



Gizzard Erosion in Broilers: The Fowl Adenovirus (FAdV)-Induced Inclusion Body Hepatitis (IBH) Poses a Significant Threat to the Broiler

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ABSTRACT

Background: Bangladesh's central and northern regions have seen gizzard erosion and feed passage syndrome in commercial broiler farms, causing significant economic losses due to poor body weight gain, high feed conversion rate, gut health issues and poor performance. This study investigated the causes of gizzard erosion and feed passage syndrome in broiler farms in these areas.

Methods: Commercial broiler farms were selected in three areas (Tangail, Sirajganj and Pabna) for farm visits and data collection because of the higher incidence of gizzard erosion and feed passage syndrome. Feed samples from certain regions were analyzed for mycotoxins and intestinal health in flocks was examined for coccidiosis and bacterial enteritis/dysbacteriosis. Gizzard erosion was observed in 52.78% of birds, with most being flaccid and moderate to severe erosion in the koilin layer. Some birds had mild-to-moderate coccidiosis lesions caused by *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella*. Tissue impressions on Fast Technology for Analysis (FTA) cards were collected from livers and gizzards with erosion and submitted for identification of fowl adenovirus (FAdV) through the polymerase chain reaction (PCR) method and phylogenetic analysis.

Result: All samples tested positive for fowl adenovirus (FAdV)-induced Inclusion Body Hepatitis (IBH) and were processed for serotype identification. Two serotypes were detected, namely FAdVD serotype 11 and FAdVE serotype 8b. All mycotoxin levels were less than the quantification limit. The TMLS and dysbacteriosis scores were mild, but individual *Eimeria* species scores were high in different areas. Therefore, we can conclude that mild coccidiosis with dysbacteriosis and fowl adenovirus-induced gizzard erosions in commercial broiler birds could be the cause of feed passage syndrome.

Key words: Dysbacteriosis, Feed passage syndrome, Fowl adenovirus, Gizzard erosion, TMLS.

INTRODUCTION

The poultry sector in Bangladesh plays an important role in the country's economy and food security. Poultry farming is one of the fastest-growing industries in Bangladesh, with a high demand for chicken meat and eggs. Bangladesh's poultry sector contributes to 1.5-1.6% of the country's GDP and employs 6 million people (BPICC, 2020). According to Light-Castle, over 53,000 broiler farms are in operation, with broiler breeds accounting for over 58.39% of total chickens (Business Inspection BD, 2022). Poultry has a significant impact on sustainable development goals by enhancing livestock production systems, but farmers face challenges like disease outbreaks, leading to significant losses due to high morbidity and mortality (Saleque, 2020). Poultry practitioners and consultants suspect that Inclusion Body Hepatitis (IBH) disease is an emerging disease in Bangladesh, posing a significant threat to broiler production (Saleque, 2020).

Inclusion body hepatitis (IBH) is caused by fowl adenoviruses (FAdV), which are categorized into 5 groups (A–E) and 12 serotypes (Hess, 2000). FAdV A–E is divided into twelve distinct serotypes and IBH mostly results from FAdV type D or E (Marek *et al.*, 2010). Since its initial description in 1963 in the USA, numerous countries worldwide have reported on the disease (Cizmecigil *et al.*, 2020). IBH is a sporadic disease caused by various bird

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adenovirus serotypes, with horizontal and vertical transmission playing a crucial role (Schachner *et al.*, 2018). In 2002, Bangladesh reported the first case of IBH in broiler parents (Biswas *et al.*, 2002). It is a recently emerging poultry disease, characterized by sudden onset and a high death rate (Memon *et al.*, 2006).

FAdV is a common infectious agent in poultry, often replicating in healthy birds without any visible signs of infection (Memon *et al.*, 2006). High virulent Fowl Adenovirus (FAdV) serotypes cause inclusion body hepatitis (IBH) in chickens, increasing mortality by up to 30% (Batista

et al., 2024). Less virulent FAdV can affect bird health and performance by infecting other parts of the body, such as the lungs, heart, pancreas and gizzard, leading to respiratory disease, hydropericardium syndrome, necrotizing pancreatitis and gizzard erosion (Dutta *et al.*, 2023; Das and Shelke, 2024).

Gizzard erosion and ulceration syndrome (GEU) are characterized by erosive lesions in the koilin layer of the gizzard and macroscopic defects in the mucosa (Gjevre *et al.*, 2013). According to research, during GEU development, birds' feed intake and growth were significantly affected and average body weight gain and feed consumption decreased by up to 12% and 14%, respectively (Wang *et al.*, 2021). In Bangladesh, the prevalence of GE (Gizzard Erosion) in broilers has reached 70.6 - 87.2% (Moula, 2020). Multiple factors play a role in the development of GEU, including congenital factors, starvation, malnutrition, feed material, toxicants (copper sulphate, biogenic amines, gizzerosine and mycotoxin, *Clostridium perfringens*, Fowl Adenovirus, *etc.* (Haque *et al.*, 2023). All these factors causing GE also can have deleterious effects on birds' intestinal integrity. Poor intestinal integrity can lead to increased feed passage syndrome, poor feed conversion and a drop in flock performance. Feed passage syndrome is a condition affecting chicken gastrointestinal health, where poorly digested coarse feed particles are more prevalent in feces. Factors such as feed quality and infectious diseases can predispose broiler birds to this condition, resulting in poor feed conversion and body weight gain (Butcher *et al.*, 2002).

