HISTOPATHOLOGICAL DIAGNOSIS AND DETECTION OF *AVIAN PATHOGENIC Escherichia coli* VIRULENCE GENES IN BROILER CHICKENS AT INDONESIA

Abstract

Colibacillosis is a disease in poultry that often occurs in poultry farms in developing countries, including Indonesia. This disease is generally caused by cage or environmental sanitation problems, as well as poor poultry husbandry patterns. Colibacillosis, caused by *Avian Pathogenic Escherichia coli* (APEC) infection, is one of the significant health problems in the poultry industry with clinical symptoms such as emaciation decreased appetite, impaired growth, diarrhea, dirty or sticky feathers around the vent, bloated intestines and white feces, especially in Indonesia. This study aims to identify histopathologically and detect virulence genes of Avian pathogenic *Escherichia coli* in broiler chickens. The methods used included organ sampling such as heart, liver, jejunum and cecum, which were then processed for histopathology preparation using Hematoxylin-Eosin (HE) staining. In addition, molecular diagnosis was performed using *Polymerase Chain Reaction* (PCR) technique to detect virulence genes, *iroN* and *hlyF*. The results showed that there were avian pathogenic *Escherichia coli* isolates in chickens suspected of colibacillosis with positive blood agar culture showing hemolysin production (β-hemolysis) and the gene encoding *hlyF* was found positive but the gene encoding *iroN* was not found. Histopathology results of liver, heart, jejunum and cecum infected with pathogenic *Escherichia coli* showed damage in the form of hemorrhage, necrosis, rupture of intestinal villi, erythrocyte accumulation, central venous congestion and fatty degeneration. Our study shows that *avian pathogenic Escherichia coli* strains can be isolated from broiler chickens suffering from colibacillosis and cause pathological changes anatomically. This study emphasizes the importance of a better understanding of this pathogen to develop effective prevention and control strategies in the poultry farming industry.

Keywords: Colibacillosis, APEC, Gene *hlyF*, Gene *iroN*, Poultry

1. Introduction

Colibacillosis is a disease in poultry that has a high economic impact. Economic losses due to colibacillosis in the poultry industry in Indonesia amount to 825 million dollars every month (1). Colibacillosis disease in poultry is caused by avian pathogenic *Escherichia coli* strains (2). Avian colibacillosis is the main disease that attacks the poultry industry throughout the world, including Indonesia (3). *Escherichia coli* bacteria are opportunistic bacteria and can develop into pathogens which can be divided into two large groups: Diarrhogenic *Escherichia coli* and Extraintestinal Pathogenic *Escherichia coli*. DEC strains are responsible for gastrointestinal infections, while ExPEC strains are responsible for diseases outside the intestinal tract such as sepsis, urinary tract infections, and meningitis. ExPEC has pathogenic strains including Avian Pathogenic *Escherichia coli* (APEC) and is responsible for colibacillosis in poultry (3,4).

It is important to improve understanding of the diagnosis, symptoms, and pathogenesis of colibacillosis, especially in developing countries such as Indonesia. The incidence of colibacillosis in poultry is very high, this can be due to predisposing factors such as stress and the assumption that the disease is caused by opportunistic infections, thus underestimating the virulence of APEC. In general, pathogenic *Escherichia coli* infections in poultry occurs in two forms, systemic and local (1,5). The local form of infection in colibacillosis can be omphalitis / yolk sac infection, swollen head syndrome, cellulitis, enteritis, venereal colibacillosis, salpingitis, egg peritonitis, while the systemic form of infection in colibacillosis is in the form of colisepticemia (2,6). This study is expected to explain the symptoms of colibacillosis seen in

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chickens. Confirmation of diagnosis with necropsy seen in this study can be done by veterinary medical personnel in the field. Direct evidence of the presence of poultry pathogenic *Escherichia coli* bacteria as the cause of colibacillosis in poultry is still lacking, so research is very important to do. Colibacillosis is a zoonotic disease, which can have a negative impact on society. people are more careful about colibacillosis which is not only caused by common *Escherichia coli*, but apparently caused by Avian Pathogenic *Escherichia coli* which has the virulence genes HlyF and IroN (7,8).

2. Materials and Methods

2.1. Study period and location

The study was conducted during December 2023-March 2024. The sampling method used purposive sampling, sampling according to the purpose of colibacillosis disease detection based on visible clinical symptoms. 60 chickens were observed, 27 chicken with no colibacillosis clinical signs, and 33 chickens showed clinical symptoms of colibacillosis. Samples came from live broiler chickens sold in traditional markets in Surabaya city, Indonesia. The traditional markets taken as samples are Wonokromo, Keputran, Pabean, Pucang, Dukuh Kupang, and Benowo. The traditional markets can represent the Surabaya area, Indonesia, with 10 chickens taken from each market.

