

**CASE REPORT: CONCURRENT ASPERGILLOSIS, STAPHYLOCOCCOSIS AND COCCIDIOSIS IN BROILER CHICKENS IN BENOA, BALI**

**Laporan Kasus: Aspergilosis, Stafilokokosis, dan Koksidirosis yang Terjadi Secara Bersamaan pada Ayam Broiler di Bena, Bali**

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**Abstract**

Co-infection of *Aspergillus fumigatus* with *Staphylococcus sp* and *Eimeria spp* that occur in poultry farming systems has the potential to increase the severity of the disease and cause significant economic losses. This case report aims to report the results of a diagnosis of coinfection of the disease in broiler chickens at one of the farms in Bena, South Kuta District, Badung Regency, Bali. The farm has a capacity of 16,000 heads with a closed cage system. The examination was carried out based on epidemiological fingerprints, anamnesis, clinical examination, pathology, bacteriological tests, mycology and parasitology. Clinical symptoms observed include anorexia, weakness, curled up, drowsiness, and being smaller than peers of his age (dwarf). Interview data showed that as many as 0.78% of the chicken population showed symptoms of disease (morbidity), with a mortality rate of 0.075%. About 1.05% of infected chickens die (CFR). The results of an anatomical pathology examination found a multisystemic form of aspergillosis characterized by many yellowish-white granulomas of various sizes in various organs, including the lungs, heart, proventriculus, ventricles, kidneys, spleen, and small intestine. On histopathological examination, there are special granulomatous lesions in which there are septated hyphae. Mycological tests showed the presence of *Aspergillus fumigatus*

*infection*. Bacteriological tests identified the presence of *Staphylococcus* sp., while parasitological tests detected the presence of *Eimeria* spp. with a value of 12,300 oocysts per gram. Based on a series of examinations carried out, the case chickens experienced aspergillosis, staphylococcosis, and coccidiosis at the same time. Coinfection of the disease needs to be a concern because it can make it difficult to diagnose and control the disease.

Keywords: *Aspergillus fumigatus*, Co-infection, *Eimeria* spp., *Staphylococcus* spp., Poultry

### Abstrak

Koinfeksi *Aspergillus fumigatus* dengan *Staphylococcus* sp dan *Eimeria* spp yang terjadi pada sistem peternakan unggas berpotensi meningkatkan tingkat keparahan penyakit serta menimbulkan kerugian ekonomi yang signifikan. Laporan kasus ini bertujuan melaporkan hasil diagnosis terhadap koinfeksi penyakit pada ayam broiler di salah satu peternakan di Bena, Kecamatan Kuta Selatan, Kabupaten Badung, Bali. Peternakan tersebut berkapasitas sebanyak 16.000 ekor dengan sistem kandang tertutup. Pemeriksaan yang dilakukan berdasarkan sidik epidemiologi, anamnesis, pemeriksaan klinis, patologi, uji bakteriologi, mikologi dan parasitologi. Gejala klinis yang diamati meliputi anoreksia, lemas, suka meringkuk, mengantuk, dan lebih kecil dari teman seusianya (kerdil). Data wawancara menunjukkan sebanyak 0,78% dari populasi ayam menunjukkan gejala penyakit (morbiditas), dengan angka kematian (mortalitas) mencapai 0,075% . Sekitar 1,05% dari ayam yang terinfeksi mengalami kematian (CFR). Hasil pemeriksaan patologi anatomi, ditemukan bentuk aspergillosis multisistemik yang ditandai dengan banyak granuloma berwarna putih kekuningan dengan berbagai ukuran di berbagai organ, termasuk paru-paru, jantung, proventrikulus, ventrikulus, ginjal, limpa, dan usus halus. Pada pemeriksaan histopatologi terdapat adanya lesi granulomatosa khas yang di dalamnya terdapat hifa berseptata. Uji mikologi menunjukkan adanya infeksi *Aspergillus fumigatus*. Uji bakteriologis mengidentifikasi keberadaan *Staphylococcus* sp., sedangkan uji parasitologi terdeteksi adanya *Eimeria* spp. dengan nilai 12.300 ookista per gram. Berdasarkan serangkaian pemeriksaan yang dilakukan ayam kasus mengalami aspergilosis, stafilokokosis, dan koksidiosis secara bersamaan. Koinfeksi penyakit perlu menjadi perhatian karena dapat menyulitkan diagnosis dan pengendalian penyakit.

