Case Report: Identification of *Eimeria tenella* Infection and Pathologic Changes in a Broiler Chicken in Kutuh Village Farm, Badung, Bali

Case Report: Identifikasi Infeksi Eimeria tenella dan Perubahan Patologinya pada Ayam Broiler di Peternakan Desa Kutuh, Badung, Bali

Ni Komang Wahyu Centika Sari[1*], I Made Kardena[2], I Nengah Kerta Besung[3], Ida Bagus Kade Suardana[4], I Putu Cahyadi Putra[5*]
[1]Student of Veterinary Professional Education, [2]Laboratory of Veterinary Pathology, [3]Laboratory of Veterinary Bacteriology and Mycology, [4]Laboratory of Veterinary Virology, [4]Laboratory of Veterinary Parasitology
Faculty of Veterinary Medicine, Udayana University, P.B. Sudirman Street, Denpasar, 80361, Bali, Indonesia
**Corresponding author*: cahyadi_putra@unud.ac.id

Abstract

Coccidiosis is a serious parasitic disease of chickens caused by the protozoan Eimeria, with *Eimeria tenella* being one of the most virulent species. The infection causes necrosis and hemorrhage of the large intestine, resulting in significant economic losses in the poultry industry. The purpose of this case report was to determine the cause of death of broiler chickens in one farm in Kutuh Village, South Kuta Subdistrict, Badung Regency. The case animal (protocol number 39/N/24) was a 20-day-old broiler from the farm with a population of 18,000 birds. The examination process included anamnesis, clinical signs, anatomical pathology, histopathology, bacteriology, parasitology (including coprology and McMaster techniques), and morphometric identification of *Eimeria tenella* using ImageJ software. The clinical signs observed included anorexia, weakness, dull feathers, and reddish feces with a porridge-like consistency. Pathological examination revealed alterations in the cecum and colon. Histopathological analysis indicated *typhlitis hemorrhagis et necrotican* and *colitis necrotican*. Bacteriological examination revealed *Escherichia coli*, a normal flora, in the caecum and colon. Morphological and morphometric analyses of the oocysts revealed that *E. tenella* is ovoid, with two wall layers, an average size of 21.670 × 19.942 µm, and an oocyst count of 188,600 per gram of feces (OPG). The cause of death of the case chickens was due to severe Eimeria tenella infection.
Keywords: Caecal coccidiosis, chicken farm, typhlitis, Kuta Selatan

Abstrak

Koksidiosis adalah penyakit parasitik serius pada ayam yang disebabkan oleh protozoa *Eimeria*, dengan *Eimeria tenella* sebagai salah satu spesies paling ganas. Infeksi ini menyebabkan nekrosis dan hemoragi pada usus besar, mengakibatkan kerugian ekonomi signifikan di industri perunggasan. Tujuan laporan kasus ini adalah untuk mengetahui penyebab kematian ayam broiler di satu peternakan di Desa Kutuh, Kecamatan Kuta Selatan, Kabupaten Badung. Hewan kasus (nomor protokol 39/N/24) merupakan seekor ayam broiler berumur 20 hari berasal dari peternakan tersebut dengan populasi 18.000 ekor. Pemeriksaan yang dilakukan meliputi anamnesis, tanda klinis, patologi anatomi dan histopatologi, bakteriologi, parasitologi (koprologi dan *McMaster*) dan idetifikasi morfometri *Eimeria tenella* dengan *ImageJ.* Tanda klinis yang teramati meliputi anoreksia, lemas, bulu kusam dan teramati feses bewarna kemerahan dengan konsistensi seperti bubur. Hasil perubahan patologi menunjukkan adanya perubahan pada sekum dan kolon. Histopatologi menunjukkan *Typhlitis hemorrhagis et necrotican* dan *Colitis necrotican.* Pemeriksaan bakteriologi menunjukkan pada sampel organ sekum dan kolon terisolasi *Escherichia coli* yang merupakan flora normal. Pemeriksaan koprologi ditemukan ookista *Eimeria spp*. Pengamatan morfologi dan morfometri dari ookista menunjukkan *E. tenella* yang berbentuk bulat telur, dua lapis dinding, ukuran rata-rata 21,670 x 19,942 µm dan ookista per gram tinja (OPG) sebanyak 188.600. Penyebab kematian ayam kasus adalah akibat terinfeksi oleh *Eimeria tenella* derajat berat.
Kata kunci: Caecal coccidiosis, peternakan ayam, thyphilitis, Kuta Selatan

