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Case report

E. coli Contamination of Drinking Water with Concurrent Coccidiosis Illness caused by a High Litter Moister at a Majalengka Broiler Chicken Farm

Kontaminasi E. coli di Saluran Air Minum dengan Koinfeksi Coccidiosis yang Dipredisposisi Kelembaban Alas Kandang di Peternakan Ayam Broiler di Majalengka

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Abstrak

Kolibasilosis dan koksidiosis termasuk penyakit yang sering menyerang unggas komersil. *Eimeria* sp. Koksidiosis adalah penyakit pencernaan yang disebabkan oleh *Eimeria* sp., protozoa yang bersifat patogen, sementara *Escherichia coli* adalah bakteri komensal yang bersifat oportunistik penyebab kolibasilosis. Kolibasilosis umumnya terjadi sebagai infeksi sekunder dari infeksi penyakit lain, misalnya koksidiosis. Penyakit kolibasilosis dan koksidiosis menyebabkan pertumbuhan ayam terhambat. Penelitian ini dilakukan untuk mengetahui gambaran patologi pada kasus kolibasilosis dan koksidiosis, serta faktor predisposisi yang dapat meningkatkan risiko kejadian penyakit tersebut. Diagnosis kolibasilosis dilakukan berdasarkan temuan gejala klinis, patologi anatomi, didukung dengan analisis cemaran *E. coli* pada air minum untuk mengetahui sumber infeksi. Diagnosis koksidiosis dilakukan berdasarkan temuan gejala klinis, temuan patologi anatomi, dan didukung oleh perhitungan OPG pada feses menggunakan metode apung. Hasil penelitian ini menunjukkan kolibasilosis menimbulkan patologi berupa adanya peritonitis yang ditandai dengan terbentuknya eksudat fibrinus pada peritoneum, dan adanya peradangan pada kantung udara. Sementara koksidiosis menyebabkan adanya lesi hemoragi pada saluran usus halus dan sekum. Pipa air yang berkerak dan ditumbuhi biofilm dapat menjadi predisposisi kejadian kolibasilosis, karena lapisan biofilm dapat menjadi tempat bertumbuhnya *E. coli* dan menyebabkan disinfeksi air minum tidak optimal. Alas kandang yang lembab dapat mendukung sporulasi ookista *Eimeria* sp. sehingga meningkatkan risiko koksidiosis. Selain itu sekam yang lembab juga mendukung pertumbuhan agen penyakit lain seperti bakteri dan jamur.

Kata kunci: ayam pedaging; koksidiosis; kolibasilosis; patologi; predisposisi

Abstract

Colibacillosis and coccidiosis are diseases that often infect broiler chicken. Coccidiosis is a digestive disease caused by *Eimeria* sp, while *Eimeria* sp. is a pathogenic protozoa. Coocidiosis itself caused by *Escherichia coli (E. coli)*, an opportunistic commensal bacterium. Colibacillosis often occurs as a secondary infection from other infectious diseases, such as coccidiosis. Colibacillosis and coccidiosis cause stunting in chicken. This study was conducted to determine pathological features in colibacillosis and coccidiosis cases in

broiler, as well as predisposing factors that can increase the risk of the diseases. The diagnosis of colibacillosis was made based on findings of clinical symptoms, anatomical pathology, supported by analysis of *E. coli* contamination in drinking water to determine the source of infection. The diagnosis of coccidiosis was made based on clinical symptom findings, anatomical pathology findings, and supported by OPG calculations in feces using the floating method. The results of this study showed that colibacillosis causes pathological changes including peritonitis which is characterized by the formation of fibrinous exudate in the peritoneum, and inflammation of the air sacs. Coccidiosis causes hemorrhagic lesions in the small intestine and cecum. Water pipes that are corroded and overgrown with biofilms can become predispose factor to colibacilosis, because the biofilm layer facilitate the growth of *E. coli* and cause ineffective disinfection of drinking water. On the other hand, moist litter can promote *Eimeria* sp. oocysts sporulation; thereby increasing the risk of coccidiosis. In addition, moist litter also promote the growth of other disease agents such as bacteria and fungi.

