Veterinary Integrative Sciences 2025; 23(2): e2025039-1-11. DOI: 10.12982/VIS.2025.039

Veterinary Integrative Sciences
ISSN: 2629-9968 (online)

Research article

Prevalence with precise diagnosis of Hepatitis-Hydropericardium Syndrome (HHS) in broiler chicken in eastern Bangladesh

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Abstract

Hepatitis-Hydropericardium Syndrome (HHS) is an increasingly prevalent poultry disease, particularly affecting broiler chickens in Bangladesh. HHS is caused by Fowl Aviadenovirus Serotype 4 (FAdV-4), characterized by the accumulation of clear or yellowish-brown fluid in the pericardium. This study, conducted over one year from March 2022 to February 2023, examined a total of 109,000 broiler birds to assess the prevalence and precise diagnosis of HHS. Diagnosis was performed systematically and ethically through physical and clinical examinations, including the evaluation of clinical symptoms and post-mortem lesions. The presence of the causal agent was confirmed via polymerase chain reaction (PCR) from liver and kidney samples, using FAdV-specific primers targeting the hexon-b gene. The study found an overall prevalence of HHS in broilers to be 10.55% (95% CI: 10.37-10.73). The disease was infrequently observed in birds younger than two weeks, with the highest prevalence noted between two to three weeks of age (15.31%). Seasonally, HHS incidence was higher during the summer (13.92%) and rainy season (12.86%), while it was significantly lower in the winter (4.35%). The case fatality rate was determined to be 58.77%, with an average mortality rate of 6.20%. These findings provide critical insights for the poultry industry in Bangladesh, enabling the development of effective strategies for the prevention and control of HHS.

Keywords: Bangladesh, Fowl Aviadenovirus Serotype 4, Hepatitis-Hydropericardium Syndrome, Polymerase Chain Reaction

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Article history; *received manuscript: 22 April 2024, revised manuscript: 18 May 2024, accepted manuscript: 31 July 2024, published online: 5 August 2024,*
Academic editor; *Korakot Nganvongpanit*

INTRODUCTION

An emerging infectious viral disease of broiler breeds named Hepatitis-Hydropericardium Syndrome (HHS) is severely impacting by the poultry industry (Chandra et al., 2000). The etiology of HHS is fowl aviadenoviruses, which is classified into the 5 species FAdV-A, FAdV-B, FAdV-C, FAdV-D and FAdV-E and it has 12 serotypes (FAdV-1 to 12) (Ahmed et al., 2020). Fowl aviadenoviruses have been associated most commonly with Type A gizzard erosions and respiratory problems, Type C hydropericardium syndrome, and Types D and E of inclusion body hepatitis (Arazi et al., 2020). In the field, aviadenovirus infections are typically not identified in the first few days of life, yet isolations after three weeks are common (Asthana et al., 2013).

The regular method of vertical transmission is through the egg, while horizontal transmission can occur through excrement, contaminated egg trays, boxes, and transportation vehicles (Hafez Mohamed Hafez, 2011). Aviadenoviruses can spread through eggs in laying hens, especially during the period of highest egg production (Philippe et al., 2007). Although virus excretion is frequently discovered in the flock between 2-4 weeks of age, chicks hatching from contaminated eggs may begin to excrete the virus in their feces as soon as they hatch (Chandra et al., 2000; Philippe et al., 2007; Sentíes-Cué et al., 2010). The virus takes 24-48 hours to incubate (Niczyporuk et al., 2010). Depending on the virulence of the virus, the immune status of the infected birds, and the presence of concurrent secondary infections, outbreaks have been known to result in mortality rates as high as 30% (Nakamura et al., 2002; Philippe et al., 2005; Sawale, et al., 2012).