Introduction

Coccidiosis is caused by protozoa of the genus Eimeria that infect the gastrointestinal tract of chickens and can cause significant economic losses in the livestock sector owing to high mortality (Rumapea et al., 2023). The life cycle of Eimeria lasts approximately 4-7 days, starting when chickens ingest active oocysts. Once ingested, Eimeria invades the intestinal lining of chickens and multiplies several times, causing tissue damage (Mares et al., 2023). Based on the location of the parasite, coccidiosis is categorized into two types: intestinal coccidiosis and cecal coccidiosis. *Eimeria tenella* is the cause of cecal coccidiosis and is the most virulent and fatal species (Sun et al., 2023). This infection causes a decrease in body weight and productivity, thereby hindering the development of chicken farms and reducing animal protein production (Putra et al., 2020).

The identification of various Eimeria species is crucial for the prevention, surveillance, and control of coccidiosis, particularly in light of the emergence of anti-coccidial resistance and concerns regarding drug residues. *E. tenella* has demonstrated reduced sensitivity to three anticoccidial drugs: toltrazuril, amprolium, and sulfaquinoxaline sodium (Oimelukwe et al., 2018). Identification can be achieved through morphological and morphometric observations, which differ between species. Morphological characteristics are discernible from the shape and structure of the oocyst, whereas morphometry, a straightforward technique for analyzing oocyst size, can be conducted using the ImageJ software (Adams et al., 2022).

In Badung Regency, Bali, the population of broiler chickens is notably higher than that of other livestock types. According to the Central Bureau of Statistics (BPS) of Badung Regency, broiler populations from 2021 to 2023 were recorded at 1,109,927, 939,448, and 835,448, respectively (BPS, 2024). This high density of chicken livestock is correlated with an increased risk of diseases caused by the Eimeria protozoa (Wardani et al. 2021). A study by Simamora et al. (2017) conducted in a specific area of the Badung Regency revealed a high prevalence of *E. tenella* (31.8 %). Several factors contribute to this high prevalence, including management practices, air humidity, dose of oocyst infection, age of chickens, nutritional status, stress during infection, and level of host immunity.

The objective of this study was to ascertain the presence of *E. tenella* infecting the cecum and colon of broiler chickens at a poultry farm in Kutuh Village. This investigation employed a comprehensive methodology encompassing pathology, histopathology, coprology, morphology, and morphometry of parasites. The findings of this study are anticipated to elucidate the specific species of *E. tenella* affecting farms, thereby facilitating the implementation of appropriate preventive and control measures.

Materials dan Methods

Animal Case

The broiler chicken (protocol 39/N/24) was obtained from Kutuh Village, South Kuta District, Badung Regency, Bali. The characteristics of the case chickens were obtained through interviews with the farmer regarding their disease history, vaccination history, and clinical signs. The chickens were observed postmortem and necropsied at the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine (FKH), Udayana University (Unud).

Epidemiological Investigations

Data were collected directly through interviews with farmers using questionnaires. The respondent was a closed-house broiler farmer in Kutuh Village who had more than 5 years of experience. The questionnaire's points included signals, anamnesis, clinical signs, the number of sick and dead chickens, maintenance management, and environmental conditions around the farm. The chicken population (18,000 chickens) and the number of sick and dead chickens were obtained from the farm owner's records. One chicken was used as a sample to trace the cause of death. Observations of the three main risk factors (host, agent, and environment) were also made at the farm location. Epidemiological analysis was performed based on the calculation of morbidity, mortality, and case fatality rates (CFR).

Anatomical Pathology (PA) and Histopathology (HP) Examination

This study involved the observation of anatomical pathology (PA) alterations, specifically focusing on the location of infected organs, as well as their distribution, size, shape, color, consistency, and other distinctive characteristics. Organs exhibiting pathological changes were collected for histopathological analysis. Histopathological preparations were conducted using organ samples cut to dimensions of 1 × 1 × 1 cm, which were subsequently fixed in 10% neutral-buffered formaldehyde (NBF). The samples were then subjected to routine hematoxylin and eosin (HE) staining for histopathological examination. The prepared slides were observed under a microscope at magnifications ranging from 100 × to 400 × (Jubb et al., 2016).

Bacteriological Isolation and Identification

Isolation and identification of bacteria for differential diagnosis of diseases that show similar clinical symptoms. This study was conducted at the Veterinary Bacteriology and Mycology Laboratory, FKH, Unud. Bacterial cultivation was started on Nutrient Agar (NA) media with the cecum and colon as the sample organs. The samples were then incubated for 24 h. The growing colonies were observed based on their shape, color, edge surface, and diameter. Bacterial colonies on NA media were cultured in MacConkey (MAC) selective media. The colonies were then tested for catalase activity and Gram staining was performed to determine bacterial morphology. Subsequently, the bacteria were tested for biochemistry, including Triple Sugar Iron Agar (TSIA), Simmons Citrate Agar (SCA), Sulfide Indole Motility (SIM), Methyl Red (MR), Voges-Proskauer (VP), and glucose (Apriani et al., 2023).