Keywords: broile; coccidiosis; colibacillosis; pathology; predisposition

Introduction

Broiler chicken farming is a broiler fattening business starting from the age of one day (DOC) until the broiler chickens are ready for harvest at the age of four to five weeks. Although broiler chicken possesses high rate productivity and efficiency, there are several challenges, such as diseases or environmental problem. One of the most prevalence disease agents that often infects broilers being *Escherichia coli* (*E. coli*) bacteria. Term used to describe disease caused by this bacterial infection is called colibacillosis. This disease can be local or systemic infection. Manifestations of colibacillosis in poultry can be found in the form of egg yolk infection, omphalitis, colisepticemia, air sacculitis, enteritis, peritonitis, swollen head syndrome, and salpingitis (Kabir, 2010; Koutsianos *et al.,* 2020; Nolan *et al.,* 2020). Even though the mortality caused by this disease is not high, chickens infected with *E. coli* show stunted growth (Panth, 2019), which can reduce the productivity of the farms.

E. coli belongs to the Gram-negative bacteria, which have characteristic bacil shaped, possess a flagella, are not acid-resistant, and unable to form spores. These bacteria are able to grow under both aerobic and anaerobic conditions (Pudjiatmoko, 2014; Koutsianos *et al.,* 2020). These bacteria are not resistant to dry conditions and can be killed easily with a disinfectant. *E. coli* can be killed at temperature of 60 ºC for 30 minutes. However, *E. coli* excreted in the feces can survive for several days to several weeks (Pudjiatmoko, 2014).

Generally, *E. coli* classified into two types based on the pathogenicity, *E. coli* commensal bacterial strains and *E. coli* pathogenic bacterial strains (Filho *et al.,* 2015), or in poultry known as Avian Pathogenic *E. coli* (APEC). While APEC are highly pathogenic, *E. coli* commensal bacterial strains are basically part of the normal microflora that can be found in the digestive tract of healthy birds. However, *E. coli* are an opportunistic bacterium, which under certain conditions can grow to become invasive. *E. coli* can become a pathogen when the amount in the digestive tract increase, or if they were found in an organ that is not its natural habitat (Amyati, 2008).

Colibacillosis often occurs as a secondary infection, co-exist with other diseases caused by viruses, bacteria, or parasite. In addition, colibacillosis can also occur as a manifestation of poor environmental management (Panth, 2019). One of disease that is commonly found to occur along with colibacillosis, and might also be a predisposing factor for the disease is coccidiosis (Kabir, 2010; Panth, 2019).

Coccidosis is a digestive disease in poultry caused by parasitic infection from the phylum Apicomplexa, more precisely from the genus *Eimeria*. Several *Eimeria* species are known often cause disease in poultry, such as *E. acervulina*, *E. maxima*, *E. tenella*, *E. necatrix*, *E. brunetti*, and *E. mitis* (Mesa-Pineda *et al.,* 2021). Each of these species has different predilection sites, and causes different manifestations (Fatoba & Adeleke, 2018; Mesa-Pineda *et al.,* 2021).

Morbidity rate of coccidiosis can reach up to 100%, with mortality rate ranging up to

40-60% (Zheng *et al*., 2023). Chicken infected by *Eimeria sp*. can be characterized by bloody diarrhea, ruffled feathers, pale comb, drowsiness, and dehydration (Fatoba & Adeleke, 2018). Economically, coccidiosis potentially causes great impact on productivity due to impaired feed intake and nutrient absorption, leading to poor productive performance (Mesa-Pineda *et al*., 2021).