The etiology of fowl adenovirus (FAdV) has been linked to gizzard erosions (Lim *et al.*, 2012). According to most scientific reports from viruses identified in Asia and Europe, FAdV-1, genotype A, is the main cause of GE in commercial broilers (Okuda *et al.*, 2004). In other cases of GE, FAdV

serotypes 8 and 11 were isolated (Okuda *et al.*, 2004). Researchers found a link between GE-affected birds and growth retardation (Mirzazadeh *et al.*, 2021). Since several factors can have deleterious effects on the birds' GIT, it becomes imperative to identify the exact causative factor to take the necessary preventive measures. In Bangladesh, most of the efforts aimed at identifying and genotyping FAdV in cases of IBH and high mortality are not associated with GE in broilers (Islam *et al.*, 2023). We conducted the study to investigate the causative factors responsible for gizzard erosion and feed passage syndrome in commercial broilers in this area.

MATERIALS AND METHODS

History of the flocks

Towards the end of February 2024, we visited Sirajganj, Pabna and Tangail (Fig 1) area broiler farms and observed that 55-60% of farms drop in performance, less feed intake, depression and decreased flock uniformity. At that time environmental conditions were good in these areas (Average temperature -25°C and humidity -65%). Although there was no outward major clinical sign of illness, average mortality was 3.19% but, besides normal droppings, 15-20% of the droppings contained orange mucoïd with undigested feed particles. All these broiler flocks were from different breeder flocks and none of them were vaccinated against FAdV. The vaccination status of breeder flocks against FAdV from which these broiler flocks came from were unknown. Farm conditions, bird behavior and health, performance data and farm management practices were examined in each flock.

Routine intestinal health examination and lesion scoring

We observed farm conditions and randomly selected twelve farms in three areas, in each farm, three live birds

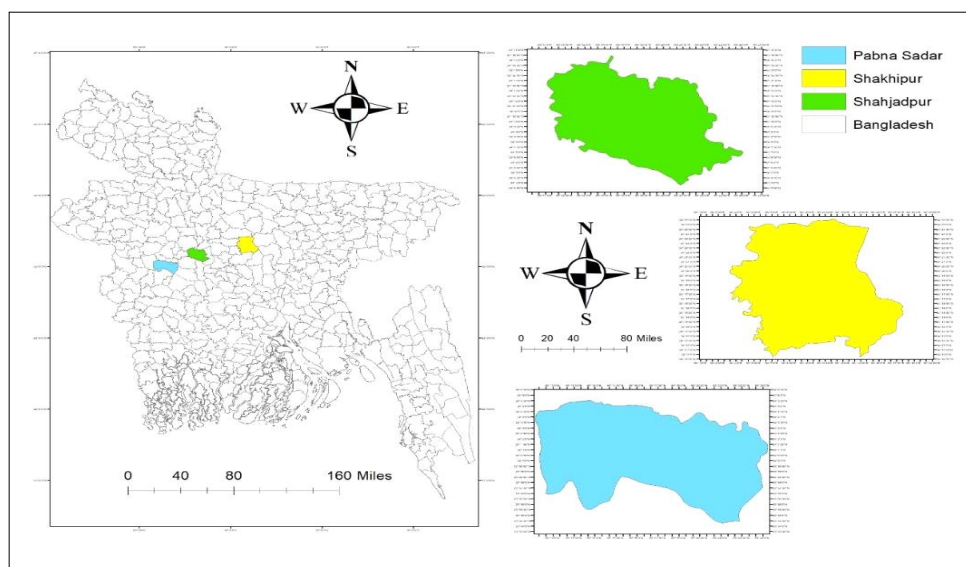


Fig 1: Location of the sampling sub-districts (Upazila) of Bangladesh marked by different colors. Map is created in ArcGIS 10.8.2. ArcGIS Enterprise, ESRI, Redlands, California, USA. IMG-KAI-00792.

were randomly sampled and euthanized humanely and the gastrointestinal tract was examined. GIT was thoroughly examined for lesions of coccidiosis (*Eimeria acervulina*, *E. maxima* and *E. tenella*) and bacterial enteritis/dysbacteriosis. Coccidiosis lesions were scored and recorded based on severity according to the method of Johnson and Reid (1970), whereas dysbacteriosis lesions were scored based on the method by Teirlynck *et al.* (2011).