Kata Kunci: *Aspergillus fumigatus*, *Eimeria* spp., Koinfeksi, *Staphylococcus* spp., Unggas

### INTRODUCTION

Broiler chickens are a type of meat-producing poultry that supports the demand for animal protein in Indonesia. Broiler chickens have tender meat and relatively rapid growth, allowing them to be harvested at 4–5 weeks of age (Kaler *et al.*, 2024). In addition, their affordable price makes this type of chicken popular and highly favored by the community. This is reflected in statistical survey results from 2020 to 2022, which showed per capita consumption levels of 6.04 kg/year, 6.55 kg/year, and 7.12 kg/year, respectively (Susanti & Putera, 2023). The increase in national chicken meat consumption is supported by population growth, increased income levels, and improved public awareness of nutrition and the benefits of consuming animal protein (Susanti & Putera, 2023). Closed-house broiler production systems are implemented to increase meat production in order to meet consumption demands. However, coinfection with various diseases can still occur in closed-house systems (Rashid *et al.*, 2019). Coinfection is an important factor affecting the economic viability of the poultry industry. Costs arising from therapeutic interventions, decreased production performance, and increased mortality result in significant financial impacts (Wernicki *et al.*, 2017).

Coinfection involving fungi of the genus *Aspergillus* with various diseases can have fatal consequences for the respiratory tract (Chu *et al.*, 2017). *Aspergillus* spp. are fungi that act as

infectious agents causing aspergillosis. Aspergillosis is commonly caused by *Aspergillus fumigatus*, *Aspergillus flavus*, and *Aspergillus niger* (Praja & Yudhana, 2018). *Aspergillus fumigatus* is the primary cause of avian aspergillosis, which is characterized by respiratory disorders (Ceolin *et al.*, 2012). A case study in broiler chickens in Tunjuk, Tabanan, Bali reported aspergillosis infection with clinical signs such as dyspnea and lethargy, accompanied by mortality in part of the population (Kaler *et al.*, 2024). *Aspergillus* spp. are cosmopolitan fungi with very small and lightweight spores, allowing them to spread easily through the air and contaminate various materials (Alvarez-Perez *et al.*, 2010). *Aspergillus* spp. are able to survive on media with high pH levels and high sugar content. *Aspergillus* spp. may exist as parasites or saprophytes; however, they can act as parasites because they are capable of producing toxins known as aflatoxins (Sharma *et al.*, 2025). *Aspergillus* spp. are commonly found in feed materials stored under high humidity conditions. *Aspergillus* spp. are considered pathogenic because they are capable of causing diseases of the respiratory tract, granulomatous inflammation of mucous membranes, the eyes, ears, skin, meninges, bronchi, and lungs (Praja & Yudhana, 2018).

Staphylococcosis is a disease caused by Gram-positive bacteria of the genus *Staphylococcus*. The clinical signs commonly observed include arthritis, synovitis, and osteomyelitis. This bacterial infection generally appears as a secondary infection that further worsens the condition of poultry (Sato & El-Gazzar, 2025). The pathogenicity of *Staphylococcus* species is associated with their ability to produce virulence factors, such as toxins and enzymes, which can damage host tissues. For example, *Staph. aureus* can cause acute septicemia affecting multiple organs and resulting in severe systemic disease (Tabar *et al.*, 2024). Coinfection of *Staphylococcus* spp. with parasitic, viral, and other bacterial infections can lead to more severe pathological changes and more extensive systemic lesions compared to single infections (Abdelhamid *et al.*, 2020).

Meanwhile, coccidiosis is a protozoal disease caused by infection with *Eimeria* spp. This disease affects the digestive tract, causing digestive disturbances and bloody diarrhea in poultry (Sari *et al.*, 2025). *Eimeria* infection can alter the composition of the intestinal microbiota in chickens. These changes include a reduction in bacterial diversity and a decrease in beneficial bacterial populations, which may ultimately increase susceptibility to secondary bacterial infections (Campos *et al.*, 2024). Various studies have reported the effects of single infections caused by *Aspergillus* spp., *Staphylococcus* spp., and *Eimeria* spp. in broiler chickens. However, studies addressing the occurrence and pathological manifestations of coinfection involving these three agents remain very limited. This case report was prepared to present the results of diagnostic confirmation of coinfection with these diseases in broiler chickens occurring at a poultry farm located in the Bena area, South Kuta District, Badung Regency, Bali Province.

## MATERIALS AND METHODS

### Case Animal

The case animal with protocol number 175/N/25 was a 13-day-old broiler chicken originating from a closed-house poultry farm located in the Bena area, South Kuta District, Badung Regency, Bali.

