

Coccidiosis and Hemorrhagic Necrotic Typhlitis in Broilers Fed without Antibiotic Growth Promoter

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Abstract

This study aims to determine the level of damage and the inflammatory response in the cecal epithelium of broiler chickens infected with *Eimeria* spp. The sample population consists of 39 broiler cadavers from Tabanan and Bangli Regencies, Bali, Indonesia, which showed clinical signs of weakness, drooping wings, and bloody stools before death. Subsequently, they were necropsied, and the swollen cecum tissue, with a color range of pink to black, was sufficiently harvested and placed in a pot filled with 10% neutral buffer formalin. After fixation for 24-48 hours, the sample was then processed routinely and stained with hematoxylin-eosin (HE) dyes. The histopathological examination revealed the occurrence of bleeding, epithelial desquamation, gland necrosis, macrophage infiltration, and various stages of *Eimeria* spp. development. The presence of *E. tenella* in the epithelium of the tissue was assumed to be associated with hemorrhage necrotic typhlitis.

KEYWORDS

Coccidiosis, Hemorrhagic Necrotic, Typhlitis, Broiler

INTRODUCTION

Several population statistics agencies have estimated an increase in the global human population to 9.6 billion by 2050. During this period, broiler production is also expected to experience a 121% growth to meet the increased need for animal protein sources (Mottet and Tempio, 2017). This predicted high demand is not in line with the prohibition on the use of antibiotics for growth promoters (AGP), as stated by Law no. 41/2014 Jo and 18/2009 on Livestock and Health. Antibiotics are drugs that can suppress the growth of pathogenic bacteria by increasing the population of the beneficial variants. They also stimulate the formation of antimicrobial compounds, such as free fatty acids and acidic substances to create an inconducive environment for the microorganisms. However, the addition of these drugs to feed is detrimental to livestock and humans because they cause resistance to bacteria. Another negative effect is that the residues in the animals' products, such as meat, eggs, and milk are harmful to the consumers (Abbas *et al.*, 2011; Nilsson *et al.*, 2012). This has led to the development of an ethical, healthy, and environmentally friendly meat production technique by the livestock sector. Changes in poultry production practices affect animal health and increase the rate of infection by several pathogens. They can also cause an increase in the incidence of coccidiosis, necrotizing enteritis, impaired nutrient absorption, low feed conversion efficiency, and death (Allen and Feterrer, 2002).

Coccidiosis is an enteric parasitic disease caused by several species of protozoa in the genus *Eimeria*. It is also one of the most common and economically important poultry infections globally (Olanrewaju and Agbor, 2014; Shirley *et al.*, 2005). The disease often occurs after the ingestion of sporulated oocysts by susceptible hosts and is characterized by diarrhea, emaciation, drooping wings, poor growth, as well as increased morbidity and mortality. Broilers reared in wet cages with high density can exacerbate its incidence (Sharma *et al.*, 2013). There are seven species of *Eimeria*, including *Eimeria tenella*, *E. maxima*, *E. necatrix*, and *E. brunetti*, which are highly pathogenic, while *E. acervulina*, *E. mitis*, and *E. praecox*, are less pathogenic (Gharekhani *et al.*, 2014; McDougald and Fitz-Coy, 2008). Furthermore, each of them has different preferences in the gastrointestinal tract to affect growth performance and gut health (López -Osorio *et al.*, 2020). *E. tenella*, which lives in the mucous membrane of the cecum during replication causes necrosis of the epithelium, bleeding, and impairs intestinal health (Zaman *et al.*, 2012). The cecum is the main compartment in the intestine for bacterial fermentation as well as a reservoir of pathogenic bacteria, and toxins that can cause oxidative stress after entering the bloodstream (Cason *et al.*, 2007). It also plays an important role in producing beneficial metabolites, such as vitamins, volatile fatty acids (VFA), lactic acid, and antimicrobial compounds through fermentation (Shang *et al.*, 2018). The majority of *Eimeria* spp. infects poultry birds aged 3-18 weeks and can cause death in chicks. In poor cage condi-

tions, mixed infections are common among the animals, and it is characterized by the occurrence of 2 or more diseases simultaneously (McDougald and Fitz-Coy, 2008).

