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## Diagnosis of *Clostridium perfringens* infection (Necrotic enteritis) in a flock of Giriraja birds

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### Abstract

Necrotic enteritis is the bacterial disease of the intestinal tract of chicken caused by *Clostridium perfringens*, type A and C. The present study was done in the backyard chicken, presented to the veterinary dispensary, Jeelakarragudem, West Godavari district, Andhra Pradesh. A poultry farmer brought 2 Giriraja birds for the necropsy and with a history of mortality of 5-week-old birds @ one bird per day for 3 days continuously in a flock of 50 birds without showing any obvious clinical signs except for off feed and listlessness. Upon necropsy, the lesions found were ballooning of small intestines, turkish towel like appearance of the mucosa of the small intestine, distended caecum with blood-tinged contents and liver with foci of necrosis. The impression smears taken from cut portions of liver and intestines showed violet-coloured gram-positive rod-shaped organisms with Grams stain. With spore stain, greenish spores and brownish red to pink vegetative forms were observed. Conclusively, the organism is presumably diagnosed as *Clostridium* species and the disease as Necrotic enteritis.

**Keywords:** *Clostridium perfringens*, giriraja birds, spores staining, necropsy

### Introduction

Gastrointestinal diseases are an important concern to the chicken industry because of production losses, increased mortality, reduced welfare of birds and increased risk of contamination of poultry products for human consumption. Necrotic enteritis is a widespread disease in chicken, imposing a significant economic burden on the poultry industry worldwide. The causative agent of necrotic enteritis is *Clostridium perfringens*, a Gram-positive spore-forming anaerobe. Necrotic enteritis usually occurs in the chicken of about 4 weeks old and is found in all poultry-growing areas of the world [2]. Necrotic enteritis is usually described in broiler chickens; however, layers and turkeys are also affected by this condition [3].

The important risk factor for the development of necrotic enteritis in poultry is, an intestinal environment that favours the growth of *C. perfringens*. The nature of the diet is a key non-bacterial factor for necrotic enteritis. Diets with especially high levels of indigestible, water-soluble, non-starch polysaccharides predispose the birds to necrotic enteritis [5]. High dietary inclusions of animal protein, such as fishmeal, shrimp meal have also been reported to increase the incidence of necrotic enteritis [4]. Apart from the feed, any factor that could cause stress in chickens like immunosuppressive viruses of chicken could predispose them to necrotic enteritis, by altering the intestinal environment [11]. Furthermore, the best-known predisposing factor for necrotic enteritis is mucosal damage caused by coccidia species [19].

Necrotic enteritis may occur as an acute clinical disease or as a subclinical condition. The classical acute clinical form of the disease is characterized by a sudden increase in flock mortality, often without premonitory signs, although sometimes wet litter is an early indicator of the disease. The course of the disease is often peracute, with death in 1 to 2 hours and with mortality rates up to 50% [11]. In the subclinical form of *Clostridium perfringens* infection, no clinical signs and no peak mortality were observed but chronic intestinal mucosal damage leads to production losses from poor digestion and absorption, reduced weight gain [7]. During the subclinical form of infection, the intestinal damage can allow the bacteria to reach the bile duct and portal blood stream and leading to colonization in the liver resulting in cholangiohepatitis. Diseased livers are enlarged and are often red or white foci [17]. Although, clinical outbreaks of necrotic enteritis may cause high levels of mortality, the subclinical form of the disease is more important than the clinical form because it can persist in the flocks without any clinical manifestations, causing the greatest economic losses to the poultry industry [2].

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Gross lesions are commonly restricted to the small intestine of birds, but can also occur in other organs, such as the liver and kidney. Upon post-mortem examination, the duodenum, jejunum and ileum are usually distended, thin walled and filled with gas and when cut opened, confluent mucosal necrosis of large parts of the small intestine, covered with a yellow-brown or bile stained pseudomembrane layer is noticed [14]. Sometimes, ulcers may be present in the form of depressions on the mucosal surface, with discoloured, amorphous material adhering to the mucosal surface [6].

During the acute outbreaks of necrotic enteritis, birds do not present obvious external signs but lesions from dead birds help in finding the disease and thereby preventing the spread of the infection. In a flock of 50 five-week-old Giriraja birds, the owner reported death of at least a bird per day with no obvious clinical signs in the last 3 days. Out of which, two birds were brought for the post-mortem examination to diagnose the cause of death. The present study is aimed to diagnose the cause of death in the chicken by a combination of conventional bacteriological methods and pathological lesions in birds.

## Materials and Methods

### Necropsy examination and sample collection:

The two dead birds were opened for necropsy examination according to standard protocols [18]. The impression smears were collected on clean glass slides, aseptically and further processed for staining.

### Gram's Staining

Impression smears taken from the collected samples were stained with Gram's stain [12] to demonstrate the bacteria.

### Spores staining

Spores staining was performed according to the standard protocol [13] with the help of standard Schaeffer & Fulton's Spore stain kit (Himedia, Mumbai, India).

