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Case Report

Identification and Pathological Finding of Colisepticemia in Broiler

Rusmin Indra[1], I Made Kardena[2], I Gusti Ketut Suarjana[3]

[1]Student of Veterinary Medicine, Udayana University
[2]Department of Pathology, Faculty of Veterinary Medicine, Udayana University
[3]Department of Microbiology, Faculty of Veterinary Medicine, Udayana University

Corresponding author: Indrarusmin@gmail.com

Abstract

The chicken was sick for 6 days, weak and disrupted growth. Necropsy confirmed that several organs was bleeding and containing fibrin exudation. The results of histopathological examination showed the presence of necrotic encephalitis, hemorrhagic tracheitis, hemorrhagic and necrotic pneumonia, hemorrhagic epicarditis and myocarditis, hemorrhagic enteritis, hemorrhagic and necrotic hepatitis, also hemorrhagic and necrotic glomerulonephritis. Microbiological examination confirmed the discovery of Eschericia coli bacterial agents that can hemolyze blood in the heart, lungs, and intestines. Parasitological examination confirmed that no parasitic agent was involved in this case. From this case study, it can be concluded that the single agent that causes pathological changes was found in the form of pathogenic E. coli bacteria (colisepticemia) which was exacerbated by unfavorable farming conditions.

***Keywords**: Escherichia coli, colisepticemia, broiler, pathological findings*

Introduction

Broiler is one of the meat-producing livestock that is useful for fulfil the needs of animal protein which is in high demand by the public (Septiani et al., 2016). The development of broiler farms is increasing rapidly with the increasing demand for chicken meat so that maintenance management must be very concerned. Livestock disease is one of the obstacles in livestock business. Disease greatly affects the growth of chickens and can also cause death and loss (Nolan et al., 2013). One of the common diseases in broiler farms is colibacillosis.

Colisepticaemia and colibacillosis are diseases caused by infection with the pathogenic agent called *Escherichia coli*. Colibacillosis is a common bacterial disease of economic importance in poultry through decreasing the infected birds productivity, increase mortality, condemnation of infected carcasses at slaughter, prophylaxis, treatment cost (Lutful, 2010) and reported worldwide. Colibacillosis in poultry is generally caused by Avian Pathogenic *E.coli*. Colibacillosis in poultry can affect all age groups and other types of poultry. Colibacilosis often occurs in

areas that are less clean and have substandard sanitation. Given the importance of knowledge about this disease, the authors try to convey the results of the pathological findings in Colibacillosis cases so that they can be used as references in the future.

Case Report

Signalement, Anamnesis, and Physical Examination

The case comes from Nyalian, District of Banjarangkan, Klungkung Regency. The number of chickens that are kept is as much as 5000. Until the 16th day of rearing, as many as ± 150 chickens died and during a visit to the farm as many as ± 50 chickens were sick. The owner and local veterinarian provided information that the area was an endemic for colibacillosis. Chickens have been given Medivac ND/AI and Gumboro vaccines.

Duration of illness in the case of chickens was about 6 days, starting to look worse 2 days before necropsy, chickens were weak because anorexia. The same age as the sick chickens showed rapid growth. During observation and inspection, the chickens were seen to be weak and featherless. The chicken also very hard to standing. When the chicken is lifted, it is gasping for breath. Examination of the integumentary system showed visible baldness in the abdominal area. Further examination of the eyes showed that the chicken looked difficult to open its eyes. During an examination of the digestive system, it was found that there was abdominal swelling, which was suspected to be filled with gasses, the area around the anus looked dirty, and yellowish diarrhea was found. There were no signs of sneezing or neurological symptoms on examination.

Laboratory Examination

Pathology Examination

Gross anatomy examination found massive massive haemorraghic and fibrin exudation like showed in Fig. 1.