Samples collected

In both visited areas, the intestinal health examination revealed that 8 out of 36 examined birds had pale, hepatomegaly and focal necrotic foci in their livers, while 19 out of 36 birds (52.78%) had moderate to severe gizzard erosions. We collected tissue impressions on the FTA card from the liver and gizzards, which showed erosion on the koilin layer. We submitted the tissue impressions to the Target Poultry Health and Diagnostic Centre in Hyderabad, India, for identification of FAdV using the polymerase chain reaction (PCR) method. All positive samples were further processed for sequencing of the Hexon gene of FAdV to identify the serotype of the virus. To find out feed-related factors for gizzard erosion, three feed samples were collected from the farms and sent to the laboratory for the following mycotoxin analysis: Aflatoxin B1, Ochratoxin A,

Citrinin, Zearalenone, Cyclopiazonic acid (CPA) and Fumonisin B1, B2 and B3.

RESULTS AND DISCUSSION

Broiler production performance declined, with a 50-60% reduction in farm output characterized by low body weight gain, decreased feed intake and suboptimal feed conversion. But there was no increase in mortality and average mortality was 3.19%. In most of the farms, 15-20% of the droppings contained orange mucoid with undigested feed particles. Feed passage (undigested maize particles in birds' feces) was observed throughout the house (Fig 2) and a decline in body weight gain was observed and was lower than the breed standards. During the examination of GIT, hepatomegaly, focal hepatic necrosis in the liver with a pale color and hydropericardium were observed (Fig 5A, B and C). The presence of gizzard erosion (GE) was the most common observation and 52.78% of birds found GE in examined for GIT assessments. Affected gizzards had reduced musculature which appeared flaccid and moderate to severe erosions of the keratinoid layer as well as inflammatory changes and ulceration underneath the gizzard mucosa (Fig 4A, B and C). During intestinal health examination, poorly digested maize particles were observed in the content of the distal ileum and cloaca part (Fig 3D).

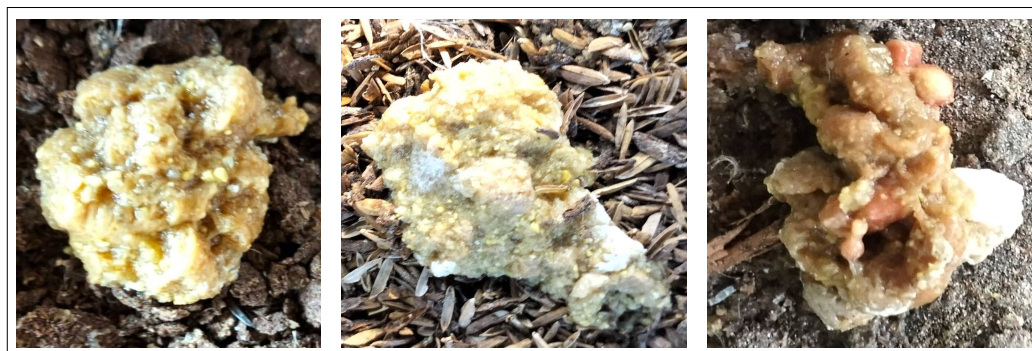


Fig 2: Undigested feed particles and orange color mucoid droppings. IMG-KAI-00793/00795/00796.

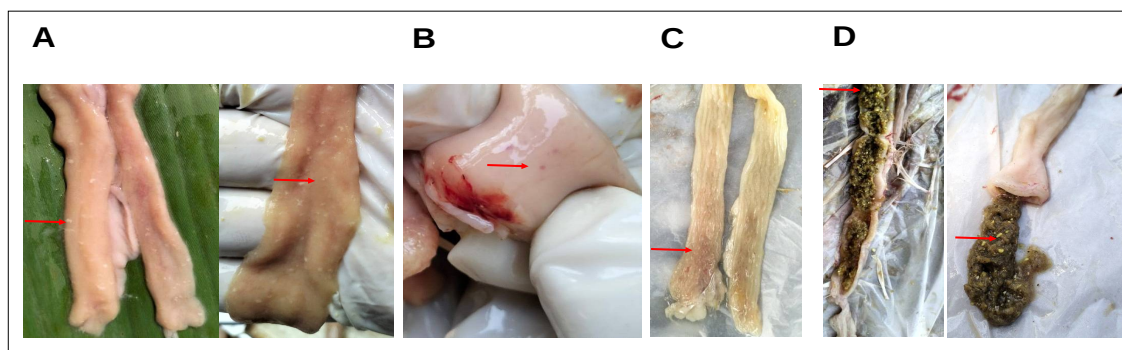


Fig 3: (A) *Eimeria acervulina* lesions-scattered white plaque-like lesions are elongated with longer axis transversely on duodenal wall. (B) *Eimeria maxima* lesions- small red petechiae are in the serosal surface of mid intestine. (C) *Eimeria tenella* lesion- Few scattered petechiae on caecal wall. (D) condition of feed passage in hindgut (undigested feed particles). IMG-KAI-00796/00797/00798/00799/00800/00801.

Proventriculus was found to be normal. In most of the birds, mild to moderate *Eimeria acervulina*, *Eimeria maxima* and *Eimeria tenella* were observed (Fig 3A, B and C) and Total means lesion scoring (TMLS) scores were 0.78, 1.67 and 0.67 in Sirajganj, Tangail and Pabna area, respectively (Fig 6). The dysbacteriosis lesion scores were found to be below 1 in the Sirajganj and Pabna, areas and 1.11 in the Tangail area (Fig 6).