Coccidiosis might occured alongside with colibacillosis, which often cause worsen chicken health condition and increase cost required for treatment. Management and environmental possess significant role as risk factor for the diseases. Indonesia which located in equator which have relatively high temperature and humidity environments all year. According to Badan Meteorologi, Klimatologi, dan Geofisika (Indonesia's National Weather Agency) along May 2023 the average air humidity were recorded more than 75%, and the average temperature can reach as high as 35°C. This condition might increases the risk of stress on broiler chicken, as well as incidence of diseases (Teyssier *et al*., 2022; Liao *et al*., 2024). The important key to encounter environment challenges is through good management meassure. It is well-known that management is a crucial factor affecting broiler producticity. While highly pathogenic diseases posses greater threat towards broiler industry, management often being the predisposing factor that might increases the risk of diseases occurrence. However, due to lack of understanding the significancy of environment control through management, this factor often ignored and considered less important. One of important management factor affecting incidence of Coccidiosis being the quality of litter. In high temperature and humidity environment, managing quality of litter might be a challenge. In higher temperature, chickens tend to drink more water, causes watery droppings, thus litter moisture might increases more rapid (Alzarah *et al*., 2021). Rarely adding litter top-up often lead to moist litter that increases risk of *Eimeria sp*. growth in broiler house. Other than management in controlling environment, management in controlling biosecurity is just as important. Managing biosecurity is not limited to preventing disease through traffic restriction and disinfecting house environment, but also through meassured water treatment program (Jacobs *et al*., 2019; Ebrahimi *et al*., 2024).

Materials and Methods

This research was conducted in May 2023 and took place at a broiler farm owned by resident in Majalengka District, Sumedang Regency, West Java. The farm is two story chicken coops, with a total population of 19,700 birds. The research was carried out by determining the type of disease based on a pathological diagnostic approach, by analyzing clinical symptoms and anatomical pathology through necropsy, and supported by microbiological examination as a method for detecting disease agents (Qandoos *et al.,* 2021; Xiao *et al.,* 2023). As a support for the diagnosis, data collection was carried out on farm records, including chicken body weight, feed consumption, number of mortalities, vaccination history, and medication history. Evaluation of the environmental conditions of the farm was carried out to explain possible predisposing factors for the emergence of disease on the farm, so that it can be used as a reference for recommendations for treatment and prevention programs for farmers.

Necropsy was performed on three broilers aged 18 days and their liver and heart were taken as samples for microbiological tests. In addition, water sample from the water reservoir and nipple water samples were taken due to suspicion of contamination of *Escherichia coli* bacteria in drinking water within alarming number. Feces samples were taken for detection of *Eimeria* sp. oocysts. Furthermore, the samples were brought to the laboratory of PT. New Hope Farm Indonesia for laboratory testing. Source water and nipple water samples were tested using bacterial culture on MacConkey's media to count number of colony forming unit formed per mL for each sample. Each sample then incubated at 37 ºC for ± 24 hours. Feces samples were tested using flotation test for detecting and counting number or oocysts per gram of feces (Qi *et al.,* 2020).

Results And Discussion

The necropsy results showed an infection in the respiratory tract, which was indicated by

the presence of exudate on the trachea (Figure 1), and white spots on the chicken's air sacs which indicated inflammation of the air sacs. Inflammation of the air sacs is a common pathological finding in colibacillosis (Abu Daud *et al.,* 2014). Air sacculitis due to colibacillosis will appear thick, cloudy, and in the more severe conditions exudate might be formed (Panth, 2019). Air sacculitis might cause abnormal breathing sounds, such as rales, sneezing, or coughing (Panth, 2019). This is match with the results of the anamnesis from breeder who stated that rales were heard in some chickens, but there were not many of them and they were only heard occasionally. Air sacculitis can also be found in cases of complex chronic respiratory disease (CCRD) caused by *Mycoplasma* infection along with by *E. coli* co-infection (Gowthaman *et al.,* 2013; Kamaruzaman *et al.,* 2021).