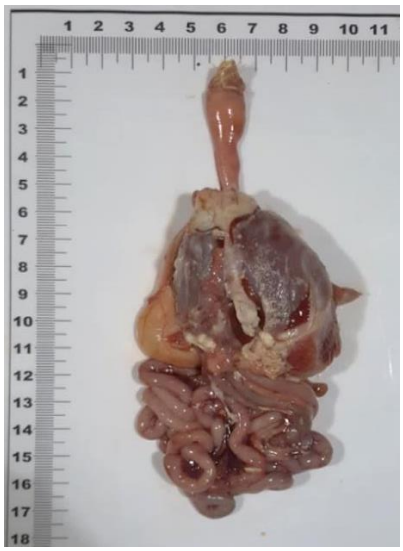

Fig. 1 haemorragic and fibrin exudation in visceral's organ.

Many organs also confirmed have several changes like showed in **Table 1.**

Table 1. Gross anatomy findings in case.

Organ	Gross anatomy changes
Brain	Haemorraghic in cerebrum
Heart	Fibrin exudation in Pericardium and heart also colour changes in heart
Trachea	Redness in trachea's lumen.
Lung	Fibrin exudation and haemorraghic in lung.
Liver	Pale and fibrin exudation
Kidney	Bleeding and discoloration to black
Intestines	Haemorraghic in intestine's lumen

Specimens used in histopathological examination were taken from organs that underwent macroscopic changes or organs that were suspected to have undergone changes based on clinical symptoms.

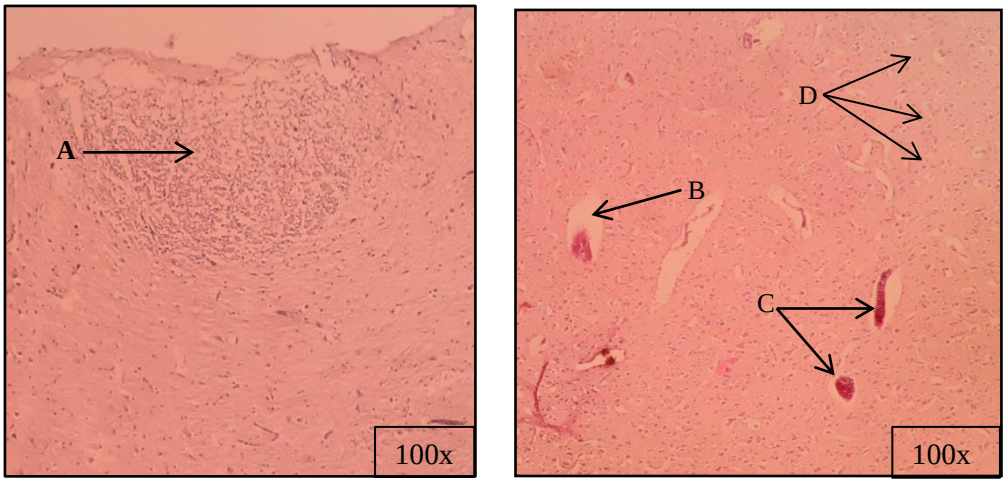

Fig.2. Brain. *Necrotic enchepalitis* (A) Inflammatory zone and necrotic in brain's tissue (H&E:100X); (B) Perivascular edema, (C) Congestion in cerebrum vascular, and (D) Gliosis in cerebrum (H&E:100X).

