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CASE OF MULTIPATHOGEN-INDUCED MORTALITY IN AN OPEN-HOUSE BROILER FARM IN DEMULIH VILLAGE, BANGLI

Kasus Infeksi Multipatogen Penyebab Kematian Ayam Broiler Kandang Terbuka di Desa Demulih, Bangli

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Abstract

The health and performance of commercial chicken are greatly influenced by poultry house environmental management. The application of open house system with poor management may act as a predisposing factor for multipathogen colonization in poultry. This condition can lead to multiple diseases due to concurrent infections involving fungal, bacterial, and protozoal agents. This study reports a case of multipathogen-related mortality in 29-day-old broiler chicken from an open house farm in Demulih Village, Bangli Regency, Bali. Diagnostic methods include anamnesis, physical examination, epidemiological data collection, gross pathology, histopathology, bacteriology, mycology, and parasitology. Gross pathological examination revealed yellowish-white nodules in the body cavity, lungs, and kidneys; surface damage of the liver and kidneys; and hemorrhage in the cecum. Histopathology showed septate hyphae and conidiophores in the lungs as well as schizonts in the cecum, indicating fungal and protozoal infection. Mycological examination using Sabouraud Dextrose Agar and subsequent macroscopic and microscopic identification confirmed the presence of Aspergillus flavus, Aspergillus fumigatus, and Mucor spp. Bacteriological examination identified colonies of Staphylococcus sp., Acinetobacter sp., and Klebsiella sp. Parasitological analysis of fecal samples revealed Eimeria spp. oocysts at a concentration of 34,450 oocysts/gram, classified as

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moderate infection. The case was diagnosed as multiple disease conditions caused by concurrent multipathogen infections of fungal, bacterial, and protozoal origin. Improvements in housing management, sanitation, feed storage, strict biosecurity, and housing system considerations are required to minimize the introduction of disease-causing pathogens.

Keywords: Multipathogen, aspergillosis, mucormycosis, multibacterial, coccidiosis

Abstrak

Kesehatan dan performa ayam komersial sangat dipengaruhi oleh manajemen lingkungan kandang. Penerapan sistem kandang terbuka dengan manajemen yang buruk dapat berpotensi menjadi faktor predisposisi kolonisasi multipatogen pada ayam. Hal ini dapat berujung pada kejadian multipenyakit yang diakibatkan oleh infeksi konkuren yang melibatkan agen jamur, bakteri, dan protozoa. Studi ini melaporkan kasus kematian multipenyakit pada seekor ayam broiler berumur 29 hari dari peternakan dengan sistem kandang terbuka di Desa Demulih, Kabupaten Bangli, Bali. Metode diagnosis meliputi anamnesis, pemeriksaan fisik, data epidemiologi, pemeriksaan patologi anatomi, histopatologi, bakteriologi, mikologi, dan parasitologi. Hasil pemeriksaan patologi anatomi menunjukkan temuan nodul putih kekuningan pada rongga tubuh, paru-paru dan ginjal, rusaknya permukaan hati dan ginjal, serta hemoragi pada sekum. Pemeriksaan histopatologi menunjukkan temuan septa hifa dan konidiofor pada paru-paru serta skizon pada sekum. Pemeriksaan mikologi melalui penanaman pada media *Saburaud Dextrose Agar* dan identifikasi dengan metode makroskopis dan mikroskopis menunjukkan ayam kasus positif pertumbuhan *Aspergillus flavus*, *Aspergillus fumigatus*, dan *Mucor* spp. Pada pemeriksaan bakteriologi teridentifikasi koloni *Staphyloccocus* sp., *Acinetobacter* sp., dan *Klebsiella* sp. Hasil pemeriksaan parasitologi pada sampel feses menujukkan temuan ookista *Eimeria* spp. dengan jumlah ookista sebanyak 34.450 ookista/gram yang tergolong infeksi sedang. Ayam kasus didiagnosa mengalami multipenyakit akibat infeksi konkuren multipatogen fungal, bakteri, dan protozoa. Perlu adanya perbaikan manajemen kandang, sanitasi, penyimpanan pakan, biosekuriti yang ketat, serta pertimbangan sistem kandang untuk meminimalisir masuknya patogen penyebab penyakit.

Kata kunci: Multipatogen, aspergillosis, mukormikosis, multibakterial, coccidiosis

INTRODUCTION

In commercial chicken farming, housing management significantly influences group size, freedom of movement, and environmental complexity, all of which can actively influence stress levels and immunity (El-Deek and El-Sabrout, 2018). Potential stressors resulting from poor rearing environments are known to threaten chicken health by disrupting immune system modulation, endangering their welfare and health due to potential disease outbreaks (Hofmann *et al*., 2020). The selection of cage design, environmental sanitation, and air circulation play a crucial role in supporting optimal health, growth, and production performance in chicken (Oloyo and Ojerinde, 2019). Poor cage conditions, such as poor sanitation, fecal accumulation, damp husks, and poor ventilation, can create environmental stress in chicken and trigger the growth of opportunistic pathogens that have the potential to infect and cause disease (Zhou *et al*., 2020; Wang *et al*., 2023). Suboptimal cage management conditions can be a significant predisposing factor for multipathogen colonization and an increased risk of disease in chicken.