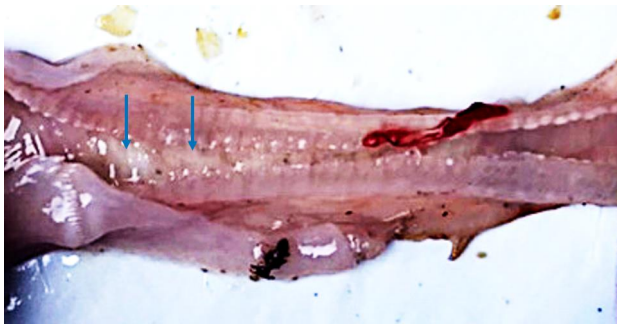

Figure 1. Exudate found in trachea (blue arrow).

The most distinctive finding leading to the diagnosis of *E. coli* was peritonitis, which was characterized fibrinous exudate build up in the peritoneum (Figure 2). Peritonitis is a typical pathological finding in colibacillosis cases (Panth, 2019). Peritonitis in colibacillosis cases is a form of lesion from local infection (Panth, 2019). Apart from peritonitis, in several cases of colibacillosis, salpingitis, enteritis, perihepatitis, and pericarditis can also be found (Koutsianos *et al.,* 2020).

Figure 2. White spots found on air sac (green arrow).

Pathological lesions which led to the diagnosis of coccidiosis were also found, such as the presence of hemorrhage in the small intestine and cecum. Supported by the presence of reddish feces and feces containing blood, these findings indicate an infection of *Eimeria* sp. in the digestive tract (Cui *et al.,* 2017; Mesa-Pineda *et al.,* 2021). Inflammation of the cecum and ileum are lesions commonly found due to *Eimeria tenella* infection (Figure 3 and Figure 4) (Cui *et al.,* 2017).

Figure 3. Hemorrhage found on ileum (purple arrow).

Figure 4. Hemorrhage found on cecum (black arrow).

Eimeria sp. infection cause inflammation and damage to the epithelial lining of the digestive tract, release of meront and oocysts of *Eimeria* sp. from intestinal epithelial cells. This process causes rupture and exfoliation of cells in the intestinal villi, lead hemorrhagic lesions to erosion in the digestive tract (Mesa-Pineda, 2021). In addition, this decreases the intestinal mucosa thickness and the height of the intestinal

villi as well, as a result absorptive surface area of the intestines decreased (Nabian *et al.,* 2018). The decrease of absorptive surface area reduces water and feed absorption, causing chickens to experience dehydration and impaired growth (Nabian *et al.,* 2018; Greenacre *et al.,* 2021).

Supporting examination were carried out by bacteria culture on MacConkey agar media. MacConkey agar is a selective bacterial growth medium which inhibits the growth of Gram-positive bacteria, and is able to differentiate Gram-negative bacteria based on lactose fermentation activity (Wanger *et al.,* 2017). Gram-negative bacteria which capable of fermenting lactose, such as *E. coli*, will grow to form pink colonies due to the production of acid during lactose fermentation process, which then reacts with the neutral red indicator contained in the agar. While Gram negative bacteria which are unable to ferment lactose such as *Salmonella* sp. and *Shigella* sp., will grow to form transparent colored colonies.

The result of culture from nipple water samples on MacConkey media shows bacterial growth exceeding 300 colonies. These results indicate high contamination of *E. coli* in nipple water, which exceed the tolerable and considered safe level of *E. coli* contain in poultry drinking water that is 50 CFU/mL (Ningtyas *et al*., 2022). In other hand, interesting result are shown in the culture result of source water sample which does not show any *E. coli* contamination. This indicates that the main critical point that predisposes to *E. coli* infection on the farm is in the drinking water pipes and nipples (Table 1). The occurrence of *E. coli* growth in large numbers in drinking water pipes can be triggered by the presence of scales or the formation of biofilms on the walls of the water pipes.