### Anamnesis, Physical Examination, and Epidemiological Investigation

Anamnesis was conducted through direct interviews assisted by an ambulatory veterinary examination team. Physical examination was performed based on direct inspection and palpation of the case animal within the affected population. Epidemiological data were obtained through direct observation of the farm and completion of questionnaires via interviews with the

broiler farmer, based on the epidemiological triad involving the interaction between host, agent, and environment.

### **Anatomical Pathology and Histopathological Examination**

Necropsy was performed to observe anatomical pathological changes in organs and to collect organ samples for histopathological examination. Preparation of histopathological slides was conducted at the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Udayana University. Organ samples fixed in 10% neutral buffered formalin (NBF) were dehydrated using graded ethanol, cleared with xylol, embedded in paraffin, sectioned at a thickness of 5  $\mu\text{m}$ , and routinely stained with hematoxylin and eosin (HE). Histopathological observations were performed using a light microscope at magnifications of 100 $\times$ –400 $\times$  (Kiernan, 2015).

### **Mycological Examination**

Mycological examination was initiated by fungal culture on Sabouraud Dextrose Agar (SDA) media using samples from the kidneys, lungs, spleen, and heart. The media were then incubated for 3–7 days at room temperature. This was followed by macroscopic and microscopic identification of fungi using 10% KOH solution and crystal violet fungal staining (McVey *et al.*, 2013).

### **Bacteriological Examination**

Bacterial culture and identification were performed using conventional methods. Culture and subculture of bacteria from lung, intestinal, liver, and heart samples were conducted on general Nutrient Agar (NA) media and incubated for 24 hours at 37°C. Subsequently, colonies grown from each sample on NA media underwent further identification. Identification was initiated with Gram staining, followed by catalase testing, Triple Sugar Iron Agar (TSIA), Sulfide Indole Motility (SIM), Methyl Red–Voges Proskauer (MR–VP), Simmons Citrate Agar (SCA), and glucose tests (Suarjana *et al.*, 2017).

### **Parasitological Examination**

Parasitological examination was performed using native and McMaster methods. Native examination was carried out using the wet smear method. Fecal samples were placed on a glass slide, added with 1–2 drops of distilled water, homogenized until a suspension was formed, and then covered with a cover slip. Enumeration of *Eimeria* spp. was conducted using the McMaster method. A total of 2 g of feces was weighed and diluted with distilled water to a volume of 30 mL, then homogenized. Saturated salt solution was added to the suspension to reach a final volume of 60 mL, filtered, and the filtrate was homogenized again. The suspension was then loaded into the McMaster counting chamber (two chambers) using a Pasteur pipette without air bubbles. Observations were conducted using a light microscope, and all oocysts present within the counting chamber area were counted. The number of oocysts per gram (OPG) was calculated using the McMaster formula (Zajac *et al.*, 2021).

### **Data Analysis**

Data obtained from the examinations were analyzed descriptively and presented in the form of figures and tables.

## RESULTS AND DISCUSSION

### Results

#### Clinical Signs and Epidemiological Investigation

The case chicken was observed to exhibit clinical signs including anorexia, huddling, a drowsy appearance, and the presence of yellowish-white nodules in the oral cavity. The case animal died after three days of observation. Based on interviews with the farmer, 0.78% of the chicken population showed clinical signs of disease (morbidity), with a mortality rate of 0.075%. Approximately 1.05% of the infected chickens died (case fatality rate, CFR). Chickens in the house had been vaccinated at the hatchery without subsequent booster schedules. The vaccines administered included Newcastle Disease (ND), Infectious Bursal Disease (IBD), Infectious Bronchitis (IB), and Avian Influenza (AI). Based on direct observations and information provided by the farmer during interviews, chickens exhibiting anorexia, lethargy, and inactivity were subsequently separated.

Broiler chickens on the farm were raised using a closed-house system. The building walls were constructed of brick and equipped with blower ventilation. The house consisted of a two-story building, with each level housing a population of 8,000 birds. The total population was 16,000 chickens, managed under an “all in–all out” system, meaning that only a single placement was performed without additional day-old chicks (DOC) introduced until harvest. The average house temperature ranged from 26–27°C with continuous lighting duration. Based on interviews with the farmer, humidity measurements were rarely conducted. In terms of house hygiene, drinkers were cleaned twice daily during feeding. The feed used was pellet-form concentrate feed (Japfa Comfeed Indonesia). Feed was stored in a warehouse separate from the poultry house. Litter was not replaced until harvest; however, it was turned over once daily and fresh rice husk was added if the bedding felt sufficiently moist or approximately every three days. The drinking water source was a private bore well located within the farm area, and the water was observed to be colorless and odorless. The area surrounding the poultry house appeared relatively clean and did not produce unpleasant odors.