The feed provided to all these flocks was formulated with plant-origin ingredients. Raw materials from animal protein sources were not used in the feed. In the feed samples submitted for mycotoxin analysis, all mycotoxins (Aflatoxin B1, Ochratoxin, Citrinin, Zearalenone (ZON), Cyclopiazonic acid (CPA), Fumonisin B1, B2 and B3) were below the limit of quantification (Table 1).

All gizzard and liver impression smear samples submitted for the detection of FAdV by PCR were found

positive for FAdV (Table 2). All positive samples were further processed for sequencing of the Hexone gene of FAdV. Phylogenetic analysis of the gene fragment indicated a resemblance to fowl adenovirus serotype 11 in two samples and serotype 8b in another two samples (Table 2 and Fig 7, 8, 9 and 10).

In recent times, gizzard erosion (GE) and ulceration syndrome (GEU) has been very common in poultry at mild to moderate levels (Contreras, 2016). It usually causes reduced feed intake, growth, poor absorption of nutrients and persistent diarrhea in birds, which leads to economic losses (Wang *et al.*, 2021). On the other hand, feed passage syndrome (FPS) has emerged as a common problem in broilers. This passage of undigested feed into feces has had a significant impact on weight gain, FCR and flock uniformity, ultimately leading to a severe loss of productivity (Butcher *et al.*, 2002). A similar observation was found in

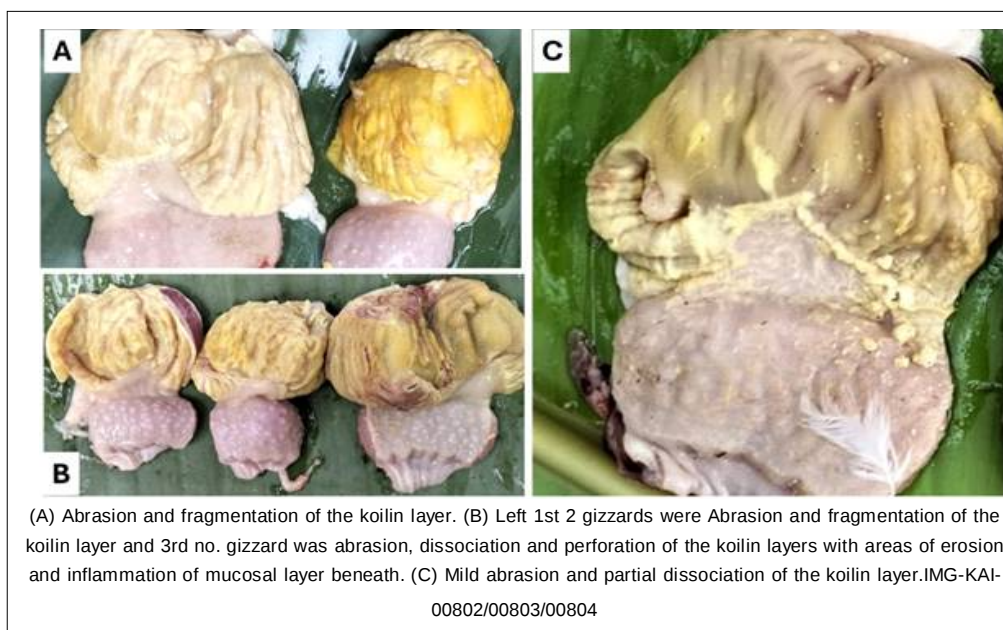


Fig 4: Gross pathology of gizzards from the field commercial broiler farms.

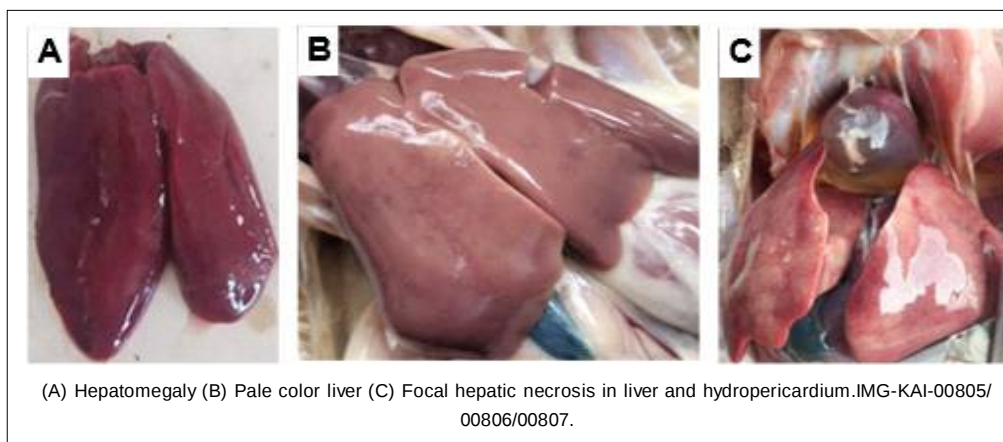


Fig 5: Gross pathology of liver and heart from the field commercial broiler farms.

the present study as well. Most of the farm's birds' body weight gain decline, low feed intake and high feed conversion ratio (FCR) were observed and were lower than the breed standards.