The complex multicausal interaction between co-infection of various pathogens and environmental factors is the main cause of high mortality in chicken, which poses a major threat to the poultry industry (Huixin *et al*., 2025; Zhou *et al*., 2020). Several opportunistic pathogens that are frequently reported include respiratory tract infections caused by opportunistic fungi such as Aspergillus sp., which produce spores (conidia) that remain viable under extreme

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conditions, manifested clinically by respiratory disorders (Sultana *et al*., 2015). Co-infection with Mucor spp., a saprophytic mold that is generally non-pathogenic, can act as an opportunistic pathogen in immunosuppressed conditions caused by Aspergillus sp. infection, which then causes mucormycosis (Skiada *et al*., 2018). Not only limited to opportunistic fungal infections, parasitic protozoa are also important factors in the complexity of concurrent infections in chicken. Eimeria spp., an internal parasitic protozoa that attacks the intestinal epithelium, can cause mucosal damage and diarrhea, thereby increasing intestinal permeability and facilitating the invasion of opportunistic bacteria into the bloodstream, which then causes Coccidiosis infection (Chapman, 2013). Damage to lung, liver, and kidney tissue due to Aspergillosis and Mucor spp. co-infection, as well as damage to the digestive tract due to Eimeria spp., facilitates the entry of opportunistic bacteria, resulting in multi-bacterial infections (Mahalingam *et al*., 2022).

This study aims to examine a case of multicausal mortality in 29-day-old broiler chicken from an open-house farm in Demulih Village, Bangli Regency, Bali. To date, reports of multidisease events involving fungal, protozoan, and bacterial infections in broiler chicken are still limited, particularly in smallholder farms with an open-house system. This situation is important to report because it can provide further understanding of the complex relationship between husbandry management and the potential for multipathogenic infections that can contribute to mortality in chicken. Therefore, these findings are expected to provide a basis for evaluating husbandry management and developing more effective disease prevention strategies.

RESEARCH METHOD

Case Animal

Animal with protocol number 170/N/2025 which is used as a study, is a 29-day-old broiler chicken,The animal originated from a chicken farm located in Demulih Village, Susut District, Bangli Regency, Bali. The animal died on July 13, 2025, and a necropsy was immediately performed at the Veterinary Pathology Laboratory to examine organ changes and to collect organ samples for testing at the Bacteriology and Mycology Laboratory and the Veterinary Parasitology Laboratory, Faculty of Veterinary Medicine, Udayana University.

Signalement, Anamnesis, and Epidemiological Screening

Data collection on signalement, anamnesis, and epidemiology was conducted through observation and interviews with the barn managers. Data collected included age, sex, clinical signs, physical examination results, barn type, husbandry management, biosecurity, population size, number of sick animals, and number of deaths.

Anatomical Pathology and Histopathology Examination

The necropsy procedure was performed at the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Udayana University. The observed pathological changes were documented and recorded. After examination, the organs were cut into 1x1x1 cm pieces and fixed in 10% Neutral Buffered Formaldehyde (NBF) solution for histopathology preparation. Some samples were taken for examination in the Bacteriology and Fungal Laboratory, as well as in the Parasitology Laboratory.

The process of making histopathological slides from tissue fixed in 10% NBF is continued with rough cutting (trimming), then immersion in 70% alcohol. Dehydration and clearing are carried out using alcohol with graded concentrations (80%, 90%, 95%, and absolute), followed by clarification using xylene. The next stage is embedding and blocking in liquid paraffin at a temperature of 58–60ºC. The resulting paraffin block is then cut thinly (4–6 µm) using a rotary

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microtome, placed on a glass slide, and stained using the Hematoxylin-Eosin (HE) method. The staining process includes deparaffinization with xylene, gradual dehydration, staining using Hematoxylin-eosin, clarification, and covering with Entellan®. Finally, the slide is observed under a microscope at 40–1000x magnification to identify microscopic changes in the tissue (Slaoui and Fiette, 2010).

Isolation and Identification of Fungi

Fungal isolation and identification were conducted in the Veterinary Bacteriology and Mycology Laboratory, Faculty of Veterinary Medicine, Udayana University. Sabouraud Dextrose Agar (SDA) was used for fungal isolation. Isolation was performed by aseptically planting 1 cm3 samples of lung, liver, kidney, and heart on the surface of the SDA medium. The medium was incubated at room temperature for 3-7 days.The samples were then observed macroscopically daily, noting the shape, size, texture, surface color, underside color, and growth period of the fungus. After isolating the fungus, the fungal identification process was carried out microscopically by first staining the fungus. The sample was placed on a glass slide and then dripped with 10% KOH or stained with methylene blue to observe the morphology of the hyphae, the shape and arrangement of the conidia, and the structure of the conidiophores (Suarjana *et al*., 2024).

Isolation and Identification of Bacteria

Bacterial isolation and identification were conducted at the Veterinary Bacteriology and Mycology Laboratory, Faculty of Veterinary Medicine, Udayana University. Trachea, lung, liver, and kidney organ samples were planted on Nutrient Agar (NA) media using the streak line method. Subcultures were then carried out from the first NA media to the second NA media to purify the colonies. Bacterial colonies were planted on MacConkey Agar (MCA) media. The next stage, the colonies were subjected to primary tests, namely catalase and gram staining tests, biochemical tests including Triple Sugar Iron Agar (TSIA), Sulfide Indole Motility (SIM), Methyl Red (MR), Voges Proskauer (VP) and Simmons Citrate Agar (SCA), and glucose tests (Suarjana *et al*., 2024).

Parasitology Examination

The examination was conducted at the Parasitology Laboratory of the Faculty of Veterinary Medicine, Udayana University. Protozoa were identified on fecal samples from the case animal placed in pots containing a 10% NBF and potassium solution. The collected fecal samples were then examined qualitatively using native, sediment, and flotation methods, and quantitatively using the McMaster method (Zajac *et al*., 2021).