#### Anatomical Pathology and Histopathological Examination

Anatomical pathology examination revealed hyperemia of the serosal membranes of the trachea, small intestine, and part of the large intestine. In addition, congestion was observed in the lungs, cecum, esophagus, heart, spleen, and kidneys. The lungs also showed hemorrhage accompanied by yellow nodules in both lobes (Figure 1). Nodules were also found in the heart, spleen, proventriculus, small intestine, and kidneys (Table 1). Histopathological examination revealed multifocal inflammatory and degenerative changes, including vascular congestion. Inflammation was characterized by infiltration of lymphocytes and heterophils, edema, depletion and proliferation of lymphoid cells in lymphoid organs, glomerulitis, necrosis, and invasion of fungal hyphal septa in the lungs (Figure 1, Table 1).

#### Mycological Examination

Based on isolation results, fungal growth was consistently observed from lung and kidney samples from the first to the fifth day of culture. Macroscopic observation of fungal cultures on SDA media on day 5 showed colonies with green to dark green coloration with white margins and yellowish to brown coloration on the reverse side. The colonies were circular, with smooth surfaces, even edges, and a velvety texture. Colony diameters ranged from approximately 2–3 cm (Figure 2A–B). Microscopic examination revealed septate hyphae with smooth walls, elongated conidiophores, club-shaped vesicle tips, uniseriate phialides, columnar conidia

attached to the tips of conidiophores, and bluish-green conidia (Figure 2C–D). Based on these morphological characteristics, the fungus was identified as *Aspergillus fumigatus*.

### **Bacteriological Examination**

The results of bacterial isolation and identification (Table 2, Figure 3) showed that bacteria isolated from lung samples were identified as *Staphylococcus* spp., whereas isolates from the intestine were identified as *Escherichia coli*. Based on pathological and histopathological findings, *Staphylococcus* spp. were determined to contribute to the cause of disease and death in the case animal. Meanwhile, *E. coli* was considered normal flora, as it was isolated only from intestinal samples without specific clinical or pathological findings.

### **Parasitological Examination**

Native examination revealed unsporulated *Eimeria* spp. oocysts with round to ovoid morphology, thin walls, and a single sporoblast. McMaster examination showed an oocyst count of 12,300 OPG (Figure 4). The morphology of these oocysts was consistent with previous findings (Brahmananda *et al.*, 2024; Ekawasti & Wardhana, 2019). The OPG value obtained in this case was classified as a mild infection. This finding is consistent with Hartady *et al.* (2024), who classified infection intensity into three categories: mild infection (<20,000 OPG), moderate infection (>20,000–60,000 OPG), and severe infection (>60,000 OPG).

### **Discussion**

Based on the series of examinations conducted, the case chicken was diagnosed with coinfection of *A. fumigatus*, *Staphylococcus* spp., and *Eimeria* spp., with *A. fumigatus* identified as the primary cause of death. Morphologically, both macroscopic observations on SDA media and microscopic fungal staining showed that *A. fumigatus* isolated from the case chicken (Figure 2) was consistent with previous studies. One such study by Putri *et al.* (2021) reported that macroscopically, *A. fumigatus* colonies initially appear as white velvety growth and later change to green to dark green with white margins, while the reverse side becomes yellowish to brown. Microscopically, *A. fumigatus* exhibits conidiophore formation with conidial heads resembling columns, consisting of flask-shaped vesicles, uniseriate phialides, and long chains of conidia.

*Aspergillus fumigatus* is the main causative agent of aspergillosis in poultry (Seyedmousavi *et al.*, 2015). This species is frequently reported to infect chicks and young chickens (Ulloa-Avellán *et al.*, 2022). This is consistent with the present case, in which the chicken was 12 days old. Transmission occurs through inhalation of spores dispersed in the air around the poultry house. Inhaled spores enter the bloodstream and subsequently cause damage to various organs, particularly the lungs (Ceolin *et al.*, 2012). The occurrence of *Aspergillus* spp. infection is closely associated with predisposing factors, especially those related to environmental conditions and housing management, such as inadequate ventilation, high humidity, dust, wet litter, or moldy feed (Prajā & Yudhana, 2018).

Although aspergillosis primarily affects the respiratory tract, it can manifest systemically in both acute and chronic forms. Acute aspergillosis caused by massive conidial inhalation is commonly observed in young poultry, whereas the chronic form is associated with immunosuppression and occurs sporadically in older birds. Chronic aspergillosis may develop following the acute phase (Arné & Lee, 2020; Seyedmousavi *et al.*, 2015). In this case, multisystemic aspergillosis was observed, characterized by yellowish-white granulomas of varying sizes in multiple organs, including the lungs, heart, proventriculus, ventriculus, kidneys, spleen, and small intestine (Table 1, Figure 1).