Due to the prohibition on the use of AGP in poultry feed, it is important to develop another coccidiostat that can control the disease as well as prevent resistance and residues in eggs and meat. Several studies reported that non-sporing bacterial probiotics and natural ingredients from plants can be used as substitutes for antibiotics as well as growth-promoting additives. Cao et al. (2018) stated that the administration of probiotics using *Bacillus* spp. can protect broilers from infection and improve their performance. Wiedosari and Wardhana (2017) also revealed that adding the powder and essential oil of *Artemisia annua* leaves to the feed of animals infected by *E. tenella* reduced the number of fecal oocysts. Therefore, this study aims to determine the level of damage and the inflammatory response in the epithelium of cecum infected with *Eimeria* spp.

MATERIALS AND METHODS

Animals

Many broilers chicken are farmed in Tabanan and Bangli districts, Bali Indonesia because the temperature and humidity strongly support the growth of chickens. However, poultry production in an effort to increase the protein needs of Indonesian people is often threatened by various diseases, one of which is the parasitic coccidiosis disease. This disease arises due to restrictions on the use of antibiotics for growth promoters (AGP) in the feed given to broiler chickens.

Samples

The sample population consists of 39 cadaveric broilers from several villages in Tabanan and Bangli Regencies. Broiler chickens

before death show clinical sign of weakness, drooping wings and bloody stools. The feed given to broilers does not contain an antibiotic growth promoter (AGP). Broiler cadaver samples aged between 2-4 weeks were then necropsied using a predetermined procedure followed by anatomical and histopathological examination to determine the intensity of damage and the inflammatory response of the cecum epithelium.

Histopathological examination

The cecum tissue is taken sufficiently and then put into a small pot that has been filled with 10% neutral buffer formalin. The formalin-fixed tissue was then processed and made histological preparations using hematoxylin-eosin (HE) dye (Kiernan, 2015). The stained cecum tissue preparations were then examined under a light microscope. The results of the examination of anatomical and histopathological pathological changes in the cecum tissue were analyzed descriptively qualitatively.

RESULTS AND DISCUSSION

Anatomical and pathological examination of the samples from January to February 2022 showed that there was swelling in the cecum with a pink to blackish-red coloration. There was also visible fresh blood in the lumen as well as necrosis on its mucosal surface. A total of 24 cadavers were obtained from Tabanan Regency, where 19 and 5 were aged 2-3 and 3-4 weeks, respectively. Meanwhile, 15 samples were collected from Bangli, where 12 and 3 of them were within the age range of 2-3 and 3-4 weeks, respectively. Table 1 shows the origin, population, age as well as the clinical signs of weakness, drooping wings, and bloody stools experienced by the broilers. Histopathological examination of the cecum tissue revealed that there was bleeding, epithelial desquamation, gland necrosis, focal macrophage infiltration, and various stages of *E. tenella* development, as shown in Figure 1.

