

Original Research Article

## Prevalence and Clinicopathological Investigation of Necrotic Enteritis at Rajshahi District in Bangladesh

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**Abstract:** *Background and Objective:* Necrotic enteritis (NE) has increased in occurrence and severity over the years. The re-emergence of necrotic enteritis has been the most significant threat for the poultry industry. It is one of the most common and economically devastating bacterial diseases in terms of performance, welfare and mortality. So, the present research work was designed to determine the prevalence with a clinicopathological investigation of necrotic enteritis in commercial broiler and Sonali chicken (the common breeds for meat purpose in Bangladesh) at Rajshahi District (the Barind Tract) in Bangladesh. *Materials and Methods:* A total of 500 (260 Sonali (Fayoumi × RIR) and 250 Broiler (Cobb 500) birds from 10 different commercial poultry farms were used to conduct the present study. The diagnosis of NE was based on history, clinical signs characteristic gross lesions and histopathological findings. Results: The overall prevalence of necrotic enteritis was 7.84 % in chickens, though the incidence rate varied from farms to farms ranging from 4.0 – 11.67%. The incidence of necrotic enteritis was almost 1.5 times in broiler chickens (9.6%) than in Sonali chickens (6.2%). The incidence of necrotic enteritis was varied with season showing peak occurrence in winter season (11.67%), and the lowest incidence was in summer season (3.2%). The major clinical signs observed were severe depression, diarrhoea (shooting type), ruffled feathers, and sudden death. The striking necropsy findings were markedly thickened enteric mucosa covered by tan-orange pseudo-membranes often referred to as a “Turkish towel” in appearance with haemorrhages occasionally and ballooning of intestine with foul smelled gas. Enterohistopathological findings included the coagulative necrosis, increased cellular infiltration in lamina propria, haemorrhage, congestion, disorganization of structure. Conclusion: The study concludes that investigation of NE in commercial chickens based on clinical signs, necropsy and enterohistopathological findings helps to determine the prevalence of NE and also help to adopt prevention and control strategies.

**Keywords:** Necrotic enteritis, enteric mucosa, necrosis, haemorrhage, Turkish towel.

## INTRODUCTION

The contribution of the poultry sector as an important tool in global efforts to overcome malnutrition and poverty in developing countries is widely recognized. Poultry often represents a farmer’s first investment in the livestock ladder (followed by goats/sheep and then cattle) as a way of increasing income and emerging from the poverty trap (FAO, 2015). Poultry has got prime importance as a source of animal protein for masses. Poultry production has undergone a substantial increase compared to the livestock industries since 1970. It contributes about 34% in total share of meat produced in world (M’Sadeq *et al.*, 2015). Poultry is the fastest growing agricultural sub-sector, especially in developing countries including Bangladesh. The global poultry sector is expected to grow as demand for meat and eggs driven by growing populations, rising incomes and urbanization. Poultry is a major asset and key to poverty alleviation, providing income and market participation, particularly for small holders and the poor, both in rural and urban areas (Mottet and Tempio, 2017). Moreover, poultry in many parts of the modern world is considered as the chief source of not only cheaper protein of animal origin but also high quality human food (Marangoni *et al.*, 2015; Haque and Gofur, 2020).