RESULTS ANDDISCUSSION

Results

Signalement, Anamnesis, and Clinical Signs

The chicken with protocol number 170/N/2025 is a 29-day-old broiler chicken originating from a broiler farm located in Demulih Village, Susut District, Bangli Regency, Bali. The farm is an open-house broiler farm with a population of approximately 500 chicken. The case chicken is one of the chicken raised as DOC (Day Old Chick) and has received the hatchery Newcastle Disease (ND) and Infectious Bursal Disease (IBD) vaccines. The case animal has shown clinical symptoms in the form of respiratory disorders with clinical manifestations of dyspnea, breathing with an open beak, symptoms of digestive disorders with manifestations of diarrhea mixed with blood, as well as non-specific clinical disorders in the form of lethargy, anorexia,

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cachexia, and dull feathers that have been seen since the chicken was 14 days old. Similar symptoms were also observed in 50 chicken in the field.

Epidemiological Screening

Of a population of approximately 500 chicken, an estimated 50 chicken were sick with similar symptoms and 15 chicken died within 14 days. The farm manager stated that the decline in activity and the onset of symptoms occurred since the onset of the rainy season with erratic rainfall over the past month. Farming is carried out in an open house. During the inspection, biosecurity was deemed quite poor, as reflected in the unkempt farming environment, high levels of moisture in the husks mixed with rain and large amounts of chicken droppings mixed with blood, the absence of isolation efforts between sick and healthy chicken, the presence of 2 dead chicken in the farming environment that had rotted with anorexia and dull feathers that were not immediately buried, poor feed storage so that the feed looked damp, livestock tended to drink puddles of rainwater, and the suboptimal restrictions on the entry and exit of officers in the farm and the absence of disinfection efforts.

Anatomical Pathology and Histopathology Examination

Post mortem anatomical pathology examination is macroscopic when the organ is firster Look Aspiration from the body cavity showed typical granuloma lesions in the form of yellowish white with a solid consistency in the body cavity, air sacs, lungs, and kidneys, as well as pericarditis in the heart, indicating a fungal infection (Mahmoud *et al*., 2021). Diffuse hemorrhages were found in the small intestine, and hemorrhages were seen in the cecum, indicating a disorder that may have occurred due to a protozoan infection.(Chapman, 2013). histopathological examination revealed histopathological changes in almost all organs (Table 1). Important findings from this histopathological examination were the presence of hyphal septa and conidiophores of Aspergillus sp. and Mucor spp. in the lungs, and the presence of Eimeria spp. schizonts in the cecum sample (Figure 1).

Isolation and Identification of Fungi

The results of fungal isolation on Sabouraud Dextrose Agar (SDA) media showed the presence of three different types of fungal colonies (Figure 2) which were confirmed through microscopic observation (Figure 3). In the first colony, the colony grew rapidly on the first day of incubation, with macroscopic observation of the colony morphology appearing white to grayish, fast growing, and cotton-like texture.(cottony)or wool(fluffy), as well as microscopic observation of the tip of the sporangiophore, there is a round to oval sporangium with thin walls that are easily broken, containing many sporangiospores that match the identification of Mucor spp. (Ellis *et al*., 2007). In the second colony, the colony grew on the fourth day of incubation, with macroscopic observation of the colony morphology being bluish green to grayish, granular textured and flat to slightly hilly surface and the results of microscopic observations showed round, rough, golden green conidia, forming long chains that match the identification of the Aspergillus fumigatus colony (Klich, 2002). In the third colony, the colony grew on the fourth day of incubation, with macroscopic observation of the colony morphology being yellowish green with a velvety to granular surface, with microscopic observation of columnar conidia resembling a dense and short brush, bottle-shaped vesicles, phialids covering the top, forming a dense and short chain that corresponds to the identification of the Aspergillus flavus colony (Samson *et al*., 2014).

Isolation and Identification of Bacteria

The results of bacterial isolation from samples of trachea, lungs, kidneys, and heart organs are presented in Table 3 and Figure 4. The isolation results show the presence of colony growth in

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lung and kidney organ samples, while no bacterial colony growth was found in the trachea and heart.Based on the attached results, it can be concluded that the bacteria isolated in the Lung colony lead to Staphylococcus sp., Kidney colony 1 leads to Klebsiella sp., and Kidney colony 2 leads to Acinetobacter sp. (El-Ghany, 2021; Mourão *et al.*, 2024; Yu *et al.*, 2024).

Parasitology Examination

The results of qualitative stool examination using native, sedimentation, and floating methods successfully found ovoid-shaped oocysts with two-layered walls, smooth and without micropyles, and oocysts containing one sporoblast and sporulation (figure 5). These oocysts were identified as Eimeria spp. (Azmy *et al.*, 2015). Quantitative examination using the McMaster method was carried out twice with the results of 33,600 oocysts/gram and 35,300 oocysts/gram, so that the average number of oocysts was 34,450 oocysts/gram, with a moderate degree of infection (Arsyitahlia *et al.*, 2019).