2.2. Isolation and identification *Avian Pathogenic Escherichia coli*

Isolation and identification of Avian Pathogenic *Escherichia coli* using *Mac Conkey agar* (MH081 - HiMedia®, *Triple sugar iron Agar* (M021 - HiMedia®), *Simmon Citrate Agar* (M099 - HiMedia®), *Sulfide Indole Motility* (M181 - HiMedia®), and *methyl red - Voges–Proskauer* (M070 - HiMedia®). *Escherichia coli* were then cultured on *Blood Agar* media with an additional 5% sheep's blood. Pathogenic *Escherichia coli* would show the formation of a clear zone around the colony which is considered to be hemolysin production (9).

2.3. Histopathology

After finding the presence of pathogenic *Escherichia coli* bacteria, a necropsy was carried out on all samples. The organ samples taken were the heart, liver, jejunum and cecum. The organ was cut lx1xl cm, then the solution was soaked in 10% Buffer Neutral Formalin (BNF) to be made into histopathological preparations using *Hematoxylin Eosin* (HE) staining (10).

2.4. Molecular diagnose Polymerase Chain Reaction

DNA extraction for Polymerase Chain Reaction was performed using QIAamp® DNA kit (QIAGEN, Germany) to detect gene encoding *iroN* (8) and *hlyF* (7). The forward primer used in iroN was AAGTCAAAGCAGGGGTTGCCCG, while the reverse primer was GACGCCGACATTAAGACGCAG with a target of 667 bp and the forward primer used in *hlyF* is GGCGATTTAGGCATTCCGATACTC, while the reverse primer was ACGGGGTCGCTAGTTAAGGAG with a target of 599 bp under thermal cycler conditions with predenaturation parameters at a temperature of 94°C for seven minutes, denaturation at 94°C for one minute, annealing at 56°C for 30 seconds, extension at 72°C for 30 seconds, cycle repeated 35 times and final extension at 72°C for five minutes. After that, the amplicons were visualized by electrophoresis using 2% agarose gel (7,8,11).

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2.5. Statistical analysis

Data analysis in this research was carried out descriptively, by identifying findings of avian pathogenic *Escherichia coli* bacteria referring to the Indonesian National Standard, SNI 7388:2009. Histopathological imaging was carried out descriptively by identifying changes in the heart, liver, cecum and jejunum of chicken sick with colibacillosis by comparing the organs in chicken with no colibacillosis clinical signs using the T test.

3. Results

3.1. Clinical examinations and necropsy

Based on observations, broiler chickens experience symptoms of disease such as lethargy, emaciation, retarted growth, dirty or sticky feathers, visible feces attached around the vent of the chicken and the consistency of greenish-white feces. The results of the macroscopic examination found petechiae on the thighs of chickens, as well as, hepatomegaly at necropsy, abnormal heart shape and the presence of fibrin membranes in the heart, the cecum looked normal, and in the jejunum found bleeding (Figure1).

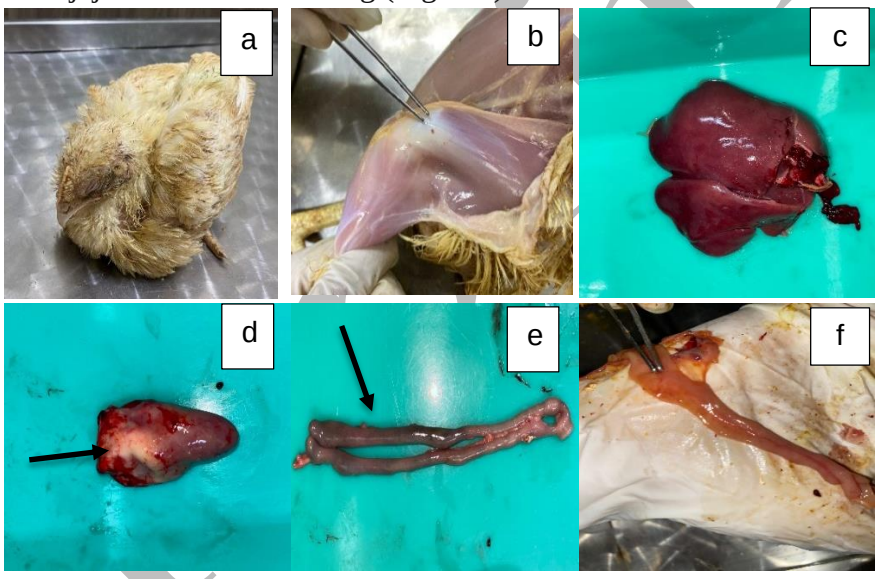


Figure 1. Necropsy (a); presence of petechiae (b); hepathomegaly (c); heart abnormalities (d); hemorrhage in the cecum (e); and hemorrhage in the jejunum (f)

3.2. Isolation and Identification Results

Based on the results of microbiological examinations conducted on broiler cloacal swab samples, the macroscopic morphology of *Escherichia coli* bacteria on MCA media showed pink colonies, small round, separate and irregular (Figure 2a). further Gram staining is carried out to determine the morphology of *Escherichia coli* bacterial cells with a short rod shape (*coccobacillus*) and appeared red (Figure 2b). Identification of *Escherichia coli* bacteria was performed physiologically using media such as TSIA, SCA, SIM, MR and VP (Figure 3c).