Serotype FAdV-4 is the cause of HHS, which primarily affects broiler chicks 3–6 weeks of age (Chandra et al., 2000). FAdV-4 affects several organs, causing the lungs, to become edematous and congested, the liver, to become discolored and swollen with zones of focal necrosis and bleeding, and the kidneys to become pale with dilated tubules due to urate deposition (Nakamura et al., 2002). Histological sections of the liver exhibit basophilic intranuclear inclusion bodies in the hepatocytes, as well as small multifocal regions of coagulative necrosis and mononuclear cell infiltration (Chandra et al., 2000; Nakamura et al., 2002). The preferred methods for diagnosis and identification of aviadenovirus serotype 4 is the polymerase chain reaction (PCR) method and clinic-pathological analysis (Chandra et al., 2000; Philippe et al., 2007).

A number of immunization approaches against FAdVs have been studied in experiments, especially to immunize broilers against HHS by targeting FAdV-4 (De Luca et al., 2022). Three types of Inclusion Body Hepatitis-Hepatitis Hydropericardium Syndrome (IBH-HHS) vaccines are available for immunization of HHS: formalin-inactivated, commercial oil-based, and montanide-adjuvanted (Aslam et al., 2012). The administration of the montanide-adjunvanted IBH-HHS vaccine may provide more effective protection for chickens against the IBH-HHSV challenge, compared to formalin-inactivated and conventional oil-based vaccines (Aslam et al., 2012). The aim of this study is to know the proper diagnostic procedure of the aviadenovirus species FAdV-C of HHS and evaluate the overall prevalence, age-wise prevalence, season-wise prevalence, mortality rate and case fatality rate of HHS in broiler chickens.

MATERIALS AND METHODS

Ethical approval

The Animal Experimentation and Ethics Committee (AEEC) at Sylhet Agricultural University, Bangladesh, has thoroughly assessed and granted the approval for the proposed experiment. The approved Animal Use Protocol is officially identified as #AUP2022103, outlining the ethical guidelines and

procedures for the implementation of the experiment. Moreover, the poultry farm owners gave their verbal permission. Participants (Farm owners) provided informed consent, ensuring they understood the study's purpose and their rights. Confidentiality measures were strictly implemented to protect participants' privacy, and efforts were made to minimize any potential harm or discomfort.

Study area

The study was conducted at scattered large and small broiler farms in Cumilla district of Bangladesh (Figure 1). All the farms were managed and maintained by local farmers.