Fecal Examinations

Fecal samples were collected from the cloaca and large intestine, placed in a pot, and 2.5% potassium dichromate solution was added. The samples were then examined at the Parasitology Laboratory, FKH, Udayana University to identify the presence of parasites. The methods used were Qualitative and quantitative analyses were also performed. The qualitative examination uses three common methods: native, sedimentation, and saturated salt (NaCl) flotation. The quantitative method used was the McMaster method (Adams et al., 2023). McMaster's quantitative examination was carried out using 2 grams of solid feces weighed using an analytical balance. The sample was placed in a 100 ml beaker, saturated salt was added until the volume reached 60 ml, stirred until homogeneous, sucked using a pipette, and put into the McMaster counting chamber. The samples were examined under a microscope at 100× magnification in both counting chambers. The number of oocysts per gram of feces (OPG) was calculated using the following formula (Dong et al., 2012):

$$OPG = \frac{n \; x \; Vcc}{Vf \; x \; Wf}$$

Notes:
Bt = Weight of feces
Vk = Volume of counting chamber
Vt = Volume of feces
n = Number of oocysts identified

Morphological and Morphometric Examination of Eimeria Oocysts

Oocysts observed during stool examination were qualitatively measured using the ImageJ software (https://imagej.net/ij/) (Adams et al., 2023). Measurements were taken to measure the main dimensions of the oocysts and to understand the variation in the oocysts. A calibration scale was used for measurements. Images of oocysts were obtained using a digital camera. Images were acquired using ImageJ software and scale calibration (µm) was performed. The edge of the oocyst was marked with a marking tool (such as “freehand selection” or “ellipse tool”). Next, oocysts were measured through the “Analyze” menu and then “Measure”) to obtain oocyst sizes, such as length, width, area, and perimeter. The data were stored in Microsoft Excel 2019, subjected to descriptive analysis, and subsequently presented in figures and tables (Robot et al., 2018).

Results and Discussion

Based on interviews, 20-day-old broilers were sick for five days with clinical signs of anorexia, weakness, dull feathers, and reddish-colored feces with porridge-like consistency. The chicken died after two days

of observation on May 14, 2024. Based on the data obtained, the total farm population was 18,000 chickens. All chickens were vaccinated against Newcastle Disease (ND), Infectious Bursal Disease (IBD), and Avian Influenza (AI). A total of 128 (from 18.000) chickens were affected, of which 28 died. This resulted in a morbidity rate of 0.71%, mortality rate of 0.15%, and CFR of 21.87%. Sick chickens were separated from healthy chickens but were not treated. Observations in the farm environment showed that the cages had a closed house system with dirt floors, and the density in the cages was quite dense with a high population. Biosecurity practices included the use of disinfectants before entering the cages.

Pathological assessment revealed extensive focal hemorrhage in the cecum and colon, affecting 100% and 90% of the cecal tissue and colon, respectively. The hemorrhage was distinctly demarcated in the cecum, presenting a flat surface with a blackish-red coloration and soft texture (Figure 1A and 1A1). Histopathological examination of the cecum and colon indicated necrosis, hemorrhage, infiltration of inflammatory cells, and the presence of oocyte developmental stages, specifically schizonts and macrogametes (Figure 1B - Figure 1F).

Bacteriological examination results showed the presence of bacterial colonies growing from colon and cecum samples that produced a white color with flat margins, convex elevation, and were 1-3 mm in size on NA media. Bacterial colonies of different sizes were planted on MAC selective media, and pink colony growth was observed on the streak line. Catalase enzyme testing showed positive results, with the formation of air bubbles. Gram staining with microscopic observation showed rod-shaped colonies and a red color. Biochemical tests on TSIA showed positive results for acid slant and acid butt, a change in color to yellow, and cracks in the media. The SCA test yielded negative results, as indicated by the absence of a color change from green to blue in the media. The SIM test showed a positive indole, namely the formation of a red ring after being tested with Kovac, positive motility, namely, blurriness in the puncture area, and negative H_2S marked by no change in the color of the media to black. The MR test was positive, with a change in color to red. The VP test was negative, with no color change. The glucose test showed positive results, with a change in color to yellow and the formation of air bubbles in the Durham tube. Based on all the tests, the bacteria identified were *Eschericia coli*.