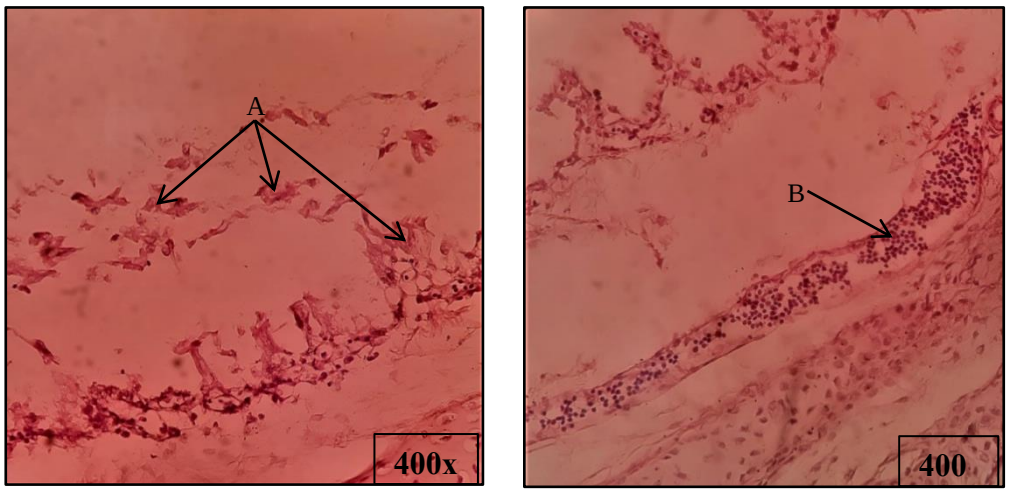

Fig.3. Trachea. *Haemorraghic tracheitis.* (A) Deciliation of lumen's epithel (H&E:400X) and (B) Congestion in submucosal vascular (H&E:400X).

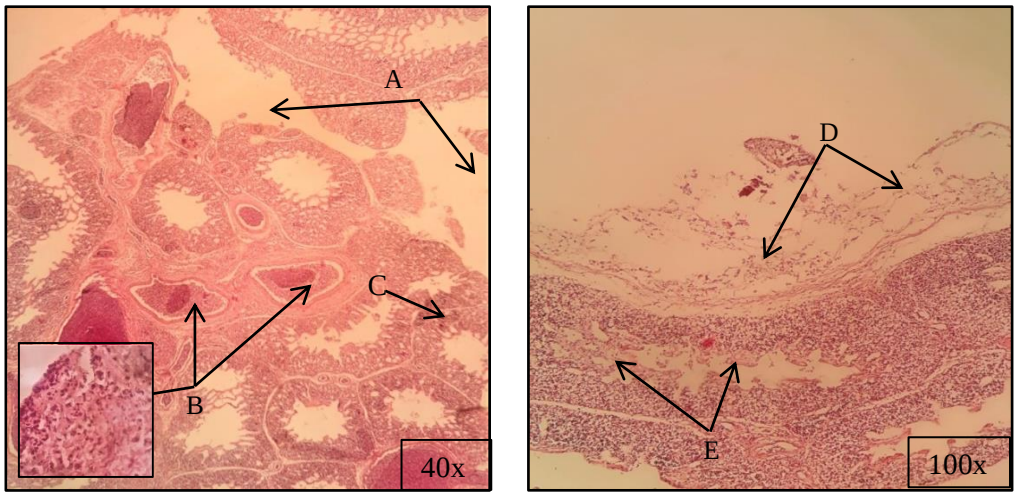

Fig.4. Lungs. *Haemorraghic and necrotic pneumonia* (A) Edema pulmonum, (B) Severe mesobronchus vascular congestion, (C) Erithrocytes accumulation in mesobrochus (H&E:40X); (D) Fibrin exudation and (E) Necrotic in parenchimal tissue (H&E:100X).