Positive results on TSIA media were characterized by Acid/Acid (A/A) reactions at both the base (butt) and slope (slant), gas production, and negative H_2 S. The Simon Citrate Agar (SCA) test for *Escherichia coli* bacteria was negative, indicated by the absence of green color changes in the media, as *Escherichia coli* did not utilize citrate as a carbon source. The Sulfide Indole Motility (SIM) test showed positive results for Indole, with motility characterized by bacterial

spread in the puncture area, and negative for sulfide. The Methyl Red (MR) test results, if positive, showed a red color change after adding 0.5% Methyl Red reagent. The Voges-Proskauer (VP) test for *Escherichia coli* produced negative results. Based on the results of the Blood Agar test (Figure 2d), the presence of β-hemolysin, forming a clear zone around the colony, was observed.

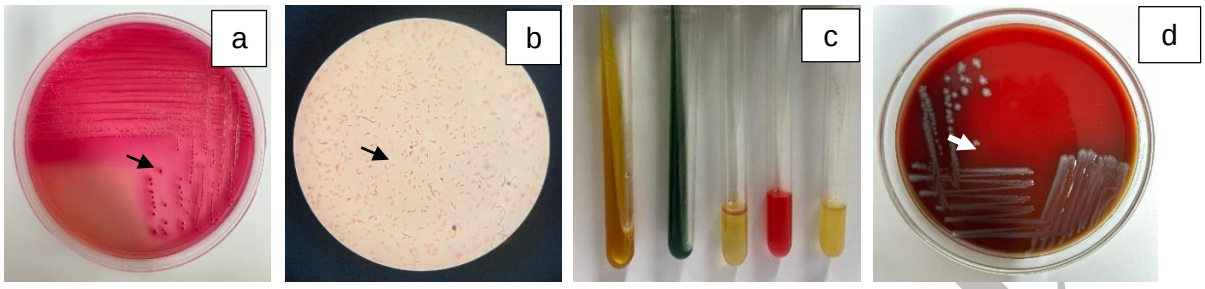


Figure 2. *Escherichia coli* colonies on *Mac Conkey Agar* (a); Gram staining on *Escherichia coli* using a microscope at 1000x (b); Biochemical test results for *Escherichia coli* (c); Hemolysin production test on Blood Agar media (β-hemolysis) (d).

3.3. Polymerase Chain Reaction (PCR) Results

Based on the PCR test, a positive result was obtained for the gene encoding hlyF at 599 bp, while iroN at 667 bp was not found. hly F gene in Avian pathogenic *Escherichia coli* is a virulence coding gene that can determine the ability of APEC to cause disease by hemolyzing, regulating outer membrane vesicles, and inducing autophagy in host cells. These results provide evidence that *Escherichia coli* found in chickens infected with colibacillosis are pathogenic to poultry (Figure 3).

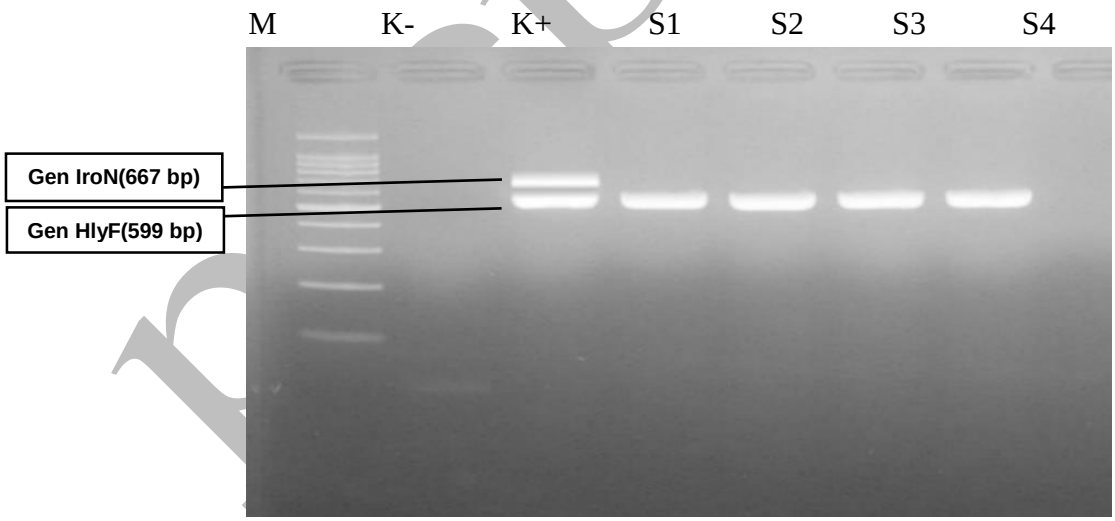
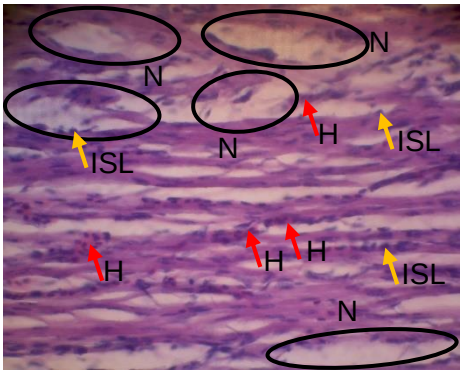