Discussion

Clinical signs in chicken are manifested chronically (± 14 days) with respiratory disorders such as dyspnea and open-beak breathing. Respiratory tract infections are a significant threat to the poultry industry because they involve various pathogens that cause respiratory disease syndrome in birds (Dyar *et al.*, 1984). Co-infections are common and often result in more severe clinical manifestations than single infections, resulting in a two-way synergistic interaction between respiratory pathogens. which are present simultaneously or compete with each other (concurrent)) which then contributed to severe clinical manifestations and high mortality (Huixin *et al.*, 2025). Other clinical manifestations in the chicken were shown by digestive disorders in the form of diarrhea mixed with blood and non-specific clinical disorders such as lethargy, anorexia, cachexia, and dull feathers. In poultry, gastrointestinal metabolism is highly dependent on intestinal integrity, which is clearly demonstrated by the overall physiological condition (Gazoni *et al.*, 2019). In this case, the synergistic interaction between respiratory disorders and impaired intestinal integrity emphasizes the complexity of the case, as both mutually exacerbated the clinical manifestations in the chicken.which is a major threat to the poultry industry (Huixin *et al*., 2025; Zhou *et al*., 2020).

Findings of anatomical pathology in the body cavity, air sacs, lungs and kidneys showed findings of granuloma lesions in the form of yellowish white nodules that are typical in cases of Aspergillosis. Zeinab *et al*. (2024) wrote that in birds with Aspergillosis cases, the lungs, air sacs, and other organs such as the kidneys are the main locations for the spread of Aspergillus sp. spores, which are indicated by manifestations in the form of typical lesions.White to yellowish granulomas of varying sizes (milli to 2 cm) involving the serosa and parenchyma of one or more organs. These findings vary depending on the acute and chronic course of the disease (Arné and Lee, 2020), where in chronic cases, on the surface of the slice, one area of necrosis or several areas can be seen, which is in accordance with the findings of the anatomical pathology of the chicken case which shows the presence of local necrosis areas in the lungs. In histopathological reading of the lung organs, the presence of septate hyphae with acute branching of around 45° which is often found in cases of Aspergillosis and hyphae with blunt branching approaching 90° with the characteristics of wide and aseptate hyphae which is often found in cases of Mucormycosis was found with the identification of fungal pathogens based on Ganesan (2022).

The presence of fungal hyphal elements found together with a granulomatous inflammatory pattern in histopathological readings indicates that the agent plays a direct role in the pathological process. The growth of fungal hyphae in the respiratory tract will cause necrosis and inflammation of the air sacs and lungs, inhibiting oxygen exchange which is manifested by

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clinical signs of difficulty breathing due to hypoxia, so that birds compensate for tissue oxygen needs by increasing the frequency of the respiratory rate (Ahamad *et al*., 2018; Nururrozi *et al*., 2020). In addition to respiratory disorders, hyphal invasion can alsospread to other organs such as the liver, heart, spleen, kidneys and intestines, even distributed to other tissues such as the pericardium, brain, and bone marrow due to the distribution of hyphae through the blood throughout the tissue (Mahmoud and Shaapan, 2021; Kousha *et al*., 2011). Based on Mahmoud and Shaapan (2021), heterophils, lymphocytes, monocytes, and a number of large cells in other organs show a chronic inflammatory pattern, where this was found in cases, confirming that the fungal infection process in chicken cases has been going on for a long time and has spread systemically.Elfdaly *et al*. (2017) stated that cases of fungal respiratory infections in poultry are generally a reflection of poor husbandry management, both on small farms and in commercial farms, which occurs chronically.

Fungal identification was performed through mycological culture on Saburaud Dextrose Agar (SDA) media. Fungal identification in this case was limited to macroscopic and microscopic observations according to the literature without molecular confirmation, so there were limitations in determining the species with high certainty. The results of lung, kidney, and liver organ cultures showed the growth of three fungal colonies, which were identified as A. fumigatus (Klich, 2002), A. flavus (Samson *et al*., 2014), and Mucor spp. (Ellis *et al*., 2007), respectively.*A. fumigatus*and A. flavus are known to produce a number of dangerous mycotoxins that can cause further complications. According to Marta *et al*. (2018), A. fumigatus is known to be the main cause of Aspergillosis in poultry associated with respiratory tract infections with the main toxin gliotoxin, while A. flavus is associated with hepatotoxic effects and decreased growth and production of poultry with the main toxin Aflatoxin. Aflatoxin and gliotoxin are known to be mycotoxins.which are carcinogenic andimmunosuppressivewhich can disrupt the function of the immune system (Kalkayeva *et al*., 2023; Arné *et al*., 2011). This can be reflected in changes that occur in the liver, kidneys, spleen, and pancreas (Table 1). Aflatoxins can cause inflammation in the liver and kidneys, disrupting the function of each organ in detoxification, filtration, and excretion (Kurniasih and Prakoso, 2019). In histopathological readings, lymphoid depletion in the spleen was shown, which may reflect a decrease in host immune capacity due to lymphocyte consumption in the chronic inflammatory response to Aspergillus spp. This can be exacerbated by the immunotoxic effects of fungal metabolites such as gliotoxins which are lymphocytotoxic (Khoufache *et al*., 2007; Latgé and Chamilos, 2019), although this cause is not limited to fungal infections alone.