Histopathological examination revealed inflammatory cell infiltration dominated by lymphocytes and granulomatous inflammation in affected organs (Figure 1). Multisystemic aspergillosis with unusual vertebral osteomyelitis in a turkey flock in Bordj Bou Arreridj Province, Algeria, has been reported. Systemic aspergillosis is characterized by numerous yellowish-white granulomas of varying sizes in multiple organs, including the lungs, air sacs, myocardium, pancreas, liver, spleen, proventriculus, gizzard, small intestine, peritoneum, and kidneys (Belalmi *et al.*, 2025).

In this case, histopathology of the trachea showed deciliation as well as edema and inflammatory cell infiltration in the submucosa. These lesions arise due to exposure to secondary metabolite toxins produced by fungi. These metabolites include aflatoxins or gliotoxins, which possess cytotoxic properties and can damage the respiratory epithelium (Jayanthi *et al.*, 2025). Although granulomas were not observed in the trachea in this case, such lesions may theoretically occur. Infected tracheal tissue often shows granulomatous inflammation, characterized by granuloma formation consisting of aggregates of macrophages, lymphocytes, and multinucleated giant cells surrounding fungal elements (Hamid *et al.*, 2021).

In this case, the lungs exhibited the most severe lesions resulting from *A. fumigatus* and *Staphylococcus* spp. infection. This was indicated by the presence of extensive granulomatous nodules throughout the pulmonary parenchyma. These nodules were likely the primary cause of death in this broiler chicken. Histopathologically, the nodules were characterized by central necrosis surrounded by a fibrous capsule infiltrated by heterophils, lymphocytes, macrophages, and multinucleated giant cells. Septate hyphae were present within the necrotic center of the granulomas (Belalmi *et al.*, 2025).

The presence of fungal hyphae in the lungs indicates that inhalation is the primary route of *Aspergillus* infection (Jayanthi *et al.*, 2025). Granulomatous inflammation typically arises as a response to persistent infectious agents, including fungi. This type of inflammation is characteristic of chronic infections that cannot be rapidly eliminated by the immune system (Arné *et al.*, 2021; Hamid *et al.*, 2021). The immune system and its functions are not fully developed in broiler chickens aged 6–13 days and only reach maturity at approximately 34 days of age (Song *et al.*, 2021). Therefore, broiler chickens exposed to high concentrations of spores, particularly during early life stages, are highly susceptible to *Aspergillus* spp. infection (Jayanthi *et al.*, 2025; Zamboni *et al.*, 2020). This supports the present case, in which the chicken was only 12 days old.

Preventive measures against *Aspergillus* spp. infection include maintaining sanitation of the poultry house and rearing environment, discarding contaminated equipment and feed, and ensuring that feed provided is free from contamination. Production equipment such as feeders and drinkers should also be routinely cleaned and disinfected (Hayani *et al.*, 2017). Rice husk litter should be used in a clean, dry, and fresh condition. Air circulation within the poultry house should be improved, and humidity must be controlled to inhibit the growth and spread of airborne spores (Kaler *et al.*, 2024).

In this case, *A. fumigatus* was determined to be the primary infection based on the observed pathological changes. Nevertheless, secondary infections with *Staphylococcus* spp. and *Eimeria* spp. were also present. Meanwhile, *E. coli* was considered a secondary infection, as it was isolated only from the intestine without specific clinical or pathological findings. *Staphylococcus* spp. were isolated from lung samples (Table 2). Hamid *et al.* (2021) reported that *Staphylococcus* spp. and *Micrococcus* spp. can act as secondary infections in aspergillosis affecting 13-day-old chickens in Malaysia, with isolates obtained from the liver, brain, air sacs, and kidneys.

*Staphylococcus* spp. are normal commensal bacteria found on the skin and mucosal membranes of animals (Tabar *et al.*, 2024). *Staph. aureus* is known to cause embryonic mortality, omphalitis and yolk sac infection, arthritis and synovitis, osteomyelitis, vesicular dermatitis, gangrenous dermatitis, and pododermatitis, while systemic infection may occur through septicemia (Tabar *et al.*, 2024). Respiratory infection occurs through inhalation of air contaminated with bacteria from dust, feces, or poultry equipment. Once inhaled, the bacteria infect the upper respiratory tract and spread to the lungs, particularly when the poultry immune system is weakened or when other respiratory infections are present (Soedarto, 2015). The isolation of *Staphylococcus* spp. from lung samples in this case further worsened the clinical condition.