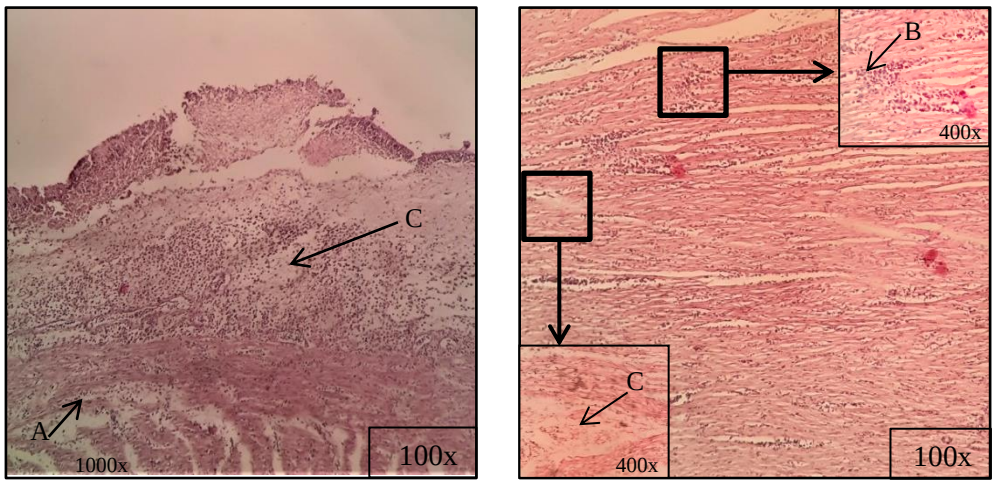

Fig.5. Heart. *Haemorraghic epicarditis et myocarditis.* (A) Infiltration of inflammatory cell in pericardium (H&E:1000X); (B) Erythrocytes accumulation in myocardium (H&E:400X); and (C) Fibrin exudation in myocardium (H&E:100X, 400X).

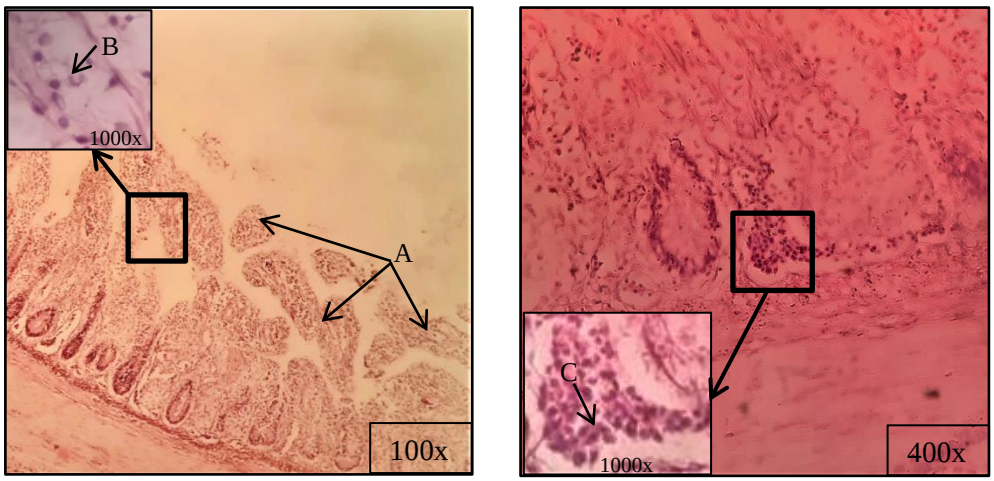

Fig.6. Intestines. *Haemorraghic enteritis.* (A) Erotion of eptelial phili in intestinal mucosa (H&E:100X); (B) Infiltration of inflammatory cells in intestinal phili (H&E:1000X); and (C) Erythrocytes accumulation in mucosal layer (H&E:400X).