Co-infection with Mucor spp., a saprophytic mold that is generally non-pathogenic and widespread in the environment, can act as an opportunistic pathogen in immunosuppressed conditions caused by Aspergillus sp., which then causes Mucormycosis infection (Skiada *et al*., 2018). In animal cases, the pathogenesis of Mucor spp. as a contagious agent in Aspergillosis cases can be explained by the mechanism of spore inhalation and consumption of contaminated feed due to moist feed storage. Primary Aspergillosis infection causes damage to lung tissue and air sacs and a chronic granulomatous inflammatory response that impacts local immunosuppression, causing the infected tissue environment to become hypoxic, acidic, and full of necrotic debris. These conditions are ideal for wider tissue invasion by Mucor spp. hyphae (Ibrahim *et al*., 2012), where the angioinvasive ability of these hyphae will then exacerbate damage to lung, liver, and kidney tissue, and facilitate systemic spread through the bloodstream.(Kousha *et al*., 2011; Mahmoud and Shaapan, 2021). In the medical world, individuals with low immunity who experience co-infection with Mucormycosis and Aspergillosis are often reported in humans with reports of liver and kidney damage that have been described in many literatures, but reports regarding this are still limited to those reported

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in poultry.This disease is life-threatening, and is most often reported in patients with immunosuppression symptoms (Addasi *et al*., 2023).Several literatures have reported cases of Mucormycosis in several birds with indications of immunosuppression without a clear cause, namely in Mallard Ducks (Anas platyrhynchos) (Dynowska *et al*., 2013), birds from the Psittacidae family (Galosi *et al*., 2022), and migratory birds (Akter *et al*., 2020), with clinical signs similar to the case animals, namely dyspnea, gasping for breath, and breathing with an open beak.

In the case chicken, anatomical pathology findings in the form of hemorrhage in the cecum and schizont findings in histopathological examination were also found, indicating that the case chicken had coccidiosis (Rumapea *et al*., 2023). The results of bloody feces examination from the case animal showed Eimeria spp. infection with OPG of 34,450 oocysts/gram which indicated that the case animal was at a moderate degree of infection (Arsyitahlia *et al*., 2019). There are nine types of Eimeria spp. species identified as causative agents of coccidiosis in chicken with seven of them being pathogenic, and based on their predilection, Eimeria can be grouped into two, namely in the cecum (caecal coccidiosis) caused by E. tenella and in the intestine (intestinal coccidiosis) caused by other types (Kahn, 2008; Jordan *et al*., 2001). Schizonts found in the cecum are indicative of asexual reproduction of E. tenella, which multiplies within host cells and causes tissue damage (Mesa-Pineda *et al*., 2021). Relatively high humidity, high air temperature, the presence of different bird categories (especially different ages) in one location, changes in feed, feed quality, and all other factors that weaken disease resistance and the general health status of birds play an important role in the distribution and prevalence of coccidiosis (Hofstad, 1984). The finding of piles of bloody feces indicated to contain sporulated oocysts supports the epidemiology of infection of case animals with coccidiosis, considering that sporulated oocysts, which are the infective stage of this enteric protozoan, are ingested and then affected by mechanical and chemical factors in the digestive tract (bile salts and trypsin) to release sporocysts, and then sporozoites, in the duodenal lumen of chicken (Jones *et al*., 1996).

Along with damage to lung, liver and kidney tissue due to concurrent infections of Aspergillosis, Mucormycosis and damage to the digestive tract due to Coccidiosis, systemic and local immune defenses will decrease, so that opportunistic bacteria...more easily reach aseptic internal organs such as the lungs and kidneys, causing multi-bacterial infections (Mahalingam *et al*., 2022). In animal cases, opportunistic bacterial infections of Staphyloccocus spp. were found in the lungs and Klebsiella sp. and Acinetobacter sp. infections in the kidneys. As two aseptic organs, the finding of these opportunistic bacteria may reflect a significant decrease in the body's defense mechanisms, either through physical barriers or cellular immune responses. Although no studies have directly described the combination of Aspergillus sp., Mucor spp., Eimeria spp. infections with Acinetobacter sp., Klebsiella sp., and Staphylococcus sp. In one case, several previous studies have shown that Eimeria can increase the presence of Klebsiella and Staphylococcus in the intestine (Lu *et al*., 2021), and there have been reports of outbreaks of Staphylococcus and Klebsiella infections in broilers without aspergillosis/coccidiosis (Abd-EL-Motelib and El-Zanaty, 1993). This supports the hypothesis that fungal or protozoal infections can create opportunities for opportunistic bacterial colonization, as found in this case. Thus, this combination of multipathogenic infections not only exacerbates disease severity but also drastically reduces the animal's prognosis.

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CONCLUSION AND SUGGESTION

Conclusion

Based on all data obtained through the methodBased on anamnesis, epidemiological data, findings of anatomical pathology and histopathology, bacteria and mycology and parasitology examinations, it can be concluded that chicken used for case study with protocol 170/N/2025 infected by multiple diseases due to concurrent fungal multipathogen infections, in the form of Aspergillosis with co-infection of Mucor spp., bacterial in the form of Staphylococcus sp., Klebsiella sp., and Acinetobacter sp., and protozoal in the form of Coccidiosis due to Eimeria spp. infection.

Suggestion

Controlling stressors and multipathogenic infections in chicken can be prevented by selecting appropriate housing management, biosecurity, sanitation, ventilation, drainage, and humidity controls to minimize the entry of disease-causing pathogens. Implementing a closed housing system can be considered to minimize the entry of pathogens into the rearing environment. This is crucial, given that the animal's immune status is significantly influenced by the environmental conditions in which it is kept. In addition, further studies are needed to comprehensively assess the effectiveness of various management strategies on the immune response and dynamics of multipathogen infections, so that the resulting recommendations have a stronger and more accurate scientific basis.