Multiple factors play a role in the development of gizzard erosion, including congenital factors, starvation, malnutrition, feed material, toxicants (copper sulphate, biogenic amines, gizzerosine and mycotoxin, such as T-2 toxin, MAS, DAS, DON) and viral/bacterial infection (Haque *et al.*, 2023).

Gizzard and koilin lesions have been observed in chick embryos and newly hatched broiler chicks, suggesting that gizzard erosion and ulceration (GEU) may be congenital and influenced by factors before hatch (Gjevre *et al.*, 2013). However, no congenital history was found in the present study and early chicks' postmortem examination did not show gizzard erosion lesions. Newly hatched chicks deprived of feed and water experienced an increase in ulcerated gizzards, from 3 to 68% on day 4 post-hatch. Restriction or deprivation of food and water may be associated with increased frequency and severity of lesions (Haque *et al.*, 2024). However, no starvation history was

found on farms in these areas, so congenital starvation as a causative factor for gizzard erosion was ruled out.

Malnutrition status, like deficiency of vitamin B6, vitamin B12, vitamin E, methionine and decreased sulfur-containing amino acids, is associated with gizzard ulceration (Gjevre *et al.*, 2013). In the current study, we analyzed all feed samples in the feed nutrition lab and proximate analysis showed that the nutrition values adhered to the breed diet standards, but we did not analyze the vitamins and minerals.

Feed-related factors of gizzard erosion such as copper sulfate, biogenic amines, gizzerosine and mycotoxins are the major ones (Masumura *et al.*, 1981; Gjevre *et al.*, 2013). High levels of copper sulfate can lead to ulceration of the gizzard lining (Luo *et al.*, 2005), while biogenic amines (histamine), found in poorly processed animal protein sources, catalyze gastric acid secretion (Barnes *et al.*, 2001). Besides this, poor-grade fish meals produce gizzerosine [2-amino-9-(4-imidazolyl)-7-azanonanoic acid], which is an even more effective stimulator of gastric acid secretion in poultry and causes gizzard erosion in chickens (Masumura *et al.*, 1981). As a result, biogenic amines and gizzerosine cause proventricular enlargement, gizzard

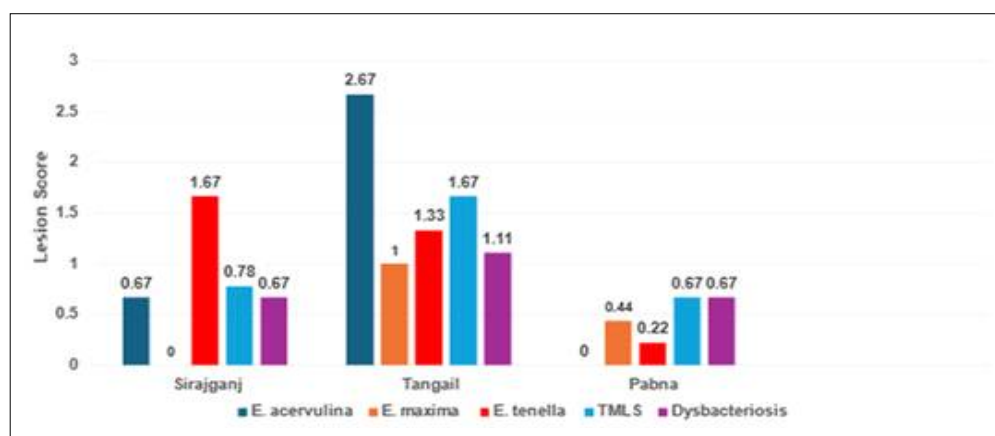


Fig 6: Coccidiosis and dysbacteriosis lesion scores. IMG-KAI-00808.

Table 1: Feed samples analysis for detection of mycotoxins level.

Parameters	Units	Broiler starter feed- T	Broiler grower feed-S-1	Broiler grower feed-S-2	Maximum permissible limits*
Aflatoxin B1	ppb	8	8	8	20 ppb
Ochratoxin	ppb	<8	<8	<8	40 ppb
Citrinin	ppb	<8	<8	<8	100 ppb
Zearalenone (ZON)	ppb	<100	<100	<100	200 ppb
Cyclopiazonic acid (CPA)	ppb	<225	<225	<225	-
Fumonisin B1	µg/kg	105.05	131.39	-	20000 µg/kg
Fumonisin B2	µg/kg	60.68	72.37	-	
Fumonisin B3	µg/kg	21.51	18.48	-	
Fumonisin (Sum of Fumonisin B1, B2 and B3)	µg/kg	187.24	222.24	-	

Permissible limit is applicable for the feed sample only. The permissible limits are applicable only for the presence of a single mycotoxin in feed. If more than one mycotoxin is observed, these permissible limits may not be relevant (Pinotti *et al.*, 2016).

erosion, sloughing of intestinal epithelia, poor weight gain and feed conversion, impaired immune response and feed passage syndrome (Butcher *et al.*, 2002). Affected birds are poorly pigmented and have low body weights and feed conversions. The study found that none of the examined farms received any animal protein sources or copper sulfate in their feed, eliminating the possibility of these factors causing gizzard erosion and feed passage syndrome.