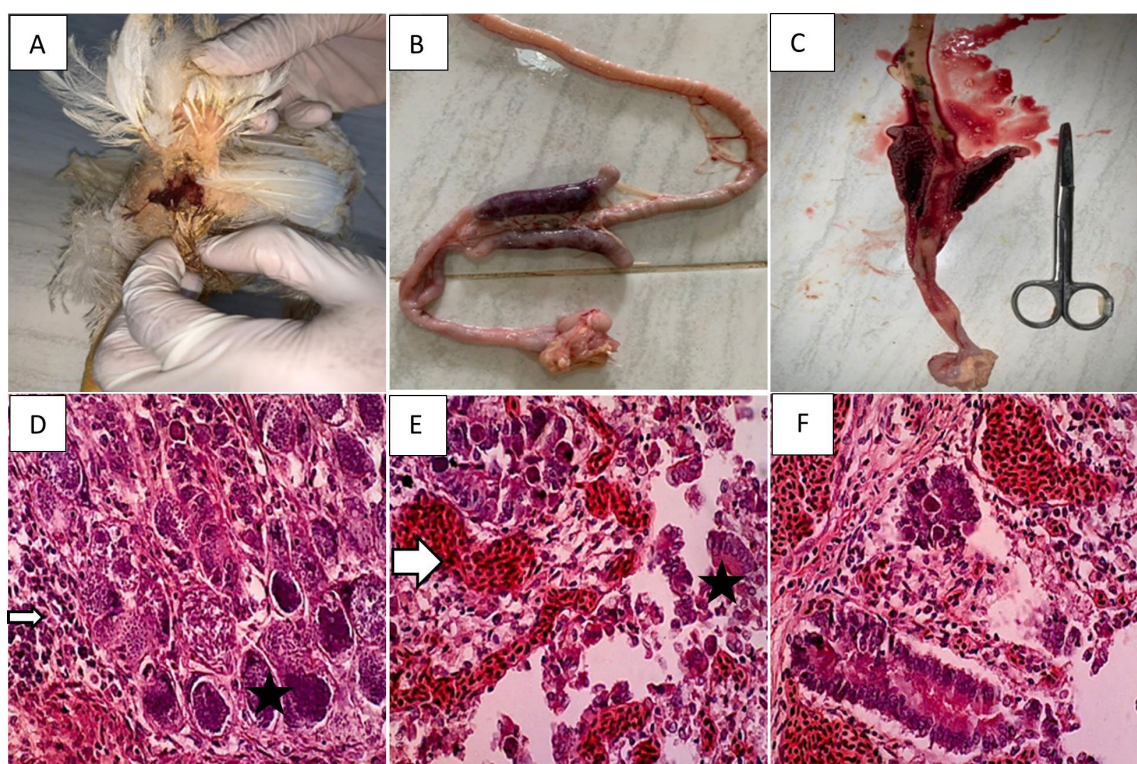


Figure 1. Photomicrograph of the cecum from broilers infected by *E. tenella*. (A) clinical signs of bloody stools, (B) a swollen cecum with dark red color, erythrocytes in the lumen, (C) several schizont formations and focal infiltration of macrophages in the caecal mucosa, (D) oocyst formations (thin arrows), hemorrhage (bold arrow) and epithelial desquamation (star), (E) necrosis of the villi. HE stained and x400 magnification.

Table 1. Origin, number, and age of broiler cadaver samples

No	Sample	Origin	Age (week)		Total (birds)
			2-3	3-4	
1	Broiler	Tabanan	19	5	24
2	Broiler	Bangli	12	3	15
Total					39

In Indonesia, coccidiosis occurs every year due to the tropical climate with temperatures ranging from 21-30°C and humidity levels that favor the sporulation of coccidia. Furthermore, between January and February in Tabanan and Bangli, the disease is more prevalent among broilers within the age range of 2-3 weeks compared to others aged 3-4 weeks. Although *Eimeria* spp can infect all age groups, it is often found at a younger age due to immature immune systems (Musa et al., 2010). Several studies revealed that the infection is also influenced by unfavorable weather conditions and its management system (Etuk et al., 2004). High environmental humidity plays an important role in the completion of the parasite's life cycle (Ahad et al., 2015). Poor management practices, such as leaving the bedding wet during the rainy season and high density also increases the occurrence of the infection (Khan et al., 2006).

Several studies reported a high rate of mortality in broilers fed without AGP in both regencies. In the United States, coccidiosis control was carried out through vaccination programs as well as the addition of chemical coccidiostats as ionophores substitute in feed. The program led to an increase in the prevalence of the disease along with necrotic enteritis (Cervantes, 2015; Newman, 2018). A comparative study on the economic impact of broilers fed with and without AGP showed poor performance in the untreated samples, which caused high production costs during the rearing period (Cardinal et al., 2019). Bozkurt et al. (2014) revealed that infected animals that were fed without the administration of the drugs did not show any clinical signs of coccidiosis. However, they experienced weight loss and decreased feed intake along with a poor conversion ratio compared to others that were given treatment containing ionophores, enzymes, probiotics, and essential oils 14 days after infection (Bozkurt et al., 2014). Belote et al. (2018) stated that the use of enramycin as a growth promoter reduced swelling and infiltration of inflammatory cells in the lamina propria when compared to poultry fed with non-AGP feed but were equally infected with *Eimeria* spp and *Cl. Perfringens*.