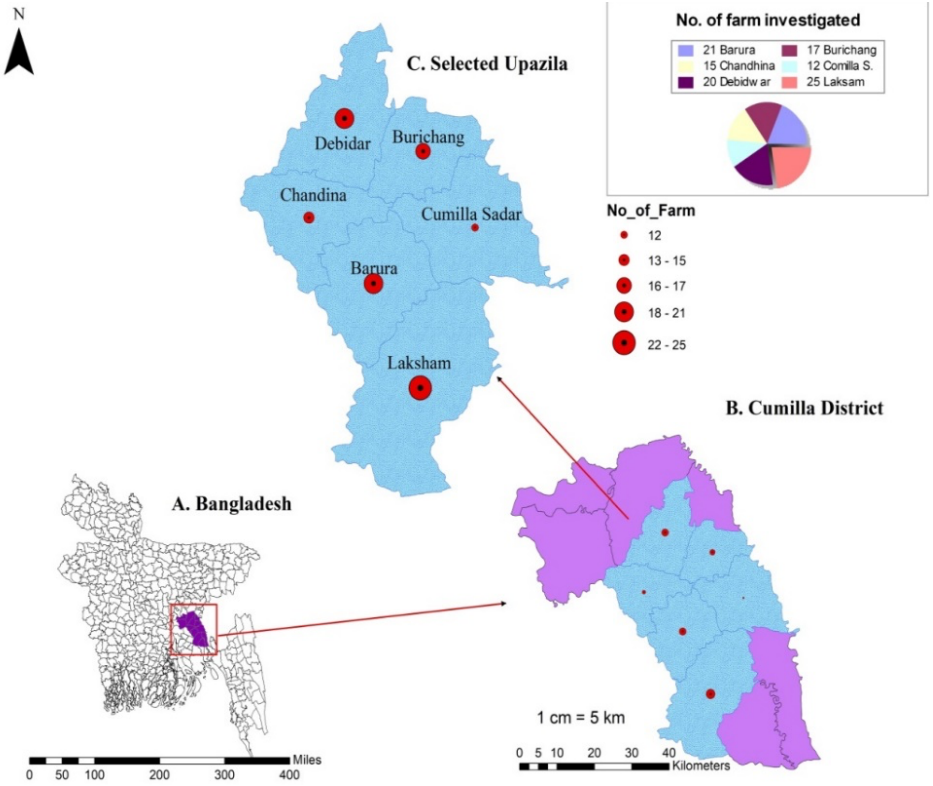

Figure 1 Study area map showing the selected upazilla and number of farm investigated during March 2022 to February 2023. The map was created using ArcMap 10.7 (ESRI, USA)

Sample Size, Study period and population

The required sample size for prevalence estimation was calculated using an equation described by Mahen et al. (2024).

$$n = \frac{Z^2 Pexp\ (1 - Pexp)}{d^2}$$

Where, Pexp = expected prevalence, n = required sample size, d = desired absolute precision, Z = 1.96 for 95% confidence interval level. To maximize the highest number of the sample this study used Pexp= 0.5 (50%). Using this Pexp with a desired absolute precision d=0.05, a required sample size of at least 384 were determined. But a total of 110 broiler farms with 109,000 bird's population were registered during the study period and Hepatitis-Hydropericardium Syndrome was recorded. The study was conducted for a period of one year from March, 2022 to February, 2023. All the farms were visited physically during the study period for the data collection and research purpose.

Table 1 Experimental Birds with number of farm investigated during the study period

Region/District	Sub Region/ Upazilla	Number of Farms	Number of Birds
Cumilla	Cumilla Sadar	12	10,000
	Debidwar	20	22,000
	Chandina	15	15.000
	Barura	21	20,000
	Burichang	17	12,000
	Laksam	25	30,000
	Total	110	109,000

Criteria for farm selection

Criteria for selecting the farms to include in the study were specific. The criteria included:

- Farms that had been established at least 12 months before the investigation.
- Farms with a minimum of 1000 birds.
- Farms that raised broiler chicken.

Diagnostic Procedure

We diagnosed the disease by using the following steps-

a. General examination

Age, size, body weight, behaviour, posture, superficial feathers, dullness and locomotion disturbance were observed by distant visual examination of the flock.

b. Physical examination

Physical examination was done by visual inspection after rounding up any broiler with abnormalities like dullness, clumsiness, off-feeding and atypical body temperature.

c. Clinical examination

Diseased birds were examined for clinical symptoms of HHS. Post-mortem examinations for HHS were also conducted on dead birds. The presented clinical manifestations of various diseases within the flock and the farmer's statement in relation to diseases were recorded carefully.

d. Laboratory Examination

Collection of sample

After a post-mortem inspection, liver and kidney samples were aseptically taken from the chickens that were suspected. In order to protect their integrity, these samples were quickly sent, via a constant cold chain, to the microbiology laboratory of Hajee Mohammad Danesh Science and Technology University in Dinajpur and the postgraduate laboratory of Sylhet Agricultural University in Sylhet for additional laboratory testing. The kidney was swollen, pale, and had multiple haemorrhages with urates deposition; the liver was enlarged, pale, friable, and haemorrhagic during the sample collection process.

PCR Amplification of the Hexon-b Region of Fowl Aviadenoviruses

Thermal Profile: Reactions were performed according to the following protocol: 95°C for 5 min, followed by 30 cycles of 95°C for 30 s, 53°C for 30 s, 72°C for 60 s, and a final elongation step of 10 min at 72°C (Yu et al., 2018). 1kb maker DNA ladder was used (Promega, USA).