Biofilms are an accumulation of microorganisms attached to any surface, which in this case is drinkin water lines, and embedded in a polymeric matrix produced by their colonies (Milanov *et al.,* 2017). Bacterial colonies that grow in the biofilms are difficult to penetrate by chlorine, therefore adding chlorine as water treatment program might not provide satisfactory results. For this reason, it is necessary to dissolve the biofilm layer first, so that chlorine agent able to penetrate through bacterial colonies and prevents further growth then the quality of drinking water may be improved. To dissolve the crusts and biofilm along the drains and nipples, flushing can be done using H_2O_2 (Khopsoh *et al.,* 2022) or using citric acid (Tsai *et al.,* 2003; Lawrence & Lawrence, 2021).

Bacterial attachement to a surface might affected by roughness of the surface. Surface roughness increases the surface area available for bacterial attachment and provides protection from water shearing force, therefore can increases biofilm formation (Yu *et al*., 2016). Thereby, routine water lines program required to prevent crusts in water lines due to deposition of medicine or chlorine residual, which might increases the surface roughness, thus prevent biofilms built-up. However, due to broiler management require chickens to be provided with water and feed in *ad libitum* manner, it is difficult to implement routine flushing program using H_2O_2 or citric acid as these are irritating and corrosive product that might impair gastrointestinal health (Samaha *et al*., 2013). Thus, H_2O_2 or citric acid might only used for flushing water line during empty house period. Iodine often used in poultry industry as an alternative less irritant disinfectant required to flush water lines daily and following medication or vaccination through drinking water. While Iodine are less reactive and irritant compared to H_2O_2, this agent still provides adequate disinfection properties yet relatively safe for chicken gastrointestinal health (Ledesma *et al*., 2018; Jacob *et al*., 2020).

Table 1. Culture result on MacConkey agar.

Media	Source Water		Nipple Water	
	Result (CFU/mL)	Standard (CFU/mL)	Result (CFU/mL)	Standard (CFU/mL)
MacConkey	0	<50	>300	<50

Note: The colony forming units of water sources and nipples sample are compared with reference standard (Ningtyas *et al*., 2022).

Supporting diagnosis of coccidiosis were carried out by applying floatation test principles to count the number of *Eimeria* sp. oocysts in the feses. This method apply the principles of different density between *Eimeria* sp. oocysts which smaller compared to salt solution, which allows *Eimeria* sp. oocysts to float (Qi *et al*. 2020). Higher salt solution concentration results in higher solution density, allows more oocysts to float compared to lower concentration solution, thus provides better oocysts recovery rate (Qi *et al*. 2020). The oocyst were later counted in the McMaster egg counting chamber. The test reveals positive results for *Eimeria sp*. oocysts with the number of oocysts as much as 1.200 OPG (oocysts per gram) (Table 2). This number is classified as a mild infection. However, the clinical findings of reddish feces and bloody stools along with the pathological findings of intestinal and cecal hemorrhage related to the presence of *Eimeria*.

Litter condition which agglomerated indicates litter was moist and had not been replaced for weeks. This finding suggests the predisposing factor for the increasing *Eimeria* growth in the chicken house. According to Djara *et al*. (2020), litter moisture is a significant factor that affects sporulation rate of *Eimeria* sp. oocysts. Later, Liao *et al*. (2024) described *Eimeria sp.* infection tend to increased as the envronment's temperature and humidity increased. Bad litter condition often become risk factor for not only *coccidiosis*, but also bacterial infection due to high moisture provides pleasureable condition for bacterial growth. For this reason, it is necessary to gradually add or replace litter in farm to prevent moist built-up in the litter.

Conclusion

Chicken in the farm were infected by *Escherichia coli* and by *Eimeria* sp. Poor drinking water quality is a predisposing factor that can increase the risk of colibacillosis. Flushing the water pipes is necessary to shed the biofilm layer so that water disinfection becomes more effective. Moist litter conditions also support the growth of bacteria such as *E. coli* and *Eimeria* oocyst sporulation, so it is necessary to add or replace the litter.

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Table 2. Culture result on MacConkey agar.

Result (OPG)	Reference value (Arsytahlia *et al.,* 2019)		
	Mild infection (OPG)	Moderate infection (OPG)	Severe infection (OPG)
1.200	<20.000	20.000–60.000	>60.000

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