Figure 3. PCR results of the hlyF gene for *Escherichia coli* isolates positive for pathogens. Sample codes S1, S2, S3, and S4; M: marker; K-: negative control; K+: positive control

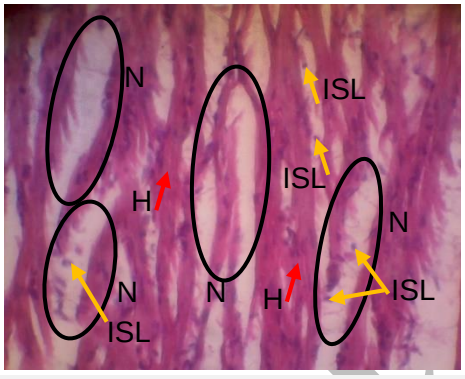
3.4. Histopathology Results

Histopathological evaluation was carried out based on the results of macroscopic pathology changes in broiler chickens with colibacillosis. The organs observed were liver, heart, jejunum, and cecum. Histopathological changes in each organ were inflammatory cell infiltration, hemorrhage, and necrosis (Figure 4). The results showed that both chicken with no colibacillosis clinical signs and colibacillosis-infected chickens showed these changes.

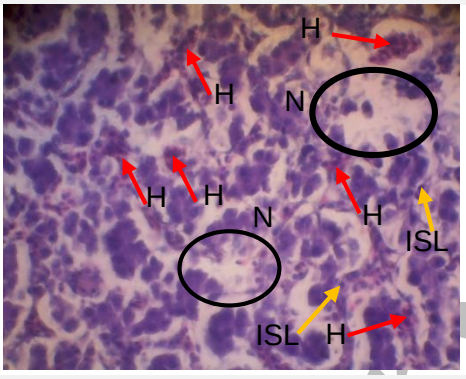
Chicken with no colibacillosis clinical signs **Chickens infected with colibacillosis**



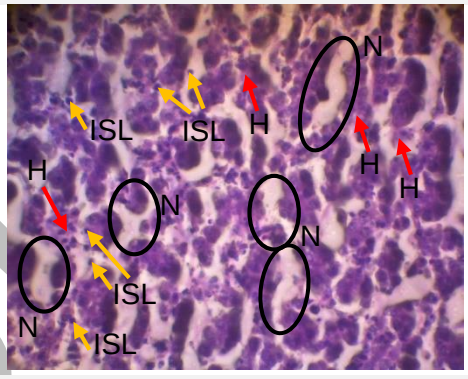
heart (400x)



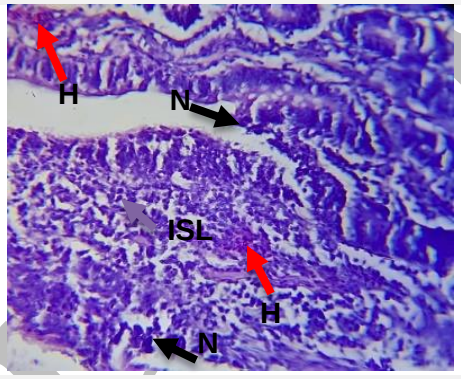
heart (400x)



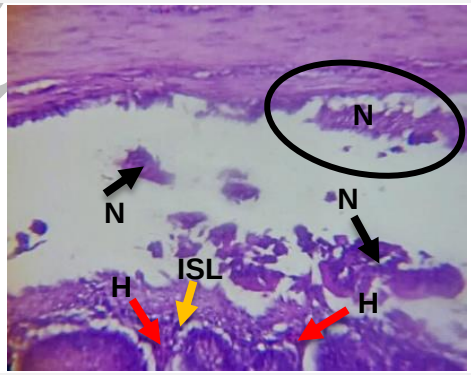
liver (400x)



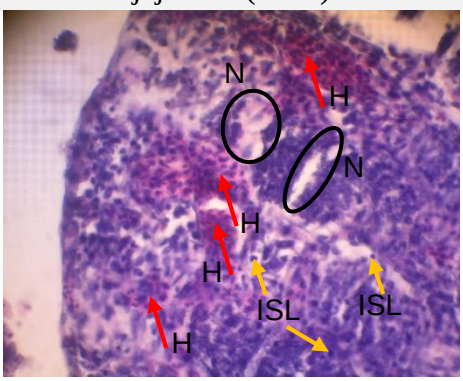
liver (400x)