Table 2 Primer for amplification of FAdV-4 gene detection for HHS

Primer	Target Gene	Sequence 5'-3'	Amplicon Size (bp)	Annealing Temp.(°C)	Reference
FAdV	*hexon-b*	F- TGGACATGGGGGCGACCTA R- AAGGGATTGACGTTGTCCA	1219	53	(Yu et al., 2018)

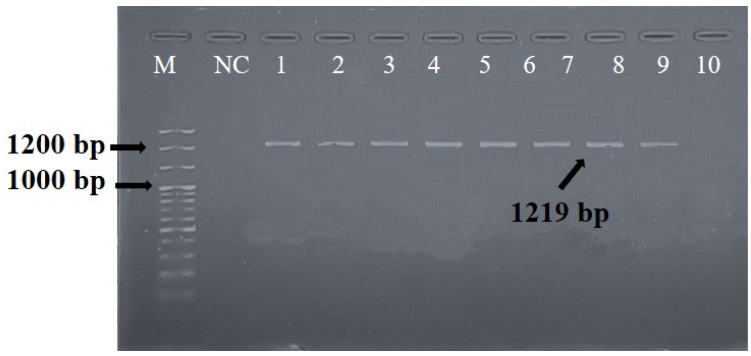

Figure 2 PCR image for identification of Fowl Aviadenovirus serotype 4 by amplification of hexon-b gene

Equation of Prevalence, Mortality, and case fatality rate

Prevalence was calculated as number of cases of disease divided by population at risk and multiple by 100. Mortality rate was calculated as number of dead of birds divided by affected population and multiple by 100 (Asha et al., 2024). Case fatality rate was calculated as number of dead of birds divided by affected birds and multiple by 100.

$$Prevalence\ Rate\ (\%) = \frac{Number\ of\ cases\ of\ disease}{Population\ at\ risk} \times 100$$

$$Mortality\ rate\ (\%) = \frac{Number\ of\ dead\ birds}{Affected\ Population} \times 100$$

$$Case\ fatality\ rate\ (\%) = \frac{\text{Case fatality}}{Number\ of\ Affected\ Birds} \times 100$$

Statistical analysis

The study involved the systematic compilation, sorting, and structuring of gathered data within Excel spreadsheets. Disease prevalence rates were calculated using established formulas. To explore associations among different explanatory variables, univariate analysis was performed using the Chi-square test. In cases where the expected count in a cell was less than 5 and occurred in at least 20% of the cells, Fisher's Exact Test was applied (Hoque et al., 2023). Confidence intervals were determined using the Binomial exact test, with a significance level set at less than 0.05 to establish statistical significance. The entire data analysis process was executed using SPSS version 26. The geographical mapping was created using the ArcMap 10.7.

RESULTS

Post mortem examination findings

The clinical signs observed in the affected birds included lethargy, stooping, and inappetence. The birds were also seen huddling with ruffled feathers. Yellow and mucoid droppings were noted, and there was a significant depression in feed conversion and weight gain.

Post-mortem examinations revealed several characteristic lesions. Hydropericardium, a typical lesion, was observed. The liver was often enlarged, pale, and friable, sometimes displaying necrotic foci and frequent hemorrhages. The kidneys were enlarged, pale, and mottled with multiple hemorrhages and urate deposits. Enlarged spleens and thymus atrophy were common findings in most of the deceased birds. Typical post-mortem lesions are shown in the figures (Figure 3) below, which were captured during our study.