Coprological method revealed a predilection for *Eimeria* spp. oocysts in the cecum and colon, with the McMaster method determining an oocyst per gram (OPG) count of 188,600. Morphological identification was conducted by observing the oocysts, which were ovoid in shape with walls comprising two layers. The oocysts appeared to be speciated by containing four sporocysts, each containing two sporozoites (Figure 2). Morphometric analysis was performed on ten oocysts, which had lengths ranging from 19 to 23 µm and widths from 17 to 22 µm, with an average size of 21.670 × 19.942 µm (Table 1). The identification results suggested that the oocysts closely resembled those of *Eimeria tenella*.

Coccidiosis can affect chickens of any age; however, the infection is more prevalent in younger chickens due to their immature immune systems (Fitri et al., 2021). The subject of this study was a 20-day-old broiler chicken. According to Arsyitahlia et al. (2019), the infection rate of *Eimeria* spp. is higher in chickens older than two weeks (30.6%) than in those younger than two weeks (0.6%). The chickens in this study exhibited a morbidity rate of 0.71% and a mortality rate of 0.15%. In a case report by Brahmananda et al. (2024), coccidiosis in Patas Village demonstrated low morbidity and mortality rates of 0.5% and 0.13%, respectively. However, this finding contrasts with the assertion by Mares et al. (2019) that morbidity and mortality can reach 80%.

Environmental conditions significantly influence the incidence of coccidiosis. In poultry farming, the use of a closed house system with a dirt floor presents particular challenges. As noted by Correia et al. (2022), soil-based cage floors are inherently difficult to clean, resulting in persistent contamination of chicken feces. This leads to increased moisture and elevated temperatures within the enclosure, thereby creating optimal conditions for the development of oocysts during their infective stage. Furthermore, feed that falls on the dirt floor is susceptible to contamination by infective oocysts, thereby increasing the risk of infection.

Eimeria tenella is the most common pathogenic species affecting the poultry industry globally, with morbidity rates reaching 100% and high mortality rates due to severe damage to the chicken digestive tract (Lawal et al., 2016; Fitri et al., 2021). Chicken case with protocol number 39/N/2024, based on pathology results, revealed hemorrhagic lesions in the cecum and colon. *Eimeria* spp. protozoa are parasitic on chicken intestinal epithelial cells, causing bloody diarrhea and nutritional disorders (Zhang et al., 2023). Among the seven *Eimeria* spp., *E. tenella* species usually invade the chicken cecum and surrounding intestinal segments (Matsubayashi et al., 2019). Lesions in the cecum are caused by the host immune response to the invasion of the intestinal tissue by *E. tenella* (Macdonald et al., 2017). *E. tenella* undergoes three life cycles: sexual (gametogonia), asexual (merogony or schizogony), and sporogony (spore formation). When villi are filled with aggregates of gametocytes and oocysts, the ends of the villi swell and detach, villi become short, and mucosal surface becomes flat. Due to mucosal damage, digestive function is impaired, proteins and electrolytes are lost, and weight gain is poor. *E. tenella* entering the merogony stage causes severe lesions. Hemorrhage and extensive tissue damage in the intestine characterize the in-depth development of the mucosa of second-generation merogony. The rupture of blood capillaries and epithelium occurs prior to merozoite release. After the release of second-generation merozoites, anemia and even death occur. The epithelium adjacent to lesions produced by second-generation merogony is invaded by merozoites that form gamonts. During the healing process, a new epithelium is formed rapidly, the number of inflammatory cells is reduced, and the number of accumulated lymphoid cells increases (Lopez-Osorio et al., 2020).

Histopathological analysis revealed alterations consistent with *colitis necroticans* and *typhlitis hemorrhagica et necroticans*, characterized by mucosal necrosis, infiltration of hemorrhagic inflammatory cells, and the presence of schizonts. These findings suggest that the oocysts have progressed to the schizont stage, a critical phase in the life cycle of *Eimeria tenella*. *Typhlitis hemorrhagica et necroticans* are marked by hemorrhage and necrosis of the cecal mucosa, respectively. Mucosal necrosis indicates cellular death due to infection, which triggers an inflammatory response. The infiltration of hemorrhagic inflammatory cells, including lymphocytes, macrophages, and granulocytes, signifies an immune response aimed at combating pathogens. The presence of schizonts, the asexual reproductive form of *E. tenella*, indicates active parasite multiplication within the host cells, leading to substantial tissue damage. Histopathological examination also reveals the gametogonia phase, where gametogenesis occurs, allowing for the differentiation of gametocytes and microgametes from gametocytes (Mesa-Pineda et al., 2021).