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Table

Table 1. Findings of organ changes in case animals

Organ	Anatomical Pathology Changes	Histopathological Changes
Brain	Congestion and hemorrhage.	*Meningitis*
Trachea	The tracheal mucosa appears reddish with multifocal hemorrhages.	*Tracheitis*
Esophagus	The esophageal mucosa appears to be hemorrhagic.	*Esophagitis*
cache	The mucosa experiences congestion and hemorrhage.	Cache congestion
Lungs	The consistency of some lobes feels brittle, while others feel hard, with uneven discoloration. Granulomas are present, appearing as yellowish-white nodules, and local necrosis.	*Necrotic granulomatous pneumonia*and found septa hyphae and conidiophores of Aspergillosis sp. and hyphae of Mucor spp.
Heart	The pericardium experiences pericarditis, with the presence of granulomas in the form of yellowish white nodules.	*Pericarditis et Myocarditis edematous et necroticans*
Heart	The consistency is crumbly with uneven discoloration and swelling.	*Hepatitis*
proventriculus	Congestion and hemorrhage occur	*Proventriculitis*
ventricle	Relatively normal	*Ventriculitis*
Small intestine	Diffuse hemorrhage in the small intestine	*Hemorrhagic enteritis*
Colon	Hemorrhage in the cecum	*Typhlitis hemorrhagica*
Kidney	The consistency is friable. Granulomas are found in the form of yellowish-white nodules. There is hemorrhage and necrosis.	*Glomerulonephritis necroticans*
Spleen	The color change is uneven without the presence of typical nodular lesions.	Depletion of splenic lymphoid cells
Pancreas	Appears to be hemorrhagic	*Pancreatitis*
Stock Exchange	Relatively normal	No inspection was performed

Table 2. Results of fungal isolation and identification

Observation	Media / Coloring	Organ	Results
Macroscopic	Natural Resources	Lungs, Kidney	Colonies are white to grayish, with a cottony or woolly texture.
		Heart	Two colonies: (1) Colonies are bluish green with white edges, granular texture and a flat to slightly ridged surface. (2) Colonies are yellowish green with a velvety to granular surface.
		Heart	Not growing

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Microscopic	KOH 10%, Methylene blue	Lungs, Kidneys	Two colonies: (1) The sporangium appears round to oval in shape with thin, easily broken walls, containing many sporangiospores, wide, non-septate hyphae and irregular branching. (2) Conidia are round, rough, large round vesicles, phialids cover the entire surface of the vesicles, hyphae appear slimmer.
		Heart	Two colonies: (1) Conidia are round, rough, large round vesicles, phialids cover the entire surface of the vesicles, hyphae appear slimmer. (2) Columnar conidia resemble dense, short brushes, bottle-shaped vesicles, phialids cover the top.

Table 3. Results of bacterial isolation and identification

Media	Organ	Results
NA	Lung 1	Colonies are small, round, yellowish white, convex elevation, flat margins.
	Lung 2	Small, round, yellowish-white colonies, convex elevation, flat margins.
	Kidney 1	Colonies are large, round, pale yellow, convex elevations, flat margins.
	Kidney 2	Colonies are medium, round irregular, opaque, flat to slightly convex elevations, wavy margins
Coloring Gram	Lung 1	Gram positive, round, clustered like grapes
	Lung 2	Gram positive, round, clustered like grapes
	Kidney 1	Gram negative, thin smooth rod
	Kidney 2	Gram negative, thick rod
Catalase Test	Lung 1	Break down H2O2 into H2O and O2
	Lung 2	Break down H2O2 into H2O and O2
	Kidney 1	Break down H2O2 into H2O and O2
	Kidney 2	Break down H2O2 into H2O and O2
MCA	Kidney 1	Lactose fermenting, large, round, mucoid, pink colonies, convex elevation, flat margins
	Kidney 2	Increased pH with a pale yellow color change, medium-sized, round, mucoid, transparent white colonies and yellow around the colonies
TSIA	Kidney 1	K/A, no H2S and gas production
	Kidney 2	A/A, no H2S and gas production
SIM	Kidney 1	No H2S production, no indole production, motile.
	Kidney 2	No H2S production, no indole production, non-motile.
MR-VP	Kidney 1	MR does not produce acid, VP forms acetonin
	Kidney 2	MR does not produce acid, VP does not form acetonin
SCA	Kidney 1	Using citrate as carbon, production of alkali
	Kidney 2	Using citrate as the sole carbon, the production of alkali.
Sugar Test	Kidney 1	Fermenting glucose, producing gas
	Kidney 2	Does not ferment glucose, does not produce gas

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Table 4. Results of microorganism isolation in animal organs in cases

Organ	Microorganisms
Lungs	*Aspergillus fumigatus*, *Mucor*spp., and Staphylococcus sp.
Kidney	*Aspergillus fumigatus*, *Mucor*spp., Klebsiella sp., Acinetobacter sp.
Heart	*Aspergillus fumigatus*, *Aspergillus flavus*
Secum	*Eimeria*spp.