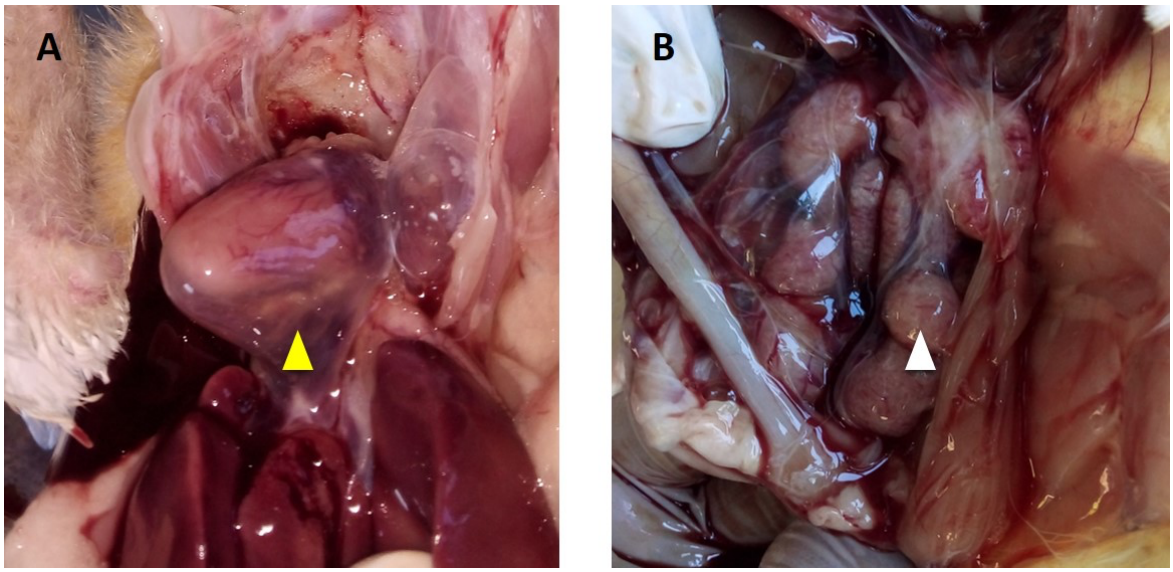

Figure 3 Characteristic features of (Pathognomonic lesions) HHS (17 days old broiler). (A) Accumulation of fluid in the pericardium (Hydropericardium, Yellow arrow head); (B) Enlarged, pale and multiple haemorrhages with urates deposition in kidney (White arrow head)

Prevalence of Hepatitis-Hydropericardium Syndrome

The prevalence of HHS in broiler chickens was investigated, and the overall occurrence of specific fowl adenovirus serotype (FAdV) associated with HHS (FAdV-C), particularly serotype 4 (FAdV-4) was found to be 10.55% (95% CI: 10.37-10.73) based on a sample of 109,000 birds. Interestingly, no cases were observed in chicks aged 0 to 14 days, but susceptibility increased significantly during the 15-21 day period, reaching a prevalence of 15.31%.

Furthermore, the study highlighted the impact of seasons on HHS prevalence. The highest occurrence was during the summer months (13.92%), followed by the rainy season (12.86%), and the lowest prevalence occurred in winter (4.35%). These findings emphasize the importance of considering both age and seasonal factors when managing HHS in broiler chicken populations (Table 3).

Table 3 The prevalence of Hepatitis-Hydropericardium Syndrome in broiler chicken

Variable	Category	No. of birds examined	Positive case	Prevalence % (95% CI)	Level of Significance
Age	0 to 7 days	11,000	0	0	**
	8 to 14 days	15,500	0	0	
	15 to 21 days	49,000	7,500	15.31 (14.99-15.63)	
	22 to 28 days	33,500	4,000	11.94 (11.60-12.29)	
Season	Summer (March to June)	39,500	5,500	13.92 (13.58-14.27)	**
	Rainy (July to October)	35,000	4,500	12.86 (12.51-13.21)	
	Winter (November to February)	34,500	1,500	4.35 (4.14-4.57)	
	Total	109,000	11,500	10.55%	

** P < 0.01

Mortality and case fatality rate of Hepatitis-Hydropericardium Syndrome

Hepatitis-Hydropericardium Syndrome (HHS) showed relatively high case fatality in this study while affecting young broiler chickens of 15-21 days old. Among the total bird population (109,000), 6,758 birds died. The overall mortality rate was 6.20%. The case fatality rate specifically for HHS-positive birds was 58.77% (6758/11500). The overall case fatality was significantly higher than the mortality (Figure 4). This rate indicates the severity of HHS in affected broilers.