Among mycotoxins, Trichothecenes are a group of Fusarium mycotoxins, including T-2 Toxin, Monoacetoxyscirpenol (MAS), Diacetoxyscirpenol (DAS), Deoxynivalenol (DON) and HT-2 Toxin, which can cause gizzard erosion (Gjevre *et al.*, 2013). Fumonisin and cyclopiazonic acid (CPA) are potent in causing GE (Gjevre *et al.*, 2013). Feeds containing mycotoxins, like aflatoxin and ochratoxin, cause syndrome in feed passage, leading to intestinal wall erosion, liver

damage, reduced bile and digestive enzyme production and poor digestion (Butcher *et al.*, 2002). In the feed samples collected from the study regions, Trichothecene was not assessed in any of the feed samples and other mycotoxins known to cause FPS (aflatoxin, ochratoxin) and GE (CPA and fumonisins) were assessed in these feed samples and were either not present or were below the limit of quantification.

Researchers have found that *Bacillus cereus* (*B. cereus*) isolates cause gizzard erosion and ulceration syndrome (GEU) in birds, potentially leading to vomiting, diarrhea, or ulceration (Ono *et al.*, 2003). *Clostridium perfringens* was discovered to be an opportunistic bacterium in commercial chickens with gizzard ulcerations and the severity of the lesions was observed to considerably increase caecal numbers (Tsilia *et al.*, 2016). In the present

Table 2: FAdV PCR and gene sequencing result.

Region	Sample type	Sample organ	IBH PCR	FAdV serotype
Sirajganj	FTA-1, CIRCLE-1,2	Liver, Gizzard	Positive	FAdVD-11
Tangail	FTA-1, CIRCLE-3,4	Liver, Gizzard	Positive	FAdVD-11
	FTA-1, CIRCLE-1,2	Liver, Gizzard	Positive	FAdVE-8b
Pabna	FTA-1, CIRCLE-3,4	Liver, Gizzard	Positive	FAdVE-8b

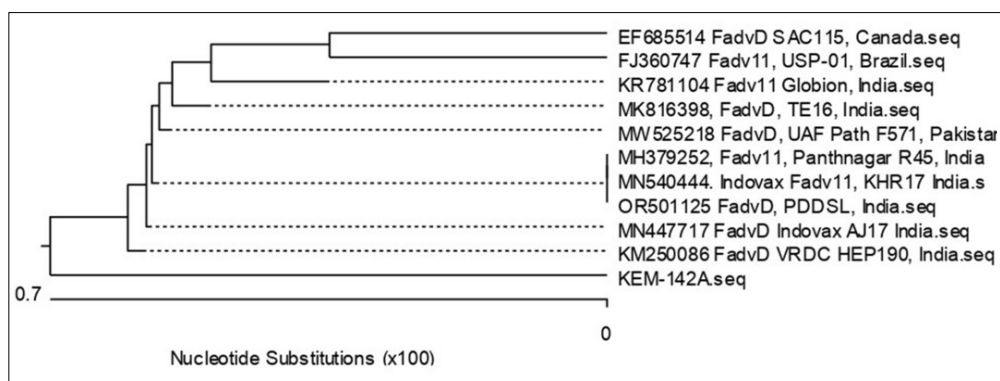


Fig 7: Phylogenetic analysis of a FAdV-positive sample's sequenced hexone gene was similar to FAdVD serotype 11. IMG-KAI-00809.

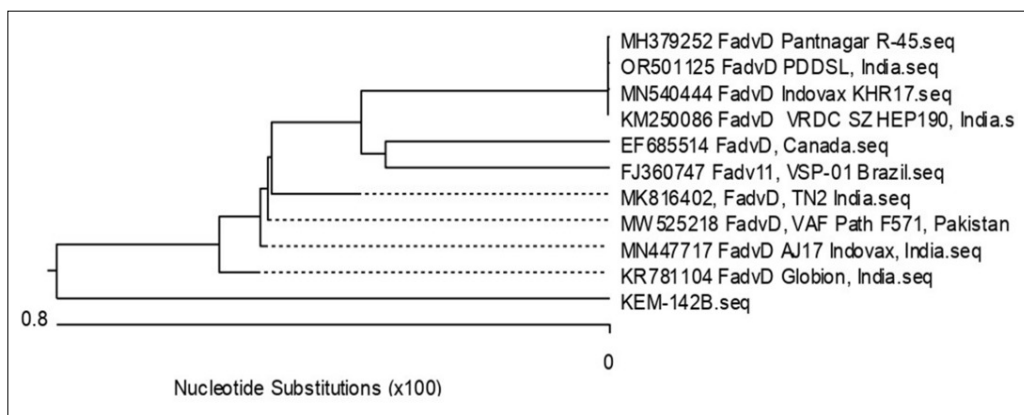


Fig 8: Phylogenetic analysis of a FAdV-positive sample's sequenced hexone gene was similar to FAdVD serotype 11. IMG-KAI-00810.

study, *Bacillus cereus* (*B. cereus*) and *Clostridium perfringens* samples were not collected from the study region and were isolated in the microbiological lab.