In this case, secondary infection with *Eimeria* spp. was classified as mild. Infection occurs through ingestion of sporulated oocysts present in the environment. Oocysts are the infective form excreted in the feces of infected poultry and subsequently undergo sporulation under suitable environmental conditions, including humidity, oxygen availability, and temperature (Brahmananda *et al.*, 2024). After ingestion and entry into the digestive tract, the oocyst wall ruptures in the ventriculus, releasing sporocysts containing sporozoites. Sporozoites are then released by digestive enzymes, penetrate intestinal epithelial cells (particularly in the small intestine and cecum), and initiate the parasite reproductive cycle within epithelial cells (Sari *et al.*, 2025). During this phase, *Eimeria* spp. reproduce both asexually and sexually, forming new oocysts that are excreted in feces and continue the infection cycle (Dewi *et al.*, 2024).

Clinical signs caused by *Eimeria* spp. reproduction include diarrhea, depression, anemia, dehydration, fatigue, and weight loss (Dauguschies & Najdrowski, 2005; Masneno *et al.*, 2023). Higher *Eimeria* spp. OPG counts in feces are directly proportional to the degree of tissue damage. High-intensity infections reaching 100,000 OPG can result in increased morbidity and mortality rates as well as reduced body weight (Rumapea *et al.*, 2023). Pathological changes caused by *Eimeria* spp. infection include hemorrhage, intestinal mucosal erosion, inflammatory cell infiltration in the lamina propria, and the presence of schizonts (Brahmananda *et al.*, 2024; Rumapea *et al.*, 2023). In contrast, in this report, erosion of the cecal and small intestinal mucosa accompanied by nodules characteristic of aspergillosis was observed during anatomical pathology examination, but schizonts were not detected (Figure 1J–L).

*Eimeria* spp. infection commonly affects chickens older than two weeks (3–18 weeks), but rarely affects chickens younger than two weeks of age (Dakpogan & Salifou, 2013; Simamora *et al.*, 2017). Chickens younger than two weeks produce limited trypsin and bile salts, thereby preventing sporozoite release from oocysts. Nevertheless, when young chickens become infected, mortality rates may be higher than in adult birds (Dakpogan & Salifou, 2013). The young age of the chicken in this case may explain why schizonts were not widely detected. The case chicken was only 13 days old, suggesting that *Eimeria* spp. had only recently begun their reproductive cycle.

## CONCLUSIONS AND RECOMMENDATIONS

### Conclusions

The case chicken was confirmed to have a coinfection with *A. fumigatus*, *Staphylococcus* spp., and *Eimeria* spp. based on a series of diagnostic examinations performed. The primary disease contributing to mortality was aspergillosis, while staphylococcosis and coccidiosis acted as secondary causes that exacerbated disease pathogenesis. This case of coinfection underscores that multiple infectious agents may occur concurrently in a single chicken and highlights the need for a more comprehensive approach to diagnosis, prevention, and therapy.

## Recommendations

Prevention of aspergillosis spread at the farm should be implemented through several measures, namely (1) optimal control of the housing environment with respect to cleanliness and ventilation, (2) strict application of hygiene and sanitation practices, (3) proper feed management to prevent contamination, and (4) reduction of stress levels that may induce immunosuppression. Molecular testing and sequencing are recommended for *Staphylococcus* spp. and *Eimeria* spp. that contributed as concurrent agents in this case.

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### Tables

Table 1. Gross pathological and histopathological changes in the case chicken

No	Organ	Gross Pathology	Histopathology
1	Trachea	Slight reddening of the serosal membrane	Edematous tracheitis
2	Esophagus	Discoloration observed in several areas of the mucosa	Esophagitis
3	Lungs	Congestion, hemorrhage, multifocal yellowish nodules in both lobes, and altered consistency	Granulomatous pneumonia
4	Heart	Congestion, nodules on the serosal membrane	Myocarditis
5	Liver	Uneven discoloration	Hepatitis
6	Kidneys	Congestion, nodules on the serosal margins	Necrotizing glomerulonephritis
7	Spleen	Nodules on the serosal membrane	Splenitis
8	Proventriculus	Yellowish nodules on the serosa	Proventriculitis
9	Small intestine	Nodules, hemorrhage on the serosa and mucosa	Enteritis
10	Cecum	Discoloration at the peripheral area	Typhlitis
11	Brain	Normal	Encephalitis
12	Pancreas	Normal	Pancreatic congestion
13	Crop	Reddish discoloration of the mucosa	Normal
14	Ventriculus	Yellowish nodules at the proventriculus–ventriculus junction	Histological preparation not performed