The infection caused by *Eimeria* spp. was quantified by determining the oocyst count in the feces of the affected chickens, which amounted to 188,600 oocysts per gram. This oocyst count indicates severe infection. This finding aligns with the assertion of Swayne et al. (2020) that severe coccidiosis can result in mortality among chickens. An infection involving 1-30,000 oocysts can lead to the general symptoms of coccidiosis and fecal bleeding. Infections with elevated oocyst counts, such as 100,000, are associated with high morbidity, mortality, and weight loss.

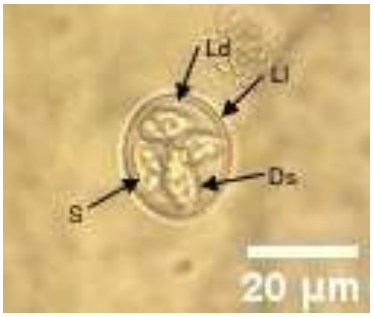

Figure 2. *Eimeria tenella*. Outer layer (Ll) and inner layer (Ld) of oocyst wall, sporocyst wall (Ds) and sporocyst (S) (Scale bar = 20µm).

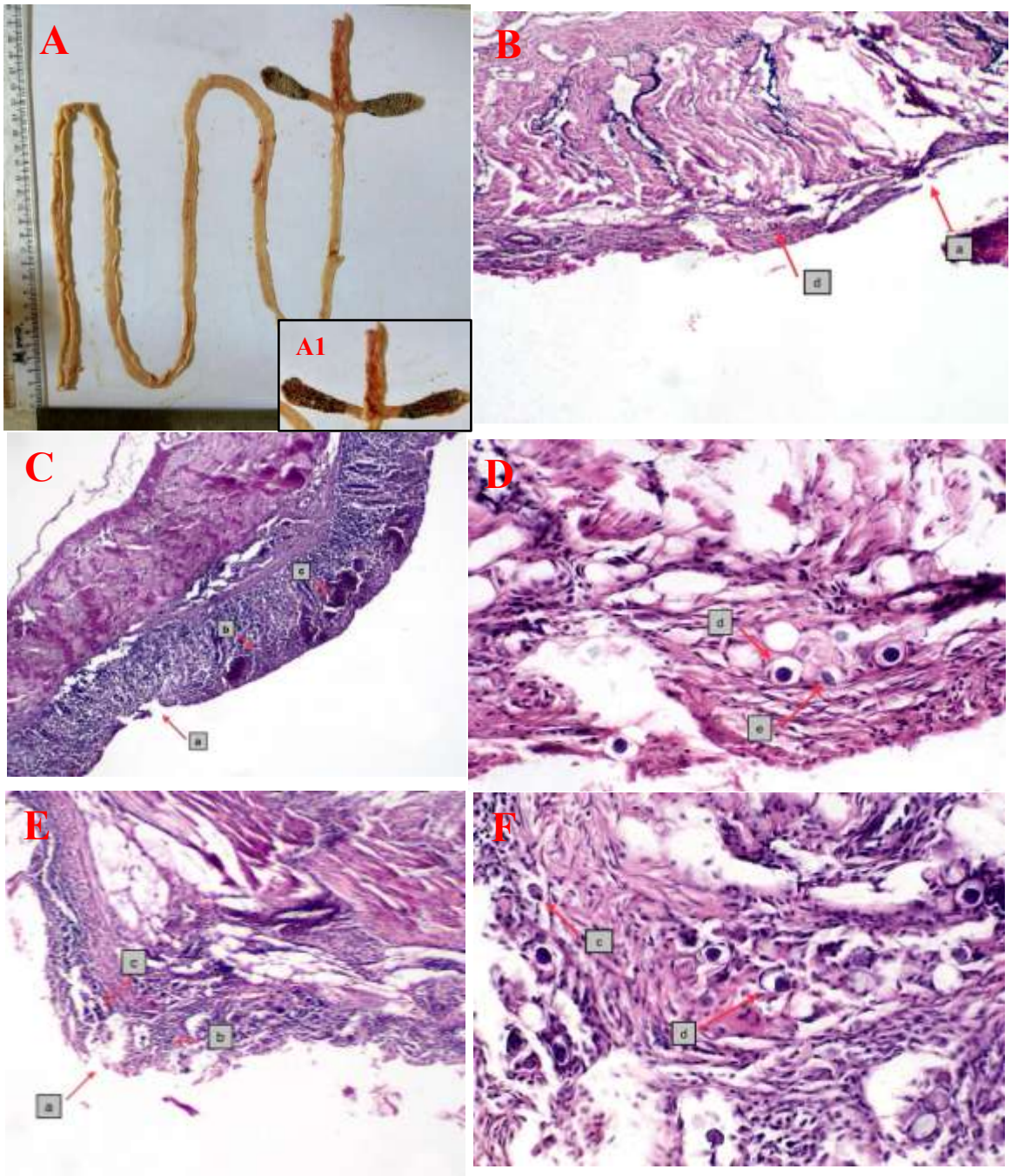

Figure 1. Anatomical pathology (PA) and histopathology (HP) of broiler chickens. (A) Changes in intestinal PA levels. (A1) PA of the reddish discolored cecum and colon. (B and C) *Typhlitis hemorrhagis et necrotican* (HE staining) at 100x magnification and (D) 400x magnification, Description: (a) mucosal necrosis, (b) infiltration of polymorphonuclear inflammatory cells and macrophages, (c) hemorrhage, (d) macrogametes, and (e) schizonts. (E) *Colitis necrotican* and observed the developmental phase of *Eimeria tenella* inside epithelial cells at 100x and (F) 400x magnification, description: (a) villous necrosis, (b) inflammatory cell infiltration, (c) schizonts, and (d) macrogametes.

Table 1. The dimensions of *Eimeria tenella* oocysts were measured using ImageJ software.

Oocyst code	Length (µm)	Width (µm)
O1	22,321	19,625
O2	20,394	19,113
O3	20,831	19,489
O4	22,727	20,907
O5	20,765	17,454
O6	22,843	20,784
O7	19,469	19,423
O8	23,249	22,953
O9	20,831	20,320
O10	23,266	19,352
Average	**21,670**	**19,942**

The morphological characteristics of *Eimeria tenella*, as determined by the structure of its oocysts, revealed an ovoid form comprising two distinct layers: an outer layer and an inner layer. The oocyst contained four sporocysts, each housing two sporozoites. According to a study by Mares et al. (2023), *E. tenella* oocysts are broad and oval shaped, featuring double-layered walls and lacking a micropylar cap. The oocyst wall is composed of two layers formed by the shedding of two specialized organelles, namely wall-forming body 1 and wall-forming body 2, during the macrogametocyte stage of Coccidia. This structural composition confers resistance to various environmental and chemical challenges, enabling oocysts to