Coccidiosis, particularly in younger broilers, can damage the intestine lining, allowing undigested nutrients to pass through feces (Ram *et al.*, 2020). *E. acervulina* and *E. maxima* are commonly associated with feed passage (Butcher *et al.*, 2002). Bacterial enteritis often occurs after viral or coccidial infections, with *E. acervulina* and *E. maxima*, causing damage to the gut lining, reducing intestinal passage and allowing overgrowth of *C. perfringens*, leading to necrotizing enteritis (Butcher *et al.*, 2002). In the present study, *E. acervulina* and *E. maxima* lesion scored 0.67, 2.67, 0 and 0.1, 0.44 in Sirajganj, Tangail and Pabna areas respectively. TMLS scores are 0.78, 1.67 and 0.67 in Sirajganj, Tangail and Pabna. In the lesion scoring of the GIT tract, no lesion related to necrotic enteritis (the causal agent of *Clostridium perfringens*) and dysbacteriosis score below 1 in the Sirajganj and Pabna areas and 1.11 slightly higher in the Tangail area. Observation is that TMLS and dysbacteriosis score was not so high except Tangail area for TMLS score and dysbacteriosis. So, it could be interpreted that coccidiosis and dysbacteriosis can also be the contributing factors for

the feed passage syndrome in affected flocks. Also, the effect of FPS increases when FAdV is infected in flocks.

FAdV, induced IBH, is another infectious factor that causes gizzard erosion (Butcher *et al.*, 2002). In Bangladesh, gizzard erosion (11% of suspected IBH cases) and hemorrhage of muscle are two of the most common symptoms (Saleque, 2020). However, new studies show that gizzard erosion is more common in IBH cases. Most cases of GE have revealed the presence of FAdV in the affected birds. Certain pathogenic strains of FAdV significantly impact the health and performance of healthy chickens, despite adenoviruses being detected in healthy chickens (Yates *et al.*, 1976). Pathogenic strains of FAdV significantly affect birds' health and performance. Factors such as chicken breed, immune system status and concurrent infections with other immunosuppressive agents influence the mortality and severity in adenoviral infections (Wani *et al.*, 2014). The status of the birds' immune systems also plays a key role in determining the severity of FAdV infection. FAdV infection manifests severely in immunocompromised birds (Fadly *et al.*, 1976). Pathogenicity of adenoviruses may vary among strains belonging to the same serotype (Absalón *et al.*, 2017). FAdV manifestations like gizzard erosion may not show

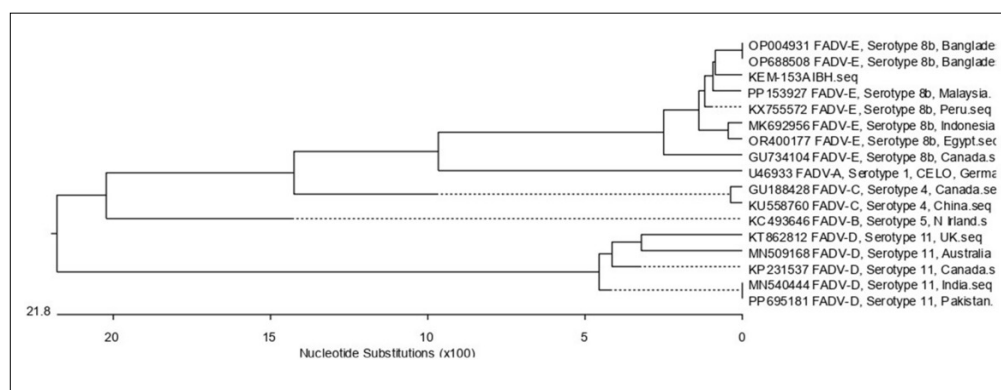


Fig 9: Phylogenetic analysis of a FAdV-positive sample's sequenced hexone gene was similar to FAdVE serotype 8b. IMG-KAI-00811.