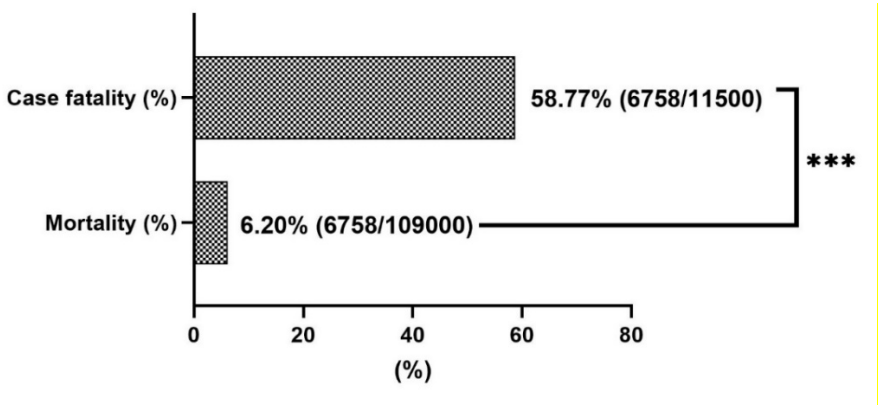

Figure 4 Mortality and case fatality rate of HHS lesion in broiler chicken. ***P<0.001

DISCUSSION

There has been an observation of hydropericardium, a common lesion, in broiler chickens. In this study, it was noted that the liver was enlarged, pale, and friable, occasionally with foci of necrosis. Liver haemorrhages were also commonly discovered. The kidneys had urate deposits, several haemorrhages, and were swollen, pale, and mottled. The post-mortem lesions that Norina (2016) discovered in December 2015 in Malacca and Johore were nearly identical to what we encountered, and were reported to the Regional Veterinary Laboratory in Johor Bahru, Peninsular Malaysia.

Polymerase chain reaction (PCR) is the most suitable and inexpensive method for the confirmative diagnosis of HHS. “FAdV” was used as a primer, and the target gene was “hexon-b” in this method. The same protocol was followed by Yu (2018) in Shandong Province, China, from July 2013 to January 2015.

Out of 109,000 birds, 11,500 were found to be positive for HHS. The overall prevalence of HHS in broilers was 10.55%. The finding was consistent with the result of Gaweł (2016), where they found 10.4% Fowl Adenoviruses (FAdV) infection in Poland from 2010 to 2014.

This study considered four distinct age groups (of 0–7 days, 8–14 days, 15–21 days, and 22–28 days old). In the 0–7 and 8–14 day age groups, the prevalence of HHS was 0%. In the 15–21 day age group, it was 15.31%, and in the 22–28-day age group, the prevalence was 11.94%. These results closely correlated with the earlier findings of Chandra (2000), especially with their discovery of the onset of HHS in broilers from 3-6 weeks of age. Broiler chickens are more susceptible to various diseases during the first 2 to 3 weeks of life, particularly infectious bursal disease. Most of the time, HHS was found with infectious bursal disease and mixed infection, which occasionally confounded the diagnosis of the disease. HHS is transmitted mostly vertically from hen to egg, but the virus could latently stay in birds for several days. It is possible that HHS was transmitted within the first 3 weeks of life and became clinically evident in the following weeks.

According to the current study, the prevalence of HHS in broiler farms is significantly higher during the summer and rainy seasons compared to winter, with 13.92% and 12.86% of birds affected respectively, suggesting that extreme weather conditions could have severe impacts on the birds’ immune system. In contrast, the winter season saw a significantly lower prevalence rate, with only 1,500 out of 34,500 birds affected, accounting for 4.35%. These findings indicate that HHS is more prevalent in the summer and rainy seasons than in winter.

The higher prevalence of HHS during the summer and rainy seasons in Bangladesh can be attributed to the high humidity and extreme heat experienced during these periods. Such harsh weather conditions are detrimental to the birds' immune systems, which could make them more susceptible to HHS. This observation aligns with the findings of Mittal (2014), who recorded similar seasonal patterns in Haryana, India. Their study, conducted from January 1997 to December 2006, documented the occurrence of IBH and HHS during the rainy and winter seasons. Although Mittal's study combined the incidence of both IBH and HHS, the seasonal trends observed reinforce the conclusion that environmental stressors, such as extreme weather conditions, play a critical role in the susceptibility of broiler chickens to these diseases.

By comparing our findings with those of Mittal (2014), we can better understand the seasonal dynamics of HHS and the importance of mitigating environmental stress to reduce the incidence of this disease in broiler farms.