Table 2. Results of bacteriological examination of lung and intestinal organs

No	Media / Examination	Lung	Intestine
1	Nutrient Agar	Circular colonies, entire margins, convex elevation, opaque yellow color, 2–3 mm in diameter	Circular colonies, entire margins, convex elevation, milky white, opaque, 3–4 mm in diameter
2	Gram stain	Cocci, purple-colored (Gram-positive) arranged in clusters (staphylococci)	Rod-shaped (bacilli), red-colored (Gram-negative) arranged singly or in short chains
3	Catalase test	Positive	Positive
4	Triple Sugar Iron Agar	Acid slant (+), acid butt (+), H <sub>2</sub> S (-), gas (-)	Acid slant (+), acid butt (+), H <sub>2</sub> S (-), gas positive (+)
5	Simmons Citrate Agar	Negative (-)	Negative (-)

6	Sulphide Indole Motility (SIM)	Motility (-), indole (-), sulphide (-)	Motility (+), indole (+), sulphide (-)
7	Methyl Red–Voges Proskauer	MR (+), VP (-)	MR (+), VP (-)
8	Glucose test	Positive	Positive
Conclusion		<i>Staphylococcus</i> spp.	<i>Escherichia coli</i>

### Figures

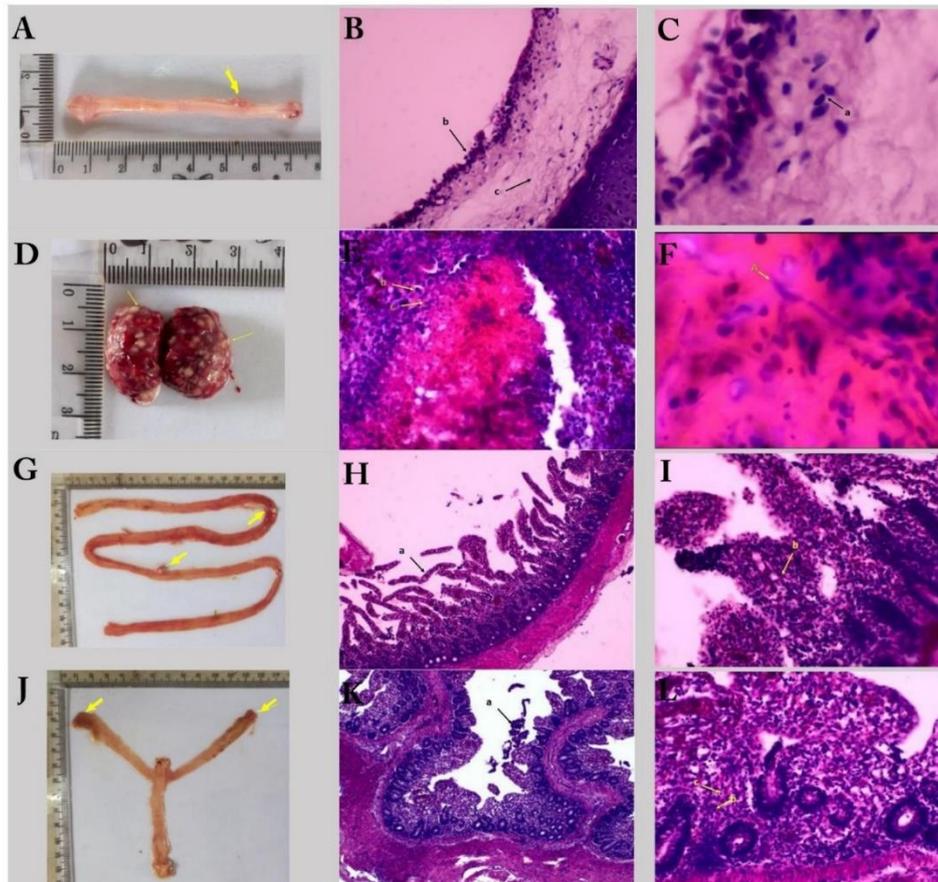


Figure 1. Macroscopic and histopathological lesions in the trachea (A–C), lungs (D–F), small intestine (G–I), and cecum (J–L). Macroscopically, the tracheal serosa exhibited mild hyperemia. Histologically, edematous tracheitis (B, C, 400× & 1000×) was characterized by inflammatory cell infiltration in the submucosa (a), epithelial deciliation (b), and edema (c). The lungs showed congestion, hemorrhage, and numerous whitish-yellow nodules (yellow arrows); microscopic examination revealed granulomatous pneumonia with inflammatory cell infiltration consisting of macrophages (b) and lymphocytes (c) (E, 400×), as well as the presence of septate hyphae within the lesions (a) (F, 1000×). In the small intestine, macroscopically visible whitish-yellow nodules were observed on the intestinal ligament (yellow arrows) accompanied by hemorrhage of the serosa and mucosa, while histopathological findings showed enteritis characterized by villous erosion (a) (H, 100×) and inflammatory cell infiltration within the villi (b) (I, 400×). The cecum exhibited peripheral reddening, with histological features of typhlitis characterized by villous erosion (a) (K, 100×) and infiltration of inflammatory cells consisting of macrophages (b) and lymphocytes (c) in the lamina propria (K, 400×). (HE Staining).