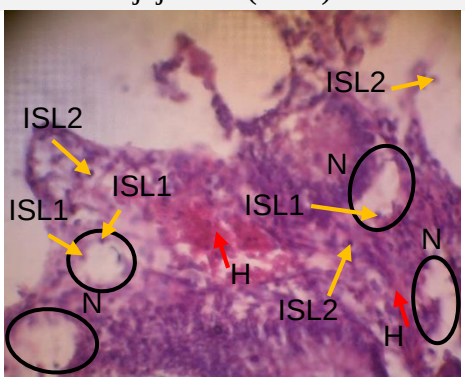
jejunum (400x)



jejunum (400x)



cecum (400x)



cecum (400x)

١٤٨ Figure 4. Results of histopathological examination (magnification: 400x)

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Based on the results of histopathological testing of colibacillosis-infected chickens and chicken with no colibacillosis clinical signs in the organs of the heart, liver, jejunum, and cecum, there were changes in lesions in the form of inflammatory cell infiltration, hemorrhage, and necrosis (Table 1). Hemorrhagic myocarditis results were found in the heart with hemorrhage, edema, and heterophils, inflammatory cell infiltration. The liver had hemorrhagic hepatitis with hemorrhage, necrosis and inflammatory cell infiltration. The intestines had hemorrhagic and necrotizing enteritis characterized by villous necrosis, hemorrhage, edema, and neutrophilic inflammatory cells.

Based on Table 2, the average number of inflammatory cell infiltration in the heart of chickens with no colibacillosis clinical signs is lower than that of chickens infected with colibacillosis, but does not show a significant difference ($P>0.05$). Likewise, inflammatory cell infiltration in the liver organ of chickens with no colibacillosis clinical signs has the same value as chickens infected with colibacillosis, which means there is non-significant difference. While the jejunum and cecum organs showed significant differences ($P < 0.05$), with inflammatory cell infiltration in chickens with no colibacillosis clinical signs higher than chickens infected with colibacillosis.

4. Discussion

Examination results have found petechiae in several chicken organs, swelling of the liver, abnormal heart bases, the presence of fibrin membranes in the heart (pericarditis), jejunum and cecum found hemorrhage. *Escherichia coli* is one of colibacillosis formes caused by the APEC strain (12). Colibacillosis in poultry is a significant challenge in poultry production,with economic losses, and mortality in poultry (13). The incidence of colibacillosis in live poultry farms and markets is due to poor sanitation, hygiene and environment (14,15). *Escherichia coli* can spread through the bloodstream (bacteremia) so that it can reach the target heart organ and colonize, causing inflammation until fibrin forms and can spread to other organs such as the liver. Emphysema is found in the liver. Emphysema is an abnormal dilation of the air spaces accompanied by damage to the alveoli that can reduce maximum expiratory airflow due to reduced elastic recoil of the lungs (16). The jejunum of chickens infected with colibacillosis can experience intestinal distension, obstruction and bleeding in the digestive tract. This can occur due to the influence of enterotoxins in *Escherichia coli* that attach to the intestine so that it can cause an increase in blood vessel capacity (17), which can be seen in (Figure 1).

Based on the results of the Blood Agar test in Figure 2c, Positive results in testing the ability of bacteria to hydrolyze blood and protein are indicated by the formation of a clear zone (transparent zone) around the colony. The formation of the hemolysis zone results as shown in Figure 2c is due to the release of active glycolipid compounds on substrates that are hydrophilic by bacterial strains. The test results on the *hlyF* virulence gene showed 100% positive result (4/4) in *Escherichia coli* isolates from chicken cloacal samples. This result is higher than previous studies on the incidence of APEC caused by the *hlyF* virulence gene in chickens by 83.3% in Bangladesh (18) and Korea by 80% (19). In Indonesia, the virulence gene was found in native chickens by 100% (11) and in ducks there was a virulence gene of 60% (7). The *hlyF* gene is found in APEC, which is a toxin that causes cells to undergo lysis and damage, motility, inducing host cell vacuolization, colonization, biofilm formation, agglutination, outer

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membrane vesicle formation, further contributing to bacterial virulence including cytolytic swelling toxin (CDT) and cytolysin factor A (ClyA) (20).

PCR test results showed negative results for the *iroN* virulence gene. These results do not show the incidence of the reported incidence of APEC affected by various *iroN* virulence genes, 100% in Bangladesh (18), 92% in China (21), 97% in Qatar, 100% in Korea (19). Another study in Indonesia on APEC chickens in irongen showed 100% (11). The iroN gene is found in APEC because the iroN gene has siderophores (aerobactin, salmochelin, yersiniabactin), which are secondary metabolites that function to absorb iron to increase bacterial growth and development. The ability of the iroN gene to enter the blood serum is very important because *Escherichia coli* causes sepsis and infections in various organs that are deficient in *iron* (20), although PCR has found the iron gene, this gene is caused by *Escherichia coli* isolated from feces, so it is more accurately called *Avian Fecal Escherichia coli* (AFEC) (8).