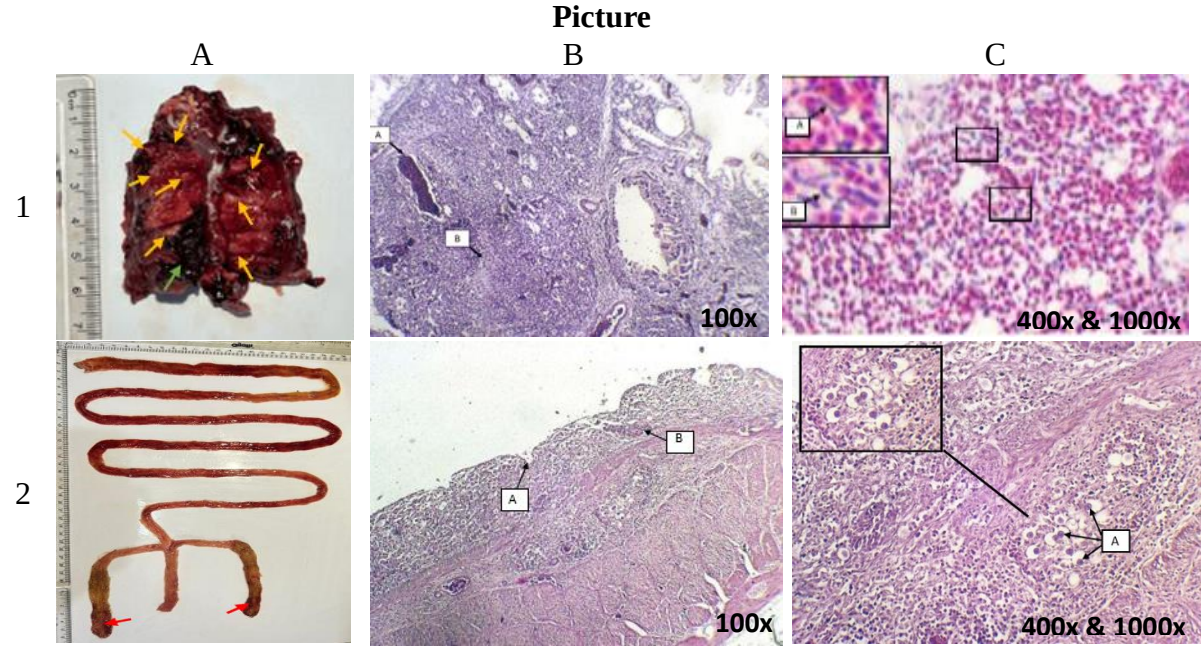

Figure 1. Anatomical and histopathological analysis of chicken case. (1A) Lung Organ, fragile in some parts and hard in some parts, there appears to be uneven color change, hemorrhage, necrosis (green arrow), and there are yellowish white nodular granulomas (yellow arrow).(1B) A)Congestion, B) Area of granulomatous inflammation with necrosis. (1C) A) Septate hyphae of Aspergillus Sp., B) Non-septate hyphae of Mucor spp. (2A) Intestinal organs, diffuse hemorrhage in the small intestine, hemorrhage in the cecum (red arrow). (2B) A) Villous erosion, B) Inflammatory cell infiltration in the lamina propria. (2C) A) Schizont of Eimeria spp.

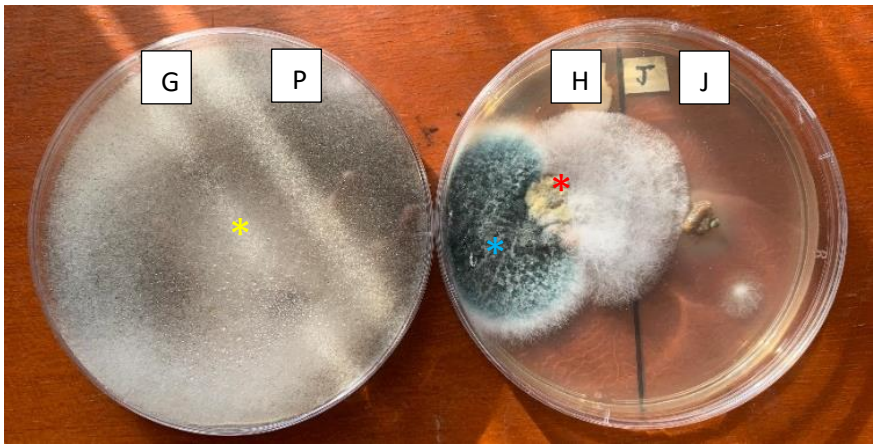

Figure 2. Results of Fungal Isolation on SDA media, macroscopically showing the characteristics of Mucor spp. colonies (yellow star), A. fumigatus (blue star), A. flavus (red star), (Description: Lungs (P); Kidneys (G); Liver (H); Heart (J))

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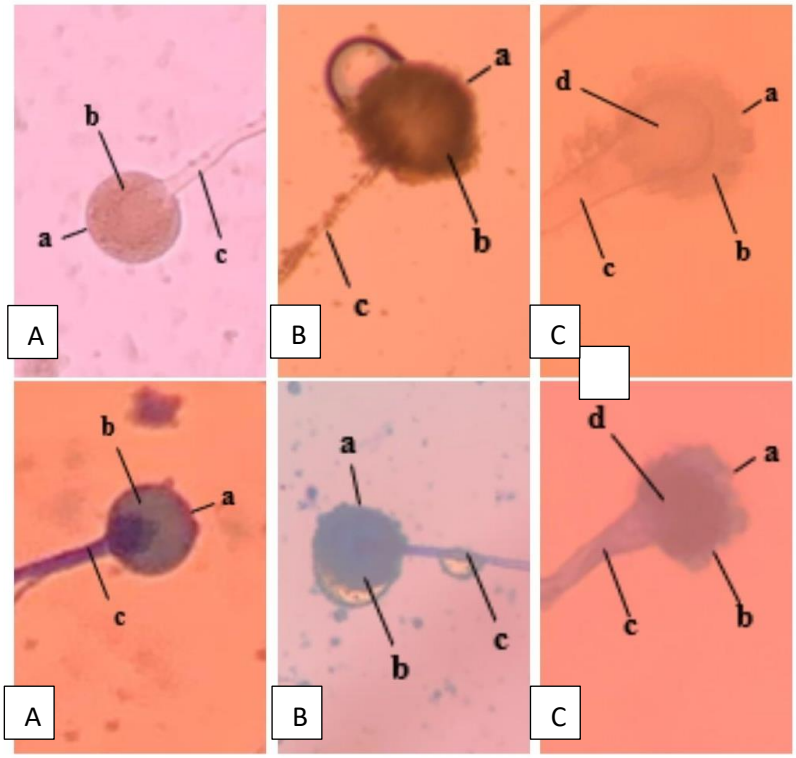