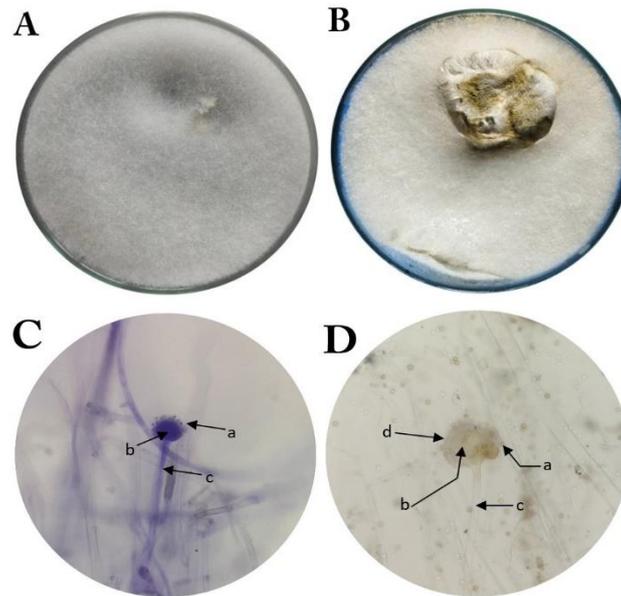


Figure 2. (A, B) Macroscopic identification of fungal cultures on SDA media; (C) microscopic examination with crystal violet staining and (D) 10% KOH solution. Description: (a) conidia; (b) vesicle; (c) conidiophore; (d) phia. Gram staining of samples showing growth of (A) *Staphylococcus* spp. from lungs.

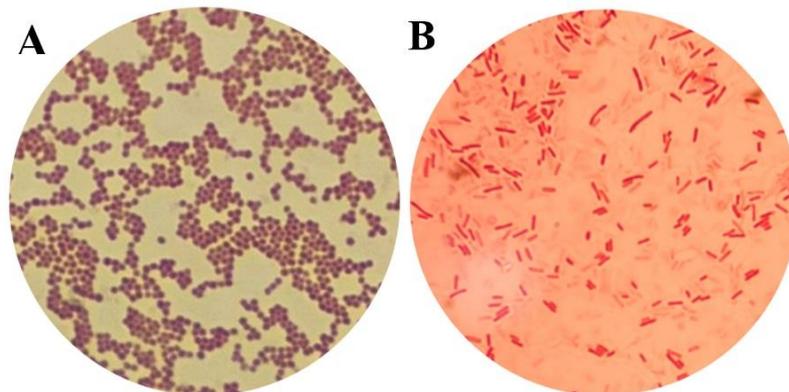


Figure 3. Gram staining of samples showing growth of (A) *Staphylococcus* spp. from lung tissue and (B) *E. coli* from intestinal tissue.

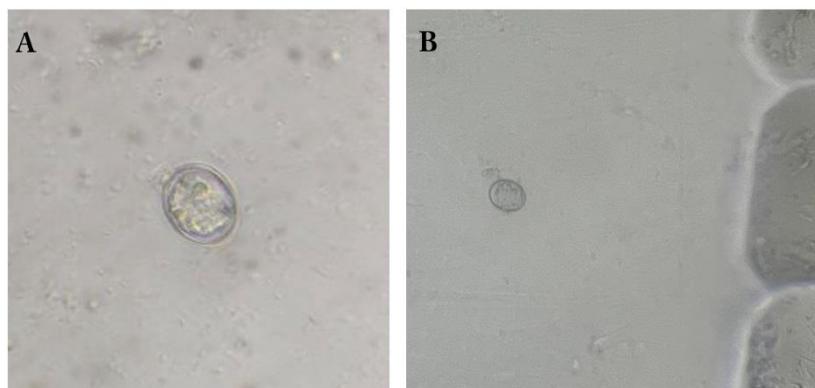


Figure 4. *Eimeria* spp. identified through native examination (A) and McMaster method (B), with an OPG count of 12.000.