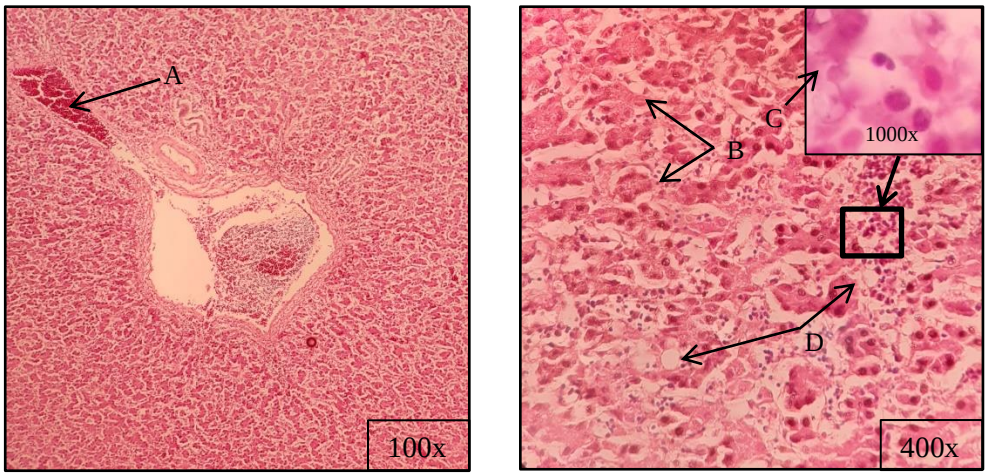

Fig.7 . Liver. *Haemorraghic and necrotic hepatitis.* (A) Congestion in central vein (H&E:100X); (B) Necrotic in parenchimal tissue (H&E:400X); (C) Erythrocytes accumulation and infiltration of PMN (H&E:1000X); and (D) fatty degeneration (H&E:400X).

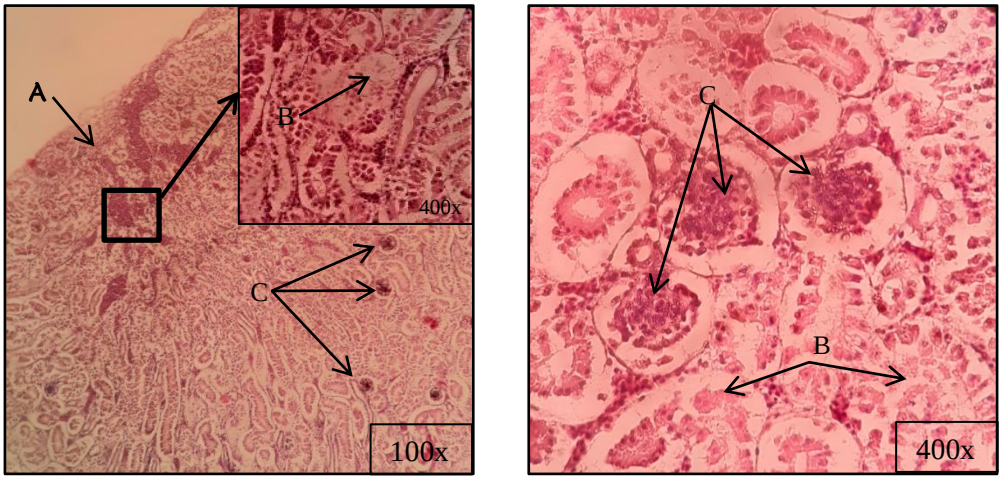

Fig.8. Kidney. *Haemorraghic and necrotic glomerulonephritis.* (A) Erythrocytes accumulation in cortex (H&E:100X); (B) Necrotic of tubular epithelials (H&E: 400X); and (C) Infiltration of PMN in glomerulus (H&E:100X,400X).

Microbiology Laboratory

Microbiological examination was carried out to culture agents from few organs (liver, intestines, and heart) that involved in this case and the result shown in Table 2.

Table 2. Culture and isolation on several agar media and staining.

Culture and Staining	Results
Nutrient Agar	Colonies are spherical in diameter ±1-3 μm, white and smooth surface.
Eosin Methylen Blue Agar	Colonies are metallic green to blackish, 1-3 mm in diameter, round, convex and flattened.
Sheep Blood Agar	White mucoid colony, hemolyzes blood (α hemolysin)
Gram staining	Bacil, short, and red (Gram negative).

The results that indicated by culture and identification led to the suspicion that the agent

involved in this case was *Escherichia coli.* After culture and isolation, primary and biochemistry test was carried out to confirm the causative agents.