Based on the results of histopathological testing of chickens infected with colibacillosis and chicken with no colibacillosis clinical signs in the organs of the heart, liver, jejunum, and cecum, there were inflammatory cell infiltration, hemorrhage, and necrosis. Myocarditis haemorrhagica was found in the heart with hemorrhage, edema, and inflammatory cell infiltration. Although the incidence of inflammation and edema is small, this pattern may be related to the role of the heart as one of the predilection organs of *Escherichia coli* bacteria. The liver experienced hepatitis with hemorrhage, necrosis and inflammatory cell infiltration. the presence of histopathological lesions in the form of hemorrhage and edema is generally caused by toxins produced by bacteria, absorbed into the bloodstream which causes endothelial cells to be damaged, while necrosis lesions that occur in most cases of colibacillosis occur due to infectious agents and/or toxins (16).

The intestine experiences hemorrhagic and necrotizing enteritis characterized by necrosis of the villi, hemorrhage, edema, and neutrophilic inflammatory cells. Inflammation that occurs in colibacillosis cases is characterized by the presence of neutrophilic inflammatory cell infiltration on microscopic examination. Inflammatory cell infiltration is a form of body defense. According to, the dominance of neutrophil inflammatory cells occurs because neutrophils are essential in the body's defense system against microorganism invasion, especially bacterial invasion. The presence of histopathological lesions in the intestine is due to pathogenic *Escherichia coli* strains adhering, colonizing and proliferating releasing toxins on the intestinal mucosa. Pathogenic bacteria that colonize the intestine along with the toxins produced can cause inflammation, damage the epithelium, haemorrhage, necrosis, edema, damage the intestinal barrier and reduce the body's immune function (22).

Organs that experience inflammatory cell infiltration are found in the digestive tract, liver, and lungs which are the initial organs that come into direct contact with *Escherichia coli* infectious agents so that the formation of defense responses such as lymphocytes, heterophils, and macrophages that infiltrate into the tissues of these organs (16), The histopathological picture of the intestine experiencing acute infection due to *Escherichia coli* toxin is characterized by the presence of heterophil in the intestinal mucus which causes congestion in the intestinal wall and an increase in macrophages and plasma cells (23). Necrosis is irreversible damage to tissue that can be caused by various factors such as infection of old cells or chemicals. Necrosis begins with a change in the morphology of the nucleus which loses its chromatin appearance, becomes

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wrinkled, the nucleus is denser, and dark in color, namely pyknosis (24). Necrosis begins with an inflammatory reaction in the liver in the form of hepatocyte swelling and tissue death (25). Colibacillosis is a common and widely occurring disease in poultry that can cause economic losses, especially in developing countries including Indonesia. Avian pathogenic *Escherichia coli* as the causative agent of colibacillosis has virulence genes such as *hly* F, IroN, *iss*, *fim* H, etc. These virulence genes play a role in APEC infection, including in host cell invasion, persistence in the bloodstream, absorption of metals from body fluids for bacterial growth or in host cell damage. The zoonotic potential of APEC as a cause of colibacillosis in poultry cannot be underestimated, this is because APEC has the ability to cause urinary tract infections and meningism in humans. several studies have shown the possibility of transmission of zoonotic APEC from poultry to humans through food. Discussion of colibacillosis is important for the poultry industry and human health, this is one way to provide education and knowledge to the public as an effort to control colibacillosis. colibacillosis can be prevented by providing understanding to farmers, treatment of chickens with mild symptoms, giving vitamins, environmental hygiene, or immediately separating infected chickens that have shown symptoms.

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Authors' Contributions

FJW played a role in the idea and led the research. AYRC, IAK, and ACA assisted with sample collection and testing. FJW and AM helped in confirming the diagnosis. ORPAN, and MAB help in reading the histopathology results. FJW, AYRC, AM, IAK, and ORPAN together wrote the article.

Ethics

Animal ethical approval was obtained from the Research Ethics Commission of the Faculty of Veterinary Medicine, Wijaya Kusuma Surabaya University, Surabaya, Indonesia, Ethical clearance number 148 - KKE.

Conflict of Interest

The authors declare that they have no conflict of interest.

Data Avalilability

Data that supports the findings of this study are available in the results of research conducted by researchers

References

1. Wibisono FJ, Sumiarto B, Kusumastuti TA. Economic losses estimation of pathogenic *Escherichia coli* infection in Indonesian Poultry Farming. Bul Peternak.

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2018;42(November):341–6.

2. Apostolakos I, Laconi A, Mughini-Gras L, Yapicier ÖŞ, Piccirillo A. Occurrence of Colibacillosis in Broilers and Its Relationship With Avian Pathogenic *Escherichia coli* (APEC) Population Structure and Molecular Characteristics. Front Vet Sci. 2021;8(September):1–13.