persist for extended periods. Morphometric analysis of ten *E. tenella* oocysts revealed dimensions ranging from 19-23 to µm in length and to 17-20 µm in width, with an average size of 21.670 × 19.942 µm. Otranto and Wall (2024) reported that *E. tenella* oocysts are oval, smooth, and colorless, measuring 14-31 x 9-25 µm (average 25 × 19 µm), and are characterized by the absence of a micropyle or residuum, but the presence of polar granules. The sporocysts are oval, equipped with Stieda bodies, and devoid of a residuum. Consistent with these findings, Swayne et al. (2020) described *E. tenella* as measuring 19.5-26.0 µm in length and 16.5-22.8 µm in width, with an average size of 22.0 × 19.0 µm.

Pathological lesions in coccidiosis are difficult to distinguish from necrotic enteritis (NE). Therefore, a follow-up examination was performed to observe the bacterial infection. However, *Clostridium perfringens* was not detected on bacteriological examination. Bacteriological examination of the cecum and colon revealed pink and rod-shaped bacteria. After biochemical testing, the bacteria were found to be *E. coli*, which is a normal flora in the intestine. According to Jakusné (2022), Gram staining of *E. coli* bacteria shows pink bacterial colonies that are short rod-shaped and composed of single or short paired bacteria. Strains of normal *E. coli* flora in the digestive tract benefit the host by producing vitamin K and preventing the growth of other bacteria (Luhung et al., 2017).

Effective management of coccidiosis is imperative for poultry farmers to mitigate economic loss. Strategies for prevention and treatment include sanitation, biosecurity measures, vaccination, prebiotics, and coccidiostats, with a preference for herbal origins to minimize residue accumulation (Ekawasti and Martindah, 2019). Vaccination involves administration of vaccines containing one or more developed Eimeria species (Abdisa et al., 2019). The use of prebiotics aids in controlling coccidiosis by safeguarding the intestinal mucosa and enhancing immune response. The recommended coccidiostats include sulfaquinoxalin, sulfadimethoxine, a combination of sulfadimethoxine and ormetroprim, clopidol, decoquinate, amprolium, a combination of amprolium and etopabat, nicarbazine, and lasaloside (a polyether ionophore). Additionally, herbal medicine is an alternative treatment option that potentially enhances the immune system, stimulates appetite, and reduces stress, thereby contributing to coccidiosis control (Ekawasti and Martindah, 2019).