Table 3. Primary and biochemistry test result

Primary Test	
Catalase Test	Positive
Oxydase test	Negative
Biochemistry Test	
Triple sugar Iron agar	Ferment carbohydrates, produce gas, H_2S negative
Sulfide Indole Motility	Indole positive, motile, H_2S negative.
Methyl Red	Positive
Simmon Citrate Agar	Negative
Glucose	Positive
Lactose	Positive

All results obtained from primary and biochemichemistry tests lead to the characteristics of pathogenic *E. coli* that can hemolyze blood.

Parasitology Laboratory

Parasitological tests were carried out to confirm the presence of parasitic agents that also infected this case. The tests carried out by soft stool tests and blood smear tests.

Table 4. Result of Stool test

Test	Result	Diagnosis	Identification
Native	Negative	Negative	Negative
Sedimentation	Negative	Negative	Negative
Floating	Negative	Negative	Negative

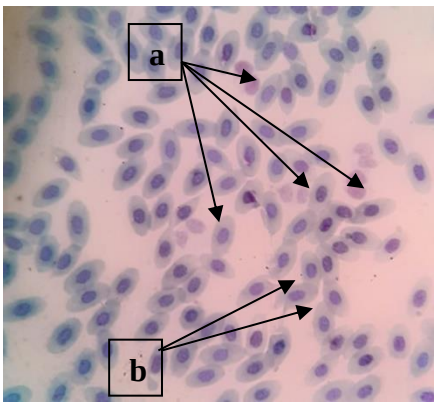

Fig.9. Result of blood smear test: found a. Heterophile dan b. Ruptured erythrocyte (No parasites founds)

Discussion

Based on anamnesis, clinical signs, and epidemiological studies, the chicken was suspected of having an *E. coli* infection. This is reinforced by the findings from the colony identification

results that Gram negative bacteria were found in the form of short rods which are the main characteristics of coli bacteria. *Escherichia coli* are Gram negative bacteria and are rod-shaped red, this is because Gram-negative bacteria have a peptidoglycan wall composition containing more lipopolysaccharides (Baehaqi et al., 2015). The suspected colonies were then isolated on selective media called Eosin Methylene Blue Agar (EMBA). The results of EMBA grew colonies with a diameter of ±1 mm with a metallic green color. Colonies of *E.coli* grow greenish red with a metallic luster on EMBA media (Dwidjoseputro, 1994). The results of the catalase test were positive indicating the bacteria in producing the catalase enzyme. The oxidase test in this examination showed a negative result indicating that this bacteria was an enteric bacteria.

The culture in sheep blood agar showed that the colonies have α-hemolysin properties because the zone formed was not clear and seemed to be spread out. α-hemolysin bacteria is an extracellular toxin that is periodically produced by isolates of pathogens in humans and animals (Minshew et al. 1978). Oxoid (2012) suggested that the virulence of a bacteria can be determined from its ability to lyse erythrocytes. Bacteria that are able to lyse erythrocytes are more pathogenic than bacteria that are unable to lyse erythrocytes. The ability of bacteria to lyse erythrocytes is determined by a substance in the form of an extracellular protein called hemolysin (McKane and Kandel, 1998).

Culture on triple sugar iron agar (TSIA) showed a change in the color of the media from red to yellow on the slant and butt, the media was lifted which indicated the bacteria forming gas. According to Lebofee (2011), the results of the TSIA test on *Escherichia coli* bacteria produce a yellow color and produce gas. This is because *Escherichia coli* in triple sugar iron agar (TSIA) able to ferment sugar. In Sulfide indole motility (SIM) test, H_2S was not formed, there was bacterial movement, and indole was positive. A positive indole test indicates that the bacteria produce the enzyme tryptophanase. While on simmon citrate agar (SCA) media, the results were negative which indicated that the bacteria were unable to utilize citrate as a source for their daily needs. In methyl red test, positive results were obtained. According to Fardiaz (1989) positive results on methyl red indicate that bacteria are able to ferment proteases into organic acids. In the test of sugars Lactose and Glucose showed positive results indicating that these bacteria were able to ferment carbohydrates (Cappucino et al., 2011). All of these test results confirmed that the bacteria isolated from the intestines, liver, and heart were pathogenic *E.coli* bacteria so that it could be said that *E.coli* was the causative agent of infection (colisepticemia).