Histopathological changes were observed in broilers aged 2-3 and 3-4 weeks with clinical signs of bloody stools, which were caused by coccidiosis and hemorrhagic typhlitis. The infection process started with the ingestion of a sporulating oocyst. The effect of coccidiosis on individual health and productivity depends on the level of severity. Furthermore, in the digestive tract, *E. tenella* destroys the epithelial lining of the host mucosa and then invades the enterocytes to initiate a multistage replication. This causes pathological changes, such as increased cell permeability, leakage of nutrients and plasma proteins, impaired digestion, and poor protein absorption (Nabian et al., 2018; Madlala et al., 2021). It also leads to morphological transformation in the intestinal mucosa in the form of a decrease in the absorption surface area (Nabian et al., 2018) as well as discomfort, and reduced productivity (Madlala et al., 2021). In the intestinal mucosa, the bacteria have an immunosuppressive effect, which causes susceptibility to various diseases (Kim et al., 2019). Peterson and Artist (2014) stated that approximately 70% of the cellular immune system of poultry is located in the digestive tract. Therefore, it is necessary to pay attention to gut health while maintaining the working sys-

tem of gut-associated lymphoid tissue (GALT) that are located in the epithelium, lamina propria, and Payer's patches.

Intestines of broilers play an important role in fermentation, transportation, absorption of water and nutrients (Stanley et al., 2014). Severe *E. tenella* infection often damages the intestinal structure (Zhou et al., 2010), reduce feed intake (Morris et al., 2007), as well as increase the risk of secondary infection and death (Wang et al., 2018). Several studies revealed that the parasite disrupts the cecum microbiota (Macdonald et al., 2017), which leads to intestinal dysfunction (Bortoluzzi et al., 2019). Furthermore, the results showed that its invasion damaged the villi of the cecum, thereby leading to epithelial sloughing, bleeding, and macrophage infiltration in the necrotic area. Desquamation of the cecal affects the surface area of the goblet cells as well as their ability to secrete glycoproteins. This secretion helps to prevent foreign pathogens and toxic materials from affecting its epithelial surface (Hansson and Johansson, 2010; Round et al., 2012). It can also stimulate the regeneration process and improve the absorption function of the cecum epithelium (van der Flier and Clevers, 2009; McCauley and Guasch, 2015). Laurent et al. (2001) stated that a decrease in goblet cells along with epithelial desquamation affected the immune response of the intestinal mucosa. Furthermore, macrophages are the main inflammatory cells involved in regulating the adaptive immune reaction by interacting directly with parasites during infection (Heriveau et al., 2000). The observation of focal macrophage infiltration and clinical signs correlates with the severity of necrosis in the cecum epithelium.

AGP substitutes in the form of herbs, probiotics, and enzymes have been used as animal feed additives, but in limited volume. Several studies reported that they can improve the productivity and health of animals. The addition of powder and essential oil of *Artemisia annua* leaves in the feed of broilers naturally infected by *E. tenella* reduced the damage to intestinal tissue compared to the untreated group (Wiedosari and Wardhana 2017). This was because the additives contain high antioxidants and have strong anti-inflammatory properties in suppressing the infection (Del-Cacho et al., 2010). The use of *Bacillus amyloliquefaciens* as a probiotic can prevent coccidiosis by stimulating the local digestive tract defense system, as characterized by mild lesions in the intestinal villi. These findings indicate that the broilers were resistant to *Eimeria maxima* infection (Tsukahara et al., 2018).

CONCLUSION

Based on the observations of clinical signs, as well as anatomical and histopathological pathological changes, the cadaveric broilers from Tabanan and Bangli Regencies had coccidiosis and hemorrhagic necrotic typhlitis with severe intensity.

CONFLICT OF INTEREST

Authors declare that they have no conflict of interest.

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