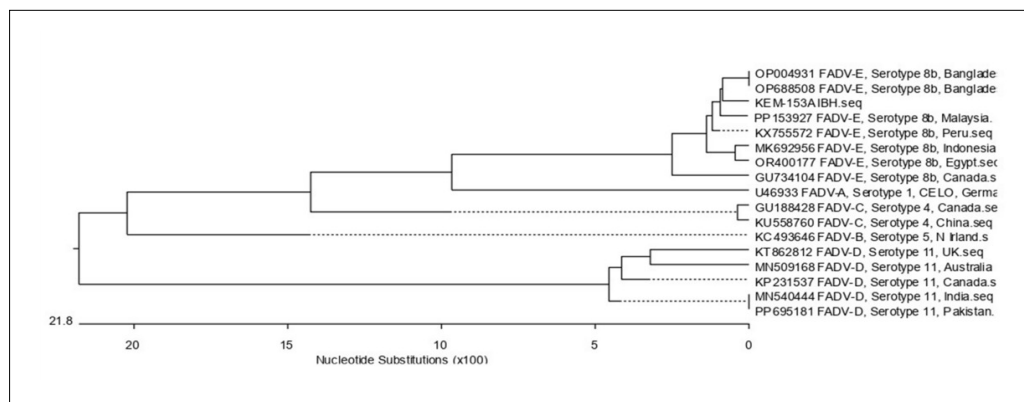


Fig 10: Phylogenetic analysis of a FAdV-positive sample's sequenced hexone gene was similar to FAdVE serotype 8b. IMG-KAI-00812.

clinical symptoms or significantly increase mortality but still impact body weight gain and feed conversion (Mirzazadeh *et al.*, 2021).

Most GE cases worldwide have identified FAdV serotype 1 (Das and Shelke, 2024; Okuda *et al.*, 2004; Ono *et al.*, 2003; Domanska-Blicharz *et al.*, 2011). In Japan and Iran, birds with GE isolated FAdV serotypes 8 and 11 (Okuda *et al.*, 2004). In India, GE cases in commercial layer chicken identified FAdV serotypes 2, 3, 4, 8 and 11 (Lim *et al.*, 2012; Chitradevi *et al.*, 2020) and commercial broilers identified fowl adenovirus-induced gizzard erosion serotypes 8a and 11 in southern India (Shankar *et al.*, 2022; Das and Shelke, 2024). Bangladesh detected three serotypes of IBH, specifically 8b (70%), 11 (10%) and 5 (10%), but did not report any gizzard erosion lesions (Islam *et al.*, 2023). Phylogenetic analysis revealed that the Bangladeshi FAdVs have a close identity with viruses from Asia, Europe and South and North America (Islam *et al.*, 2023). This study found that all the tissue impression samples were positive for FAdV. This, in line with other researchers' findings, suggests that FAdV plays a role in causing GE in broiler chickens.

The present study detected FAdV serotypes 8b and 11 in the tissue impression samples, which contrasts with the majority of studies that found FAdV serotype 1. Some studies have demonstrated that FAdV-induced GE has an economic impact on the affected flock, leading to impaired body weight gain and increased mortality, while others have revealed poor performance and lower mortality (Mirzazadeh *et al.*, 2021; Das and Shelke, 2024). In the present study, though there was no significant increase in mortality, all the affected flocks had lower body weight gain than the breed standards. Feed passage syndrome (FPS) is also caused by multiple etiological factors and FAdV is one of them (Butcher *et al.*, 2002). Any condition or factors affecting GIT will impair the digestion process leading to the excretion of poorly digested feed particles in the feces. FAdV has been identified from GIT of broilers having enteric disease conditions and feed passage problems (Das and Shelke, 2024). A similar observation was found in the present study as well. Feed passage was observed in all the visited farms with varying severity. There are many common causes of gizzard erosion and FPS and adenovirus is one of them. Gizzard erosion caused by adenovirus causes problems in the GIT tract in the form of feed passage syndrome and other factors play a role. This results in low body weight gain of birds, high FCR, poor performance and economic losses to the farms.

CONCLUSION

There are many factors involved in GE and FPS and some of them are similar. Both GE and FPS have adverse performance effects and the economic impact is high in broiler production. While other factors like mycotoxins, biogenic amines, gizzerosine, copper and so on primarily contribute to GE, FAdV stands as one of the primary causes of GE at the moment. While previous studies in Bangladesh

have identified FAdV, they did not concentrate on GE lesions associated with FAdv. The detection of FAdV serotype 8,11 specifically for GE is the first time in Bangladesh, which aligns with many studies that have been held in other countries around the world. This study will also play an important role in Bangladesh's poultry IBH vaccine development. In this case, feed passage syndrome appears to be caused by the combined effects of FAdV, coccidiosis and dysbacteriosis. To prevent and control the feed passage syndrome in commercial broiler birds, a holistic approach is necessary. In this case, vaccination against fowl adenovirus and management of in-feed anticoccidial and intestinal health can help lower feed passage syndrome, leading to better performance in commercial broiler flocks.

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Disclaimers

The views and conclusions expressed in this article are solely those of the authors and do not necessarily represent the views of their affiliated institutions. The authors are responsible for the accuracy and completeness of the information provided, but do not accept any liability for any direct or indirect losses resulting from the use of this content.

Informed consent

All animal procedures for experiments involving field visit observations and diagnostic purposes that were not approved by the Committee of Experimental Animal care and handling techniques were also not approved by the University of Animal Care Committee.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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