## Results and Discussion

The post mortem examination was performed on the dead birds. The post-mortem lesions were mostly confined to the small intestine. The small intestine is distended and filled with a foul-smelling brown fluid (Fig.1). The inner intestinal lining is covered by a brownish membrane called as pseudo membrane. The small intestine is greatly thickened due to extensive velvet-like/ turkish towel like necrosis of the intestinal mucosal lining (Fig.2). The intestinal mucosa is covered by a loose to tight yellow- green layer with deep cracks (Fig.3). The liver shows necrotic hepatitis with small, white, pinpoint like necrotic foci (Fig.4). Similar lesions were also observed by Lovland and Kaldhusdal (2001) [9]. According to Olkowski *et al.*, (2006) [14] the characteristic gross lesion of necrotic enteritis is the presence of pseudo-membrane attached to the intestinal mucosa, primarily the small intestine and the pseudo membrane may be partly or entirely detached from the viable mucosa, leaving behind small depression or a more extended smooth surface. The membrane may be white, yellow, green, brown or red. Yellow or green discoloration is most commonly found, with the affected gut segment dilated and soft with fluid contents, or turgid and rigid with dry and sparse luminal contents. A detached pseudo membrane is occasionally found in the gut lumen. Loveland and Kaldhusdal, (2001) [9] reported that birds dying with NE often show a dark liver with pinpoint

foci of necrosis, pale kidneys with prominent lobular outlines, and dark and dry pectoral musculature indicating dehydration. Long *et al.*, (1974) [8] opined that there are three main types of liver lesions in necrotic enteritis with the most common and most characteristic being necrotic hepatitis which is similar to our recordings.

Olkowski *et al.*, (2008) [15] reported that the pathogenesis in Necrotic enteritis is due to virulent *C. perfringens* strains that are able to secrete collagenolytic enzymes which damage the mucosa of the intestine by causing necrosis and thus exposing the (ECMMs) extra cellular matrix molecules. Martin & Smyth, (2010) [10] opined that because of the damage induced by the predisposing factors, the *C. perfringens* toxins and the collagenolytic enzymes, virulent *C. perfringens* strains are able to bind to exposed ECMMs, to colonize the intestine and thus to induce more severe lesions.



**Fig 1:** Marked distention of duodenum in necrotic enteritis affected chicken.



**Fig 2:** Pseudo membrane formation in the mucosa of duodenum of affected chicken



**Fig 3:** The intestinal mucosa is covered by a loose yellow- green layer



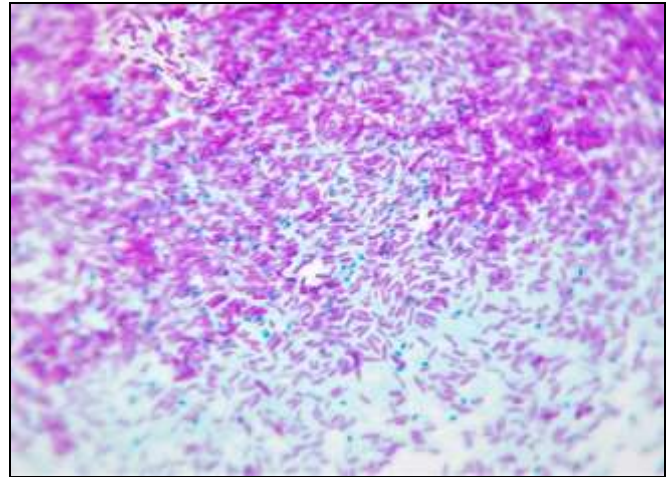
**Fig 4:** Necrotic hepatitis with whitish foci of necrosis in liver of chicken affected with necrotic enteritis.

The grams staining of intestinal mucosal impression smears revealed Gram positive rods (Fig.5) which were similar to the observations of Anderson *et al.*, (1983) [1] who opined that the differences in cell wall composition of Gram-positive and Gram-negative bacteria account for the Gram staining differences. Gram-positive cell wall contains a thick layer of peptidoglycan with numerous teichoic acid cross-linking, which resists the decolorization and hence stains blue.



**Fig 5:** Grams stain- The presence of gram positive, violet-coloured rods (100x).

The endospore staining of the impression smears revealed bright green spores and brownish red to pink coloured vegetative cells (Fig.6). Oktari *et al.*, (2017) [13] reported that the vegetative forms stain pink/red because they take up the counterstain (Safranin) while the endospores take up the green from the Malachite green. This is because, during smearing and heat fixing, the malachite green penetrates the endospore with the help of the heat from the steam, and during the water-rinse, the dye is not easily washed away and for the vegetative forms, the dye is easily washed away because of their fragile outer covering, hence they take up the last stain which is the counterstain, hence they appear brownish red to pink and thus stating similar observations to our study.



**Fig 6:** Spore staining- brownish red to pink vegetative forms and bright green spores (100x)

### Conclusion

From this study it can be concluded that the pathognomonic lesions in combination with simple staining techniques are sufficient for the presumable diagnosis of necrotic enteritis in the field conditions, where there is very little scope for higher diagnostic techniques. By following these techniques, the disease can be identified at the earliest and subsequently, the mortality in poultry farms can also be controlled.

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