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Avian necrotic enteritis (NE) is a major disease of chickens caused by toxins of *Clostridium perfringens* type A and C, with typical hallmark in small intestinal necrosis (Keyburn *et al.*, 2008; Umar *et al.*, 2016). Necrotic enteritis has increased in occurrence and severity over the years. In clinical form, necrotic enteritis causes high mortality and in subclinical forms, affects growth and feed conversion. It is one of the most common and economically devastating bacterial diseases of chickens in terms of performance, welfare and mortality (Wade and Keyburn, 2015). The disease was previously controlled by the administration of antibiotic growth promoters (Lanckriet *et al.*, 2010a), before the ban of antibiotics usage in poultry breeding by European Union (Casewell *et al.*, 2003). There is an emerging need for NE alternative control strategies particularly after problems related to the spread of antibiotic-resistant microorganisms and antibiotic residues have appeared. The use of feed additives such as organic acids, essential oils, probiotics, prebiotics and symbiotics can partially decrease the necrotic enteritis occurrence in broilers without complete disease control (Timbermont *et al.*, 2010; Jerzsele *et al.*, 2012). Recently, several studies have been focused on the development of vaccines against necrotic enteritis as active supernatants, formalin-inactivated toxoids and modified toxins either in I/M or S/C administration, in single or multiple dosage vaccination programs (Mot *et al.*, 2013). Avian necrotic enteritis was first described by Parish in 1961 (Parish, 1961), and since then it has been reported to occur in almost all poultry-producing countries in the world including Australia (Gardiner, 1967; Nairn and Bamford, 1967), North America (Bernier and Filion, 1971; Helmboldt and Bryant, 1971), South America (Baldassi *et al.*, 1995), Europe (Jylling and Mørch, 1969; Köhler *et al.*, 1974), Asia (Kwatra and Chaudhury, 1976; Oda *et al.*, 1977; Tsai and Tung, 1981) and Africa (Dosoky, 1990; Rahamathulla *et al.*, 1994). Until recently, it has been controlled rather effectively by the presence of antimicrobial growth promoters in the feed. Concerns about increased antibiotic resistance in human pathogens have led to restrictions in the use of these compounds in animal feed. Consequently, it has been a common perception in many countries that necrotic enteritis is a reemerging disease although few studies have been done to investigate the prevalence of the disease in Bangladesh. Therefore the present study is designed to determine the prevalence with a clinicopathological investigation of necrotic enteritis in commercial broiler and Sonali chicken (the common breeds for meat purpose in Bangladesh) at Rajshahi District (the Barind Tract) in Bangladesh.

## MATERIALS AND METHODS

### *Experimental chickens*

The investigation was carried out at the pathology lab of the Faculty of Veterinary and Animal Sciences, University of Rajshahi, Rajshahi, Bangladesh. We studied a total of 500 birds (sick and dead birds; 260 Sonali (crossbred of Fayoumi female and RIR (Rhode Island Red) male, developed in 1986) and 250 Broiler (Cobb 500) birds) to conduct the present study. These birds were procured from 10 different commercial poultry farms located in and around Rajshahi district (the Barind Tract of Bangladesh) in the period from September, 2019 to December, 2020.

### *Clinical signs and relevant histories*

The flock history including flock size, breed, age, sources from which the day old birds were collected, rearing system, history of vaccination, history of medication, date of outbreak occurred, number of birds affected, number of birds died, previous outbreaks of the diseased were recorded. The clinical signs of the affected flocks were recorded during the physical visit of the farm and the farmer's complaints in connection to it was also considered and noted.

### *Necropsy and Histopathological Examination*

The diagnosis of the disease was based principally on history, clinical signs and characteristic gross lesions. Histopathology of selective samples was performed to substantiate the diagnosis. Both sick and dead birds were examined systematically at necropsy following standard procedure (Charlton, 2000). The birds were also brought to laboratory during physical visit of farms. The clinical history and signs were carefully considered before the attempt of postmortem examination. The physical appearances of the carcasses and the visible gross morbid lesions of the intestines were recorded. The tissue samples were collected during the course of necropsy and preserved at 10% formalin solution as soon as possible to avoid the alteration of the tissues through autolysis. The autolysed tissues were avoided for histopathological examination. The fixed samples were processed, embedded in paraffin, sectioned with a thickness of 5  $\mu$ , and stained with routine Hematoxylin and Eosin stain following a well recommended procedure (Gridley, 1960). The characteristic histopathological lesions were observed under light microscope and recorded.