Conclusion

A chicken case with protocol number 39/N/24 was diagnosed with severe coccidiosis with an OPG of 188,000. Based on morphological, morphometric, and predilection identification in the cecum and colon, *E. tenella* was identified. Pathological changes observed in the animals included *typhlitis hemorrhagis et necrotican* and *colitis*

necrotican. In low-morbidity infections, sick chickens should be separated and administered anticoccidiostat to avoid recurrence and spread to other chickens. Molecular examination and sequencing are required to confirm the identity of the species and strain of *E. tenella*.

Acknowledgments

The authors express their gratitude to the teaching lecturers and laboratory assistants at the Veterinary Pathology Laboratory, Veterinary Parasitology Laboratory, Veterinary Bacteriology and Mycology Laboratory, and Veterinary Virology Laboratory, Faculty of Veterinary Medicine, Udayana University, for their invaluable knowledge, the facilities provided, and permission granted for their use.

Bibliography

Abdisa, T., Hasen, R., Tagesu, T., Regea, G., & Tadese, G. (2019). Poultry coccidiosis and its prevention, control. *Journal of Veterinary and Animal Research*, *1*(1), 1.

Adams, D. S., Ruiz-Jimenez, F., Fletcher, O. J., Gall, S., & Crespo, R. (2022). Image analysis for Eimeria oocyst counts and classification. *Journal of Applied Poultry Research*, *31*(3), 100260.

Apriani, A., Ni Wayan Desi Bintari, N. W. D. B., Noor Andryan Ilsan, N. A. I., Febry Istyanto, F. I., Rochmanah Suhartati, R. S., Dewi, R. K., ... & Safari, W. F. (2023). Bakteriologi untuk mahasiswa kesehatan. PT. Masagena MAndiri Medica. ISBN: 978-602-71107-6-2

Arsyitahlia, N., Ardana, I. B. K., & Apsari, I. A. P. (2019). Prevalensi infeksi *Eimeria* spp. pda ayam pedaging yang diberi pakan tanpa antibiotik growth promoters (AGP) di Kabupaten Tabanan, Bali. *Indonesia Medicus Veterinus*, *8*(2), 186-192.

BPS (Badan Pusat Statistik). 2024. Populasi Ternak di Kabupaten Badung 2021-2023. https://badungkab.bps.go.id/indicator/24/162/1/populasi-ternak-di-kabupaten-badung.html. [Diakses 1 Mei 2025]

Brahmananda, W. G. A., Putra, I. P. C., Kardena, I. M., Mahatmi, H., & Astawa, I. N. M. (2024). Coccidiosis in broiler chickens raised in close house in Patas Village, Gerokgak, Buleleng. *Buletin Veteriner Udayana*, 16(4),1079-1092.

Correia, S., Suratma, N. A., & Oka, I. B. M. Prevalensi dan intensitas infeksi *Eimeria* spp april-mei 2021 pada ayam petelur lebih tinggi daripada ayam pedaging di Tembuku, Bangli, Bali. *Indonesia Medicus Veterinus*. *11(3),* 343-349.

Dong, H., Zhao, Q., Han, H., Jiang, L., Zhu, S., Li, T., ... & Huang, B. (2012). Prevalence of coccidial infection in dairy cattle in Shanghai, China. *Journal of Parasitology*, *98*(5), 963-966.

Ekawasti, F., & Martindah, E. (2019). Pengendalian koksidiosis pada ayam melalui pengobatan herbal. *WARTAZOA*. 29(1): 001-012.

Fitri, D. Y., Agustin, A. L. D., Kholik, K., & Janah, M. (2021). Deteksi koksidiosis pada ayam layer di Sesaot, Kecamatan Narmada, Kabupaten Lombok Barat. *Media Kedokt. Hewan*, *32*(3), 97.

Jakusné, Sz. (2022). Varying morphology of *E. coli* - an experimental study. *International Journal of Current Microbiology and Applied Sciences*, *11*(6), 262–265.

Jubb, K. V. F., Kennedy, P. C., & Palmer, N. (2016). *Pathology of domestic animals, 6th edition, vol 1*. Elsevier. Missouri.

Lawal, J. R., Jajere, S. M., Ibrahim, U. I., Geidam, Y. A., Gulani, I. A., Musa, G., & Ibekwe, B. U. (2016). Prevalence of coccidiosis among village and exotic breed of chickens in Maiduguri, Nigeria. *Veterinary World*, *9*(6), 653-659.