Colisepticemia is a condition of *Escherichia coli* microorganisms and their toxins have spread in tissues or organs that can cause organ damage or inflammation (Tarmudji, 2003). Colisepticemia occurs when large amounts of pathogenic serotype *E. coli* enter the blood circulation and infect various tissues through lesions in the intestines or respiratory tract that spread because birds inhale dust from cages that have been contaminated with bacteria. Bacteria enter the lower respiratory tract and adhere to the epithelial surface (Tabbu, 2000). Rukmana (2003) reported the symptoms that occur in colibacillosis infection are the presence of watery yellow feces. Symptoms caused by colibacillosis also show clinical signs of emaciation, anorexia, diarrhea, disturbed growth, dirty and sticky feathers in the cloaca (Tarmudji, 2003).

In poultry, coli infections usually begin from the respiratory tract. This theory relevant with findings of deciliation and infiltration of polymorphonuclear inflammatory cells in the villi mucosa to the trachea submucosa. In lungs, occurs edema, heavy congestion in the mesobronchus, necrosis of the parenchymal tissue, and observed the presence of fibrin and Polymorphonuclear (PMN) cell infiltration. The same thing was reported in the study of Kumar et al. (2004). The color of kidneys also observe to changes to black. Histopathological examination revealed tubular cell necrosis, erythrocyte accumulation in the renal cortex, and inflammatory cell infiltration in the glomerulus. This is justified by Berata et al. (2015) who

revealed that in the kidney will be found infiltration of heterophilous cells, and necrosis. The predominant inflammatory cell infiltrate is heterophilic polymorphonuclear cells which indicate acute infection and the appearance of inflammation caused by bacterial agents. Its happen because kidney is the first organ to filtering toxins produced by *E. coli* (Tabbu, 2000).

Pudjiatmoko (2014) reported that the incidence of coli in poultry will cause pericarditis with fibrin, peritonitis, and thickened air sacs. In heart, examination founds the presence of fibrin exudate and PMN cell infiltration. In liver, founds a layer of fibrin and histopathologically found congestion in the portal vein, accumulation of erythrocytes outside the blood vessels in the sinusoids and fatty degeneration. This is suitable with the findings of Vegad (2007) which states that broiler chickens infected with *E.coli* at the age of less than 30 days will show changes in form of thick layer of fibrin covering the liver and heart, which if severe condition will turn to yellowish and be predisposed to cheesy degeneration. Meanwhile, according to Kumar et al. (2004) the formation of thin fibrin layer in the heart and liver in coli-artificial infection can be found on first day after infection.

Histopathological observations of the brain showed congestion, perivascular edema and an increase in glial cells, while the intestine had erosion of the intestinal mucosal villi and heterophilic infiltration of the intestinal mucosal phili. According to Gyles et al. (2004), the histopathological changes found edema as the initial change, and the first infection was characterized by airsacculitis with serous fluid to fibrin exudate, heterophilous cell infiltration, and a predominance of macrophages. In some tissues such as the liver and digestive tract can be found lymphocyte cells. This infiltration occurs because the digestive tract and liver are the initial organs that come into contact with infectious agents so that as a form of body defense, lymphocytes, heterophils, and macrophages will infiltrate into digestive tissues and organs (Agustina et al., 2010).

Ruptured of erythrocytes membrane was observed on blood smear stained with Giemsa. This ruptured is most likely caused by hemolysins produced by *E. coli bacteria*. α-hemolysin acts by forming a cytolytic pore, causing damage to the plasma membrane of the host erythrocytes (Carter and Wise, 2004). However, no parasites agent found in blood smear examination.

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