## RESULTS

### **Overall prevalence of necrotic enteritis in chickens**

To determine the prevalence of necrotic enteritis, a total of 510 birds from 10 different poultry farms located in and around Rajshahi district was studied. The overall prevalence of necrotic enteritis was 7.84 % in chickens, though the incidence rate varied from farms to farms ranging from 4.0 – 11.67% (Table 1).

### Effect of breed and season on prevalence of necrotic enteritis in chickens

We studied 250 broilers and 260 Sonali chickens to determine the prevalence of necrotic enteritis in two different breeds of chickens common used for meat purpose in Bangladesh. The incidence of necrotic enteritis was almost 1.5 times in broiler chickens than in Sonali chickens (Table 2).

To determine the effect of season on prevalence of necrotic enteritis in chickens, we investigated the chickens of different farms during the whole year. We divided a year into three major seasons; namely Summer (March-June), Rainy (July- September), and Winter (October-February). The incidence of necrotic enteritis was varied with season in chicken (Table 3), showing peak occurrence in winter season, and the lowest incidence was in summer season.

#### Clinical signs

The major clinical signs observed during physical visit of the farms and also detected from the farmer's complaints. The affected birds showed severe depression, diarrhoea (shooting type), huddling, reluctant to move, ruffled feathers, and sudden death. Death recorded inspite of history of a good bodily condition and good appetite. Orange colored feces (Fig. 1C) was also an objection from the farm owners. The main complaints of the most farmers were the quick wet litter, shooting type diarrhoea and subsequently death.

#### Necropsy findings

The striking gross morbid lesions found at necropsy were abnormally thickened enteric mucosa with varying degrees of haemorrhages, ballooning of the intestine, expulsion of foul smelling gas when opened. The intestine often looked like friable (Fig 1B). Large amount of necrotic enteroepithelial debris in the lumen including flecks of blood occasionally was also seen. The mucosa is usually covered with a tan to yellow pseudomembrane often referred to as a "Turkish towel" in appearance (Fig 1A).

#### Histopathological findings

The lesion of necrotic enteritis affected intestines of broiler and Sonali chickens were histopathologically characterized as severe necrosis of enteroepithelial cells with marked desquamation, increased cellular infiltration in lamina propria, haemorrhage, congestion, disorganization of structure, and fibrin mixed with cellular debris adherent to intestinal mucosa (Fig 2 & 3).

**Table 1: Overall prevalence of necrotic enteritis in chickens at different poultry farms**

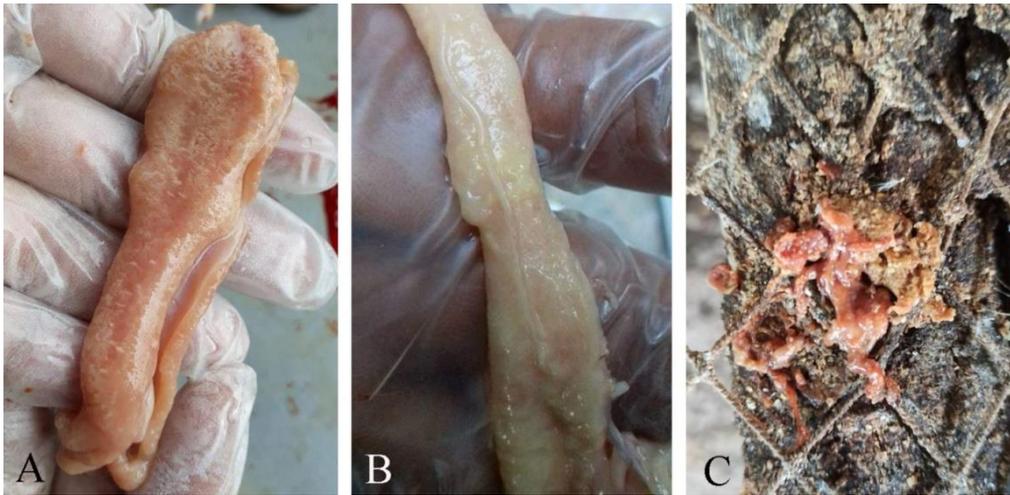
Sl. no. of farms	No. of birds randomly examined	No. of (+ve) cases	Prevalence of the cases at different farms (%)	Overall prevalence among examined cases
1	50	3	6.0	7.84 %
2	50	3	6.0	
3	50	5	10.0	
4	50	3	6.0	
5	50	4	8.0	
6	50	4	8.0	
7	50	2	4.0	
8	50	5	10.0	
9	50	4	8.0	
10	60	7	11.67	
Total	510	40	-	