López-Osorio, S., Chaparro-Gutiérrez, J. J., & Gómez-Osorio, L. M. (2020). Overview of poultry Eimeria life cycle and host-parasite interactions. *Frontiers in veterinary science*, *7*, 384.

Luhung, Y. G. A., Suarjana, I. G. K., & Gelgel, K. T. P. (2017). Sensitivitas isolat *Escherichia coli* patogen dari organ ayam pedaging terinfeksi koliseptikemia terhadap oksitetrasiklin, ampisilin dan sulfametoksazol. *Buletin Veteriner Udayana*. *9(1),* 60-66.

Macdonald, S. E., Nolan, M. J., Harman, K., Boulton, K., Hume, D. A., Tomley, F. M., ... & Blake, D. P. (2017). Effects of *Eimeria tenella* infection on chicken caecal microbiome diversity, exploring variation associated with severity of pathology. *PLoS one*, *12*(9), e0184890.

Mares, M. M., Al-Quraishy, S., Abdel-Gaber, R., & Murshed, M. (2023). Morphological and molecular characterization of *Eimeria* spp. infecting domestic poultry *Gallus gallus* in Riyadh city, Saudi Arabia. *Microorganisms*, *11*(3), 795.

Matsubayashi, M., Inaoka, D. K., Komatsuya, K., Hatta, T., Kawahara, F., Sakamoto, K., ... & Kita, K. (2019). Novel characteristics of mitochondrial electron transport chain from *Eimeria tenella. Genes*, *10*(1), 29.

Mesa-Pineda, C., Navarro-Ruíz, J. L., López-Osorio, S., Chaparro-Gutiérrez, J. J., & Gómez-Osorio, L. M. (2021). Chicken coccidiosis: from the parasite lifecycle to control of the disease. *Frontiers in Veterinary Science*, *8*, 787653.

Ojimelukwe, A. E., Emedhem, D. E., Agu, G. O., Nduka, F. O., & Abah, A. E. (2018). Populations of *Eimeria tenella* express resistance to commonly used anticoccidial drugs in southern Nigeria. *International Journal of Veterinary Science and Medicine*, *6*(2), 192–200.

Putra, R., Sugito, S., Zuhrawaty, Z., Hasan, M., Rusli, R., Hamzah, A., ... & Herrialfian, H. (2020). 6. The number of erythrocytes and hematocrit value of broilers that infected by *Eimeria tenella* and given neem leaf extract combined with Jaloh extract. *Jurnal Medika Veterinaria*, *14*(2).

Robot, R., Sangari, J. R., & Toloh, B. H. (2018). Avicennia marina leaf morphometric digital data visualization in Tongkaina and Bintauna coastal areas. *Jurnal Ilmiah Platax*, *6*(1), 42-53.

Rumapea, S., Suratma, N. A., Ayu, A. A. A. M. A., Adi, M., Besung, I. N. K., & Kencana, G. A. Y. (2023). Coccidiosis in broiler chicken caused by *Eimeria tenella*. *Veterinary Science and Medicine Journal*, *5(1)*, 221-231.

Simamora, S., Apsari, I. A. P., & Dwinata, I. M. (2017). Prevalensi protozoa *Eimeria tenella* pada ayam buras di wilayah Bukit Jimbaran, Badung. *Indonesia Medicus Veterinus*, *6*(3), 254-261.

Sun, H., Su, X., Fu, Y., Hao, L., Zhou, W., Zhou, Z., ... & Shi, T. (2023). Pathogenicity and drug resistance of the *Eimeria tenella* isolate from Yiwu, Zhejiang province, eastern China. *Poultry Science*, *102*(8), 102845.

Swayne, D. E., Boulianne, M., Logue, C. M., McDougald, L. R., Nair, V., & Suarez, D.L. (2020). Disease of Poultry, 14th edition. Wiley-Blackwell. USA.

Wardani, R. A., Indrasanti, D., & Sufiriyanto, S. (2021). Pengaruh kepadatan kandang dan kebersihan terhadap tingkat infeksi koksidiosis kelinci di Kecamatan Kembaran Kabupaten Banyumas. *Jurnal Ilmiah Ilmu-Ilmu Peternakan*, *24*(2), 109-120.

Zhang, Y., Duan, B. T., Zhao, Y. J., Cui, K. L., Xu, T., Zhang, X. S., ... & Bai, R. (2023). Pathogenic mechanism of *Eimeria tenella* autophagy activation of chicken embryo cecal epithelial cells induced by *Eimeria tenella*. *Poultry Science*, *102*(4), 102535.