3. Aleksandrowicz A, Khan MM, Sidorczuk K, Noszka M, Kolenda R. Whatever makes them stick – Adhesins of avian pathogenic *Escherichia coli*. Vet Microbiol. 2021;257(November 2020):2–8.

4. Da Rocha DT, De Oliveira Salle F, Borges KA, Furian TQ, Do Nascimento VP, De Souza Moraes HL, et al. Avian pathogenic *Escherichia coli* (APEC) and uropathogenic *Escherichia coli* (UPEC): Characterization and comparison. J Infect Dev Ctries. 2021;15(7):962–71.

5. Panth Y. Colibacillosis in poultry: A review. J Agric Nat Resour. 2019;2(1):301–11.

6. Ananda BES, Besung INK, Adi AAAM. Colisepticemia Infection in a 20 Days Old Broiler Chicken in Timuhun Village, Klungkung. Vet Sci Med J. 2023;5(9):197–210.

7. Kendek IA, Putri MFR, Wibisono FJ, Effendi MH, Tyasningsih W, Ugbo EN, et al. Molecular detection of hlyF gene on multidrug resistance of avian pathogenic *Escherichia coli* isolated from ducks on wet markets of Surabaya, Indonesia. Biodiversitas. 2024;25(3):1246–53.

8. Putri MFR, Kendek IA, Wibisono FJ, Effendi MH, Rahardjo D, Tyasningsih W, et al. Molecular detection of iron gene on multidrug resistant avian fecal *Escherichia coli* isolated from broiler on traditional markets, Surabaya, Indonesia. Biodiversitas. 2023;24(12):6454–60.

9. Ramos S, Silva V, Dapkevicius M de LE, Caniça M, Tejedor-Junco MT, Igrejas G, et al. *Escherichia coli* as Commensal and Pathogenic Bacteria among Food-Producing Animals : Health Implications of Extended Spectrum β -Lactamase (ESBL) Production. Animals. 2020;10(2239):2–15.

10. Siswandy S, Rahmi E, Masyitha D, Fitriani F, Gani FA, Zuhrawaty Z, et al. Histology, Histomorphometry, and Histochemistry of the Liver of Free-Range Chickens (Gallus gallus domesticus) During the Period Before and After Hatching. J Agripet. 2020;20(2):193–202.

11. Ramaditya NA, Besung INK, Mahardika IGNK. Detection and Sequencing Genes Iron, Iuta, and Hlyf in Avian Pathogenic Eschericia Coli. Bul Vet Udayana. 2019;11(2):229–38.

preprint

12. Newman DM, Barbieri NL, de Oliveira AL, Willis D, Nolan LK, Logue CM. Characterizing avian pathogenic *Escherichia coli* (APEC) from colibacillosis cases, 2018. PeerJ. 2021;9:1–24.
13. Kika TS, Cocoli S, Ljubojević Pelić D, Puvača N, Lika E, Pelić M. Colibacillosis in Modern Poultry Production. J Agron Technol Eng Manag. 2023;6(6):975–87.
14. Saha O, Hoque MN, Islam OK, Rahaman MM, Sultana M, Anwar Hossain M. Multidrug-resistant avian pathogenic *Escherichia coli* strains and association of their virulence genes in Bangladesh. Microorganisms. 2020;8(8):1–24.
15. Ievy S, Hoque MN, Islam MS, Sobur MA, Ballah FM, Rahman MS, et al. Genomic characteristics, virulance, and antimicrobial resistance in avian pathogenic *Escherichia coli* MTR_BAU02 strain isolated from layer farm in Bangladesh. J Glob Antimicrob Resist. 2022;30(June):155–62.
16. Solfaine R, Rahmawati I, Desiandura K, Yuriska. Study of Laboratory Diagnosis of Colibasilosis Infection In Local Hen In Surabaya. J Appl Vet Sci Technol. 2023;4(1):33–40.
17. Meha MH konda, Ketut Berata I, Kardena IM. Pathology Severity Level Of Intestine And Lungs Of Pigs That Infected. Indones Med Veterinus. 2016;5(1):13–22.
18. Hossain FE, Islam S, Islam MA, Islam S, Ahmed F. Detection of virulence genes of APEC (avian pathogenic *Escherichia coli*) isolated from poultry in Noakhali, Bangladesh. Bioresearch Commun. 2021;7(1):967–72.
19. Jeong J, Lee JY, Kang MS, Lee HJ, Kang S Il, Lee OM, et al. Comparative characteristics and zoonotic potential of avian pathogenic *Escherichia coli* (Apec) isolates from chicken and duck in south korea. Microorganisms. 2021;9(5).
20. Kathayat D, Lokesh D, Ranjit S, Rajashekara G. Avian pathogenic *Escherichia coli* (Apec): An overview of virulence and pathogenesis factors, zoonotic potential, and control strategies. Pathogens. 2021;10(4):1–32.
21. Subedi M, Bhattarai RK, Devkota B, Phuyal S, Luitel H. Antibiotic resistance pattern and virulence genes content in avian pathogenic *Escherichia coli* (APEC) from broiler chickens in chitwan, Nepal. BMC Vet Res. 2018;14(1):4–9.
22. He L, Wang C, Simujide H, Aricha H, Zhang J, Liu B, et al. Effect of Early Pathogenic *Escherichia coli* Infection on the Intestinal Barrier and Immune Function in Newborn Calves. Front Cell Infect Microbiol. 2022;12(February):1–13.
23. Ali IH, Jabir MS, Al-Shmgani HSA, Sulaiman GM, Sadoon AH. Pathological and Immunological Study on Infection with *Escherichia Coli* in ale BALB/c mice. J Phys