**Table 2: Prevalence of necrotic enteritis in two different breeds of chicken**

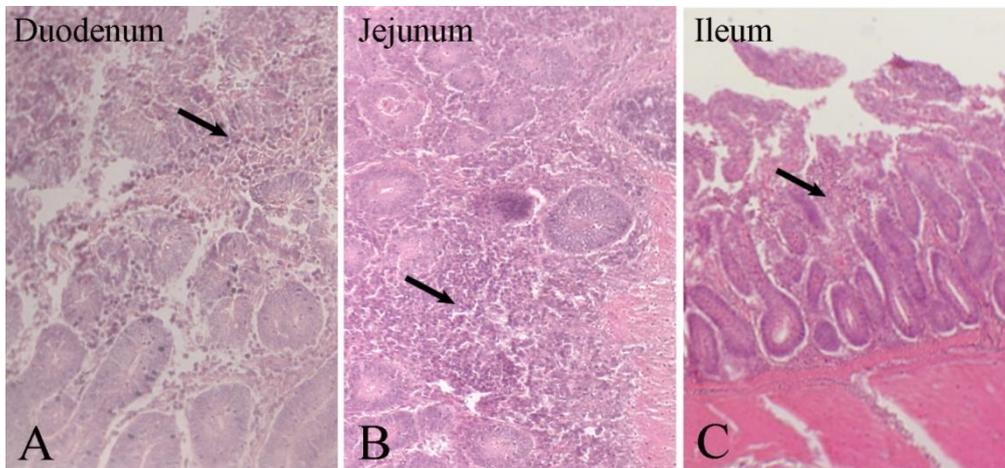
Breed	No. of chicken examined	No. of (+ve) cases	Prevalence (%)
Broiler (Cobb 500)	250	24	9.6
Sonali (Fayoumi × RIR)	260	16	6.2
Total	510	40	7.84 % (Overall)

**Table 3: Prevalence of necrotic enteritis in different seasons in chickens**

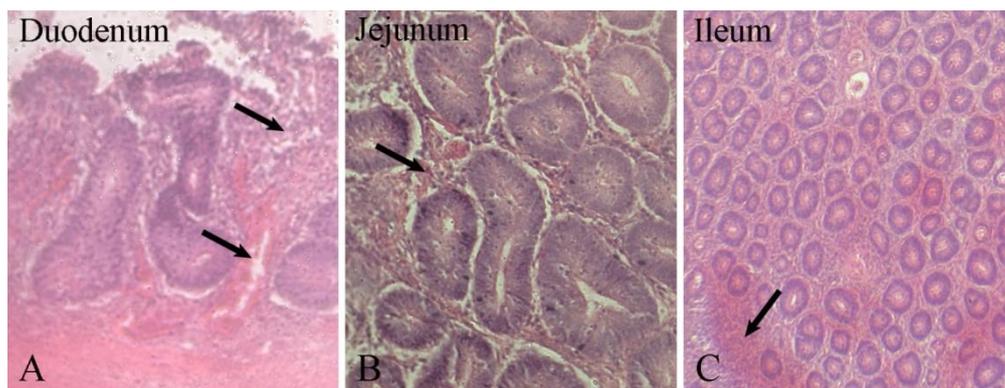
Summer (March-June)			Rainy (July- September)			Winter (October-February)		
Total chicken examined	Total infected chicken	Prevalence (%)	Total chicken examined	Total infected chicken	Prevalence (%)	Total chicken examined	Total infected chicken	Prevalence (%)
125	4	3.20	145	8	5.52	240	28	11.67