During the study period, out of 109,000 broiler birds, 6,758 were found dead, resulting in an average mortality rate of 6.20%. This rate is consistent with the findings of Sawale (2012), who reported mortality rates ranging from 6% to 10% in Maharashtra State, India. Sawale's study encompassed both Inclusion Body Hepatitis (IBH) and HHS, providing a broader context for comparison. In our study,

out of 11,500 birds affected by HHS, 6,758 succumbed to the disease, yielding a case fatality rate of 58.77%. This is closely aligned with the observations of Mittal (2014), who reported a case fatality rate of 59.45% for the combined occurrence of IBH and HHS. The similarity in case fatality rates underscores the severe impact of these diseases on poultry populations and highlights the critical need for effective management and control strategies.

By comparing our findings with those of Sawale (2012) and Mittal (2014), it is evident that HHS poses a significant threat to broiler chickens, with mortality and case fatality rates remaining high across different geographic regions and study conditions. These comparisons reinforce the importance of ongoing surveillance, improved diagnostic techniques, and targeted interventions to mitigate the effects of HHS in broiler flocks.

We worked in a highly chicken populated area consisting of broiler, layer, and color birds. We communicated with over 600 broiler farms, and only 110 broiler farmers agreed to cooperate with our research willingly. If we added more farms to our work, the result would be more thorough. Another limitation is that we got very little information from breeder farms about whether the virus could transmit vertically or not, as parent stocks were not vaccinated during this study period. During this work, there was no vaccine for HHS available in Bangladesh, not only for local commercial broiler farms but also for controlled breeder farms. This study emphasizes the need for developing and distributing the vaccine for FAdV-4 as soon as possible.

CONCLUSIONS

This study presents a detailed assessment of Hepatitis-Hydropericardium Syndrome (HHS) prevalence and diagnosis in broiler chickens in eastern Bangladesh, identifying an overall prevalence of 10.55%. The highest incidence occurred in broilers aged 15-21 days and during the summer and rainy seasons, highlighting the influence of environmental stressors on disease susceptibility. The study's rigorous diagnostic approach, combining clinical and post-mortem examinations with PCR confirmation, ensures accurate identification of HHS. A notable case fatality rate of 58.77% underscores the severe impact of HHS on affected flocks. Comparisons with previous studies from different regions validate the broader relevance of these findings. The absence of an HHS vaccine in Bangladesh emphasizes the urgent need for effective vaccination strategies. In conclusion, this research underscores the critical need for targeted interventions, including the introduction of FAdV-4 vaccines, enhanced surveillance, and improved diagnostic methods to mitigate HHS's impact on the poultry industry in Bangladesh. These steps are vital for ensuring poultry health and the sustainability of broiler farming in the region.

ACKNOWLEDGEMENTS

We are grateful to all authors who involved directly to this project. We would like to thank Department of Microbiology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University (HSTU), Dinajpur-5200, Bangladesh and Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University (SAU), Sylhet-3100, Bangladesh, who provided us laboratory support for the molecular detection of Fowl Aviadenoviruses serotype 4. In addition, the cooperation of farmers for conducting this research is inevitable.

AUTHOR CONTRIBUTIONS

Md Nurnoby Islam designed and supervised the study. Md Masuk Rahman Kingshuk, Md Shajedur Rahman, Nazmi Ara Rumi, Mst Aireen Akter, and Hemayet Hossain conducted the experiment. Md Masuk Rahman Kingshuk wrote the manuscript and analyzed the data. Md Nurnoby Islam revised the manuscript.

CONFLICT OF INTEREST

There is no conflict of interest among the authors.

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How to cite this article;

Md Masuk Rahman Kingshuk, Md Shajedur Rahman, Nazmi Ara Rumi, Mst Aireen Akter, Hemayet Hossain and Md Nurnoby Islam. Prevalence with precise diagnosis of Hepatitis-Hydropericardium Syndrome (HHS) of broiler chicken in eastern Bangladesh. Veterinary Integrative Sciences. 2025; 23(2): e2025039-1-11.