preprint

Conf Ser. 2018;1003(1):10.

24. Gelis TN, Etriwati, Erwin, Nazaruddin, Zainuddin, Muttaqien. Histopathology of rat's (rattus norvegicus) kidney After metal material wire implantation. J Ilm Mhs Vet Fak Kedokt Hewan Univ Syiah Kuala. 2020;4 (2)(2):101–6.

25. Taunde PA, Bianchi M V., Mathai VM, De Lorenzo C, Gaspar BDCB, Correia IMSM, et al. Pathological, microbiological and immunohistochemical characterization of avian colibacillosis in broiler chickens of mozambique. Pesqui Vet Bras. 2021;41:1–8.

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Table 1. Results of observations of histopathological changes in chickens infected with colibacillosis and chicken with no colibacillosis clinical signs

No.	Sample	Chicken with no colibacillosis clinical signs	Chickens infected with colibacillosis
1.	Heart	**(N).** visible rupture of tissue experiencing necrosis **(H).** Haemorragic **(ISL).** There was also heterophils, inflammatory cell infiltration in the heart muscle fibers	**(N).** Rupture of tissue experiencing necrosis **(H).** Haemorragic **(ISL).** There was also heterophils, inflammatory cell infiltration in the heart muscle fibers
2.	Liver	**(H).** Haemorragic **(N).** Necrosis (**ISL).** Inflammatory cell infiltration	**(H).** Haemorragic **(N).** Necrosis (**ISL).** Inflammatory cell infiltration
3.	Jejunum	**(H).** Haemorrhage in the lamina propria **(N).** necrosis of the epithelial layer **(ISL).** Inflammatory cell infiltration of the lamina propria	**(H).** Hemorrhage in the submucosa **(N).** There is very visible rupture/necrosis of the villi in the muscularis **(ISL).** Inflammatory cell infiltration of the submucosa
4.	Cecum	(H). Haemorrhage in the crypts of Liberkuhn (N). Necrosis of the Lieberkuhn crypts (ISL). Inflammatory cell infiltration in the crypts of Lieberkuhn	**(H).** Haemorrhage in the tunica mucosa **(N).** Necrosis of the Lieberkuhn crypts **(ISL1).** Inflammatory cell infiltration in the crypts of Lieberkuhn. **(ISL2).** Inflammatory cell infiltration of the tunica mucosa, The mucous membrane that lines the digestive tract and is the innermost layer of the digestive tract.

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Table 2. Average (± Standard Deviation) number of histopathological lesions in chicken with no colibacillosis clinical signs and those infected with colibacillosis which experienced inflammatory cell infiltration, hemorrhage and necrosis.

		Chicken with no colibacillosis clinical signs	Chickens infected with colibacillosis
Heart	Inflammation	1,60 ± 0,54[a]	2,60 ± 0,54[a]
	Hemorrhage	4,20 ± 1,09[a]	6,20 ± 3,56[b]
	Necrosis	4,40 ± 1,67[b]	5,20 ± 1,09[b]
Liver	Inflammation	2,60 ± 0,54[c]	2,60 ± 0,54[c]
	Hemorrhage	4,20 ± 1,09[c]	6,60 ± 3,20[d]
	Necrosis	4,40 ± 1,67[d]	6,00 ± 2,00[d]
Jejunum	Inflammation	0,20 ± 0,00[d]	2,60 ± 0,89[e]
	Hemorrhage	0,80 ± 0,44[e]	2,20 ± 0,83[e]
	Necrosis	1,20± 0,45[e]	2,60± 0,54[e]
Cecum	Inflammation	3,00 ± 0,00[e]	2,60 ± 0,54[f]
	Hemorrhage	4,20 ± 1,09[e]	6,20 ± 3,56[f]
	Necrosis	4,40 ± 1,67[f]	6,80 ± 1,78[f]

[a,b,c,d,e,f] superscripts of the same letter in the same column indicate insignificant differences (P>0,05), while superscripts of different letters in the same column indicate significant differences (P<0,05), N = Significant, NS = Non-Significant

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