**Figure 1:** The characteristic necropsy findings of necrotic enteritis in chicken. A) Turkish towel appearance in duodenum; B) friable intestine; and C) orange color feces



**Figure 2:** Enterohistopathologic micrographs of intestine of necrotic enteritis affected Broiler chicken at 2-3 weeks of age showing (arrow) A) structural destruction of duodenum; B) coagulative necrosis in jejunum; and C) coagulative necrosis and disorganization of villi of ileum (20x)



**Figure 3:** Enterohistopathologic micrographs of intestine of necrotic enteritis affected Sonali chicken at 5-6 weeks of age showing (arrow) A) coagulative necrosis and congestion in duodenum; B) haemorrhage in jejunum; and C) congestion in ileum (20x)

## DISCUSSION

Necrotic enteritis is one of the world's most common and financially crippling poultry diseases affecting approximately 40% of commercial broiler flocks and causing 2% to 50% mortality (Loos Scarth, 2006). Necrotic enteritis can lead to a significant effect on performance through its ability to impair nutrient absorption, growth rate, and feed conversion as well as animal welfare (Paiva and McElroy, 2014). It has been estimated that NE costs the poultry

industry \$2-6 billion globally as a result of reduction in bird performance and disease treatment (McReynolds *et al.*, 2004; Van der Sluis, 2000; Timbermont *et al.*, 2011). In the present study, necrotic enteritis in commercial chickens (commonly used for meat purpose) was investigated based on clinical signs, necropsy and histopathological findings. The prevalence of NE was varied from farm to farm and the types of birds, also varied in different seasons. Overall prevalence of necrotic enteritis among examined cases was 7.84 % in this study, nearly same to the findings of Miah *et al.* (2011) where they reported 8 % and they used only the jejunal samples.

The genetics of birds appears to have some influence on susceptibility to NE as different lines of birds have different degrees of susceptibility (Jang *et al.*, 2013) to NE and this may result from subtle difference in immune responses to *C. perfringens* (Hong *et al.*, 2014; Kim *et al.*, 2014). This is the first time to compare the prevalence rate of NE between broiler and Sonali chickens, at least in Bangladesh. We found the prevalence of necrotic enteritis 9.6% in broiler chickens and 6.2% in Sonali chickens. It is seemed that the prevalence rate of NE in broiler chickens was around 1.5 times higher than in the Sonali chickens. Various factors may be involved to discriminate the prevalence rate between the two breeds of chicken. The physical, environmental, and feed changes that can predispose birds to NE have been documented (Williams, 2005; Allaart *et al.*, 2013; M'Sadeq *et al.*, 2015).

Generally, NE is not typically known as a seasonal disease, although prevalence of necrotic enteritis varies with season in chicken (Nairn and Bamford, 1967). Hermans and Morgan (2007) reported that the disease was most often reported during the months October to February in on chicken farms. Kaldhusdal and Skjerve (1996) suggested that univariate regression analysis in south-eastern Norway indicated that NE occurred more often during the months October–March than during the months April–September, whilst in Canada it mostly appeared in July–October (Long, 1973). In the United Kingdom, the peak incidence of NE is during winter with a lower incidence during the warmer seasons (Hermans and Morgan, 2007). In the present investigation, we found peak occurrence in winter season (11.67%), and the lowest incidence was in summer season (3.2%). The NE incidence also varied with season in turkey. Kaldhusdal *et al.* (2021) observed the peak occurrence among grow-outs hatched during February-March and the lowest incidence in turkeys hatched in July-August. Arias (2021) reported that prevalence of NE increases during the rainy seasons and in winter, when relative humidity is higher.

