosis and inclusion bodies—findings consistent with the current observations.

5. Conclusion

This study confirms the occurrence of IBH & HPS outbreaks in broiler flocks within the Faridpur and Narsingdi districts of Bangladesh, primarily diagnosed through gross and histopathological evaluation. These diseases contribute significantly to economic losses in the poultry industry due to immunosuppression, poor growth performance, and high mortality. Based on field observations, disease outbreaks appeared to be more frequent during the winter months, approximately two months prior to the peak reporting time.

The findings underscore the need for enhanced awareness and preventive strategies among poultry farmers to mitigate risk factors associated with IBH and HPS. In addition, further research is warranted, particularly focusing on the immunopathology of the disease, to facilitate vaccine development and more effective control measures in endemic regions.

Compliance with ethical standards

Acknowledgments

The authors acknowledge the help and cooperation of the farmers, field assistant for providing the necessary data to carry out this work.

Disclosure of conflict of interest

The authors declare that they have no conflict of interests regarding the publication of this article.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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