Figure 3. Results of microscopic identification of fungi with 10% KOH and Methylene blue. (A) Mucor spp. (Description: (a) sporongium, (b) sporongiospore, (c) conidiophore); (B) A. fumigatus (Description: (a) conidia, (b) phialids, (c) conidiophore); (C) A. flavus. (Description: (a) conidia, (b) phialids, (c) conidiophore, (d) vesicle);

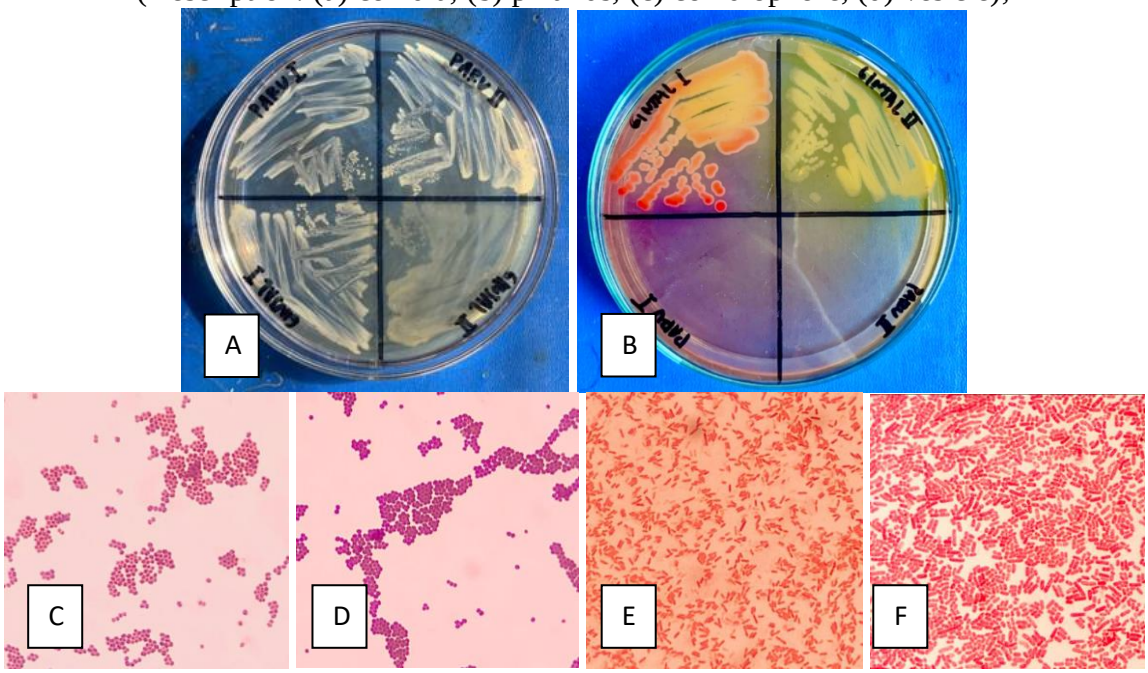

Figure 4. Isolation of Lung and Kidney Bacteria. (A) Bacterial culture on NA media (B) Bacterial culture on MCA (CD) Gram staining of Lung 1 and Lung 2 colonies, round gram-positive, Staphyloccocus sp. (E) Gram staining of Kidney colony 1 thin smooth gram-negative rod. (F) Gram staining of Kidney colony 2 thick gram-negative rods.

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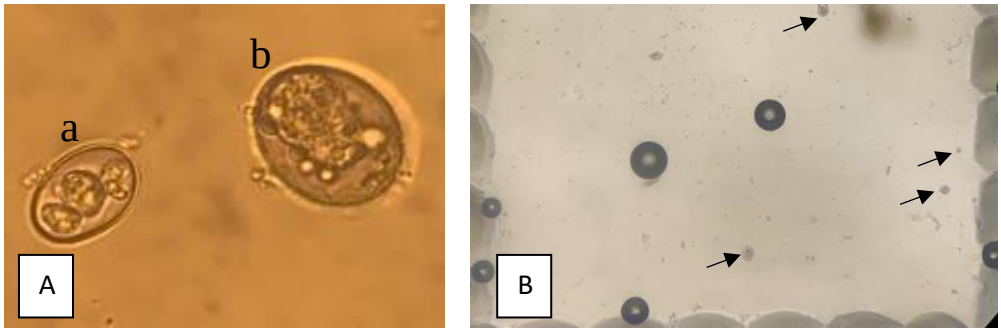

Figure 5. Fecal examination with protozoa identification results in the form of Eimeria spp. oocysts. (A) Qualitative test, found sporulated oocysts (a) and non-sporulated oocysts (b). (B) McMaster quantitative test with an OPG count of 34,450 oocysts/gram.