Coccidial infection is a well-documented predisposing factor for NE (Dierick *et al.*, 2021). Colonization of the small intestine by coccidia leads to the mucosal damage, which can provide a surface for *C. perfringens* to proliferate (Shane *et al.*, 1985). Lesions produced by *Eimeria brunetti* can be similar to those in necrotic enteritis, but uncomplicated coccidiosis is seldom as acute or severe (Fraser *et al.*, 1998). Coccidiosis can readily be confirmed by direct faeces or mucosal scrap examination under microscope, where insignificant numbers of coccidial organisms were identified.

The major clinical signs of the affected birds (diarrhoea, depression, huddling and sudden death) were more or less similar to those described by many authors (Al-Sheikhly and Al-Saieg, 1980; Calnek, 1997). The presence of wet droppings in the broiler house is a useful early warning sign that bird health is not as optimal as it could be (LaVorgna *et al.*, 2014). The most farmer's complaints were the quick wetting of litters. Wet litter is also sometimes an early indicator of the disease (Riddell and Kong, 1992). *Orange colored* feces including frothy *diarrhea* is characteristic of NE (Abd El-Hack *et al.*, 2021). Diarrhoea in such cases could result from a combination of fluid loss from localized inflammation and decreased fluid absorption due to disruption of the enteroepithelial barrier (Barker and Van Dreumel, 1993). Diarrhoea has been identified as a common clinical signs relating to CP infection among the poultry professionals (Carrier, 2000). Birds displaying clinical signs generally die within a few hours, and in acute form of NE, birds usually die without premonitory signs (Kocher and Choct, 2008).

Diagnosis of necrotic enteritis in Bangladesh is almost entirely depends upon the post-mortem examination. The small intestine of infected birds was friable, hyperemic, thin walled and dilated, *i.e.*, increased diameter (ballooning of the intestine) due to deposition of excess gas. The striking postmortem lesions found in the present study were markedly thickened enteric mucosa covered by tan-orange pseudo-membranes often referred to as a “Turkish towel” in appearance with haemorrhages occasionally. Such postmortem findings are documented in NE by different authors (Broussard *et al.*, 1986; Charlton, 2000; Vegad and Katiyar, 2003; Olkowski *et al.*, 2006).

The enterohistopathological findings of the present study are more or less similar to the findings described earlier by several authors (Charlton, 2000; Vegad and Katiyar, 2003; Olkowski *et al.*, 2006; Miah *et al.*, 2011; Gulbeena, 2013; Abid *et al.*, 2016) who reported necrotic enteritis in chickens. Histopathological examination showed a strong inflammatory reaction to *C. perfringens* of NE. The lamina propria was infiltrated and hyperemic with several inflammatory cells. These areas were edematous, permitting the extensive disorder of the structural integrity between the enterocytes and the lamina propria. NE is characterized by necrosis and inflammation of the intestine (Van der Sluis, 2000). Necrosis and coagulation of necrosis of the mucosa was observed in the small intestine. Hemorrhage or patchy congestion is present over all the lamina mucosa, particularly in the vicinity of the crypts. The crypts are usually

misshapen and inflated by pink mucus and necrotic cellular debris. The specific lesions in intestine and absence of significant number of *Eimeria spp.* on direct microscopic examination of feces and tissue debris clearly differentiated the NE from coccidiosis.

## CONCLUSION

This study provides an overall scenario of pathological lesions and prevalence of necrotic enteritis of Sonali and Broiler chickens in the Barind Tract of Bangladesh. Investigation of NE in commercial chickens based on the findings as stated above with certainly assist in proper diagnosis and control the diseases and to adopt effective strategies against the outbreak of necrotic enteritis.

**Conflict of Interest:** The authors declare that they have no conflict of interest.

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