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CO-INFECTION OF AVIAN INFLUENZA H5N1 AND COCCIDIOSIS IN A 25-DAY-OLD BROILER: A CASE REPORT

Koinfeksi Flu Burung H5n1 dan Koksidiosis pada Ayam Broiler Umur 25 Hari: Laporan Kasus

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Abstract

Avian Influenza (AI) is a highly pathogenic contagious disease in poultry caused by influenza A virus, particularly the H5N1 subtype. This disease significantly affects poultry health and causes considerable economic losses in poultry farming. This case report describes a 25-day-old broiler chicken from Demulih Village, Bangli, Bali, which showed symptoms of anorexia, weakness, greenish diarrhea, and rapid death. Epidemiological analysis revealed a morbidity rate of 33.3%, mortality rate of 22.7%, and case fatality rate of 68.3%. Anatomical pathology examination showed multiorgan hemorrhages, while histopathology revealed non-suppurative inflammation with lymphocytic infiltration in the brain, lungs, heart, pancreas, and intestine. Virus isolation using embryonated chicken eggs, along with hemagglutination (HA) and hemagglutination inhibition (HI) tests, confirmed Avian Influenza H5N1 infection. In addition, fecal examination identified *Eimeria* spp. oocysts, indicating coccidiosis. These findings highlight that H5N1 infection can be aggravated by parasitic co-infections, leading to increased mortality. Preventive measures should include routine vaccination, strict biosecurity, and good husbandry management to reduce the risk of disease spread in the future.

Keywords: Avian influenza, broiler chickens, coccidiosis, H5N1

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Abstrak

Avian Influenza (AI) adalah penyakit menular yang sangat patogen pada unggas yang disebabkan oleh virus influenza A, khususnya subtipe H5N1. Penyakit ini berdampak besar terhadap kesehatan unggas dan menyebabkan kerugian ekonomi yang signifikan dalam industri peternakan ayam. Laporan kasus ini menggambarkan seekor ayam broiler berumur 25 hari dari Desa Demulih, Bangli, Bali, yang menunjukkan gejala anoreksia, lemas, diare kehijauan, dan kematian yang cepat. Analisis epidemiologis menunjukkan angka morbiditas sebesar 33,3%, mortalitas 22,7%, dan case fatality rate 68,3%. Pemeriksaan patologi anatomi memperlihatkan perdarahan pada berbagai organ, sedangkan histopatologi menunjukkan peradangan non-supuratif dengan infiltrasi limfosit pada otak, paru-paru, jantung, pankreas, dan usus. Isolasi virus menggunakan telur ayam berembrio, serta uji hemaglutinasi (HA) dan hemaglutinasi inhibisi (HI), mengonfirmasi infeksi Avian Influenza H5N1. Selain itu, pemeriksaan feses mengidentifikasi ookista *Eimeria* spp., yang mengindikasikan adanya koksidiosis. Temuan ini menunjukkan bahwa infeksi H5N1 dapat diperberat oleh ko-infeksi parasit, sehingga meningkatkan angka kematian. Langkah pencegahan harus mencakup vaksinasi rutin, biosekuriti yang ketat, dan manajemen pemeliharaan yang baik untuk mengurangi risiko penyebaran penyakit di masa mendatang.

Kata kunci: Avian influenza, ayam broiler, koksidiosis, H5N1

INTRODUCTION

Avian influenza (AI) is an infectious disease caused by influenza A viruses of the Orthomyxoviridae family, characterized by high morbidity and mortality in poultry, particularly the H5N1 subtype, which is classified as highly pathogenic avian influenza (HPAI) (Isnawati et al., 2019). This disease poses a serious threat to the poultry industry as it can cause significant economic losses due to high mortality rates, reduced productivity, and socioeconomic impacts on farmers (Krammer et al., 2025). In addition, the H5N1 strain of AI has zoonotic potential, increasing the urgency of prevention and control efforts against it. On the other hand, coccidiosis is one of the parasitic diseases that commonly affects broiler chickens, especially at a young age. This disease is caused by protozoa of the genus Eimeria, which infect the intestinal mucosa, causing epithelial damage, bloody diarrhea, reduced growth, and predisposition to secondary infections (Shirley et al., 2007). Coccidiosis often acts as a comorbid factor that exacerbates the condition of chickens with other infectious diseases, as intestinal damage can reduce both local and systemic immunity (Fitri et al., 2021).

Double infections between AI H5N1 and coccidiosis in broiler chickens are rarely reported, but it is important to note that they can exacerbate clinical symptoms, accelerate mortality, and complicate diagnosis in the field. Viral infections can suppress the immune system, whereas damage to the intestinal mucosa caused by Eimeria can disrupt the nutritional status and increase susceptibility to AI (Lillehoj & Trout, 1996). Therefore, case reports on these co-infections are important to provide an overview of the clinical manifestations, pathology, and epidemiological implications for poultry health management.

Thus, this case report aims to describe the clinical findings, anatomical pathology, and histopathology in 25-day-old broiler chickens infected with the AI strain H5N1 accompanied by coccidiosis, so that it can serve as a scientific reference in understanding pathogen interactions and assist in developing disease prevention and control strategies in broiler chicken farms.

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RESEARCH METHODS

Case Animal

The case animal with protocol number 157/N/25 was a 25-day-old broiler chicken from a broiler farm in Demulih Village, Susut District, Bangli Regency, Bali, Indonesia. The clinical signs observed in this case included anorexia, lethargy, dull feathers, sleepy-looking eyes, nasal discharge, dyspnea, and white-greenish diarrhea with a watery consistency. The chicken died 7 h after being removed from the farm. The case history of the chickens was obtained through an interview with one of the farm guards regarding the history of disease, vaccination, and clinical signs of the birds.

Epidemiological Investigation

Data collection was conducted through direct interviews with one of the farm guards to obtain information on the chicken population, the number of sick chickens, and the number of dead chickens in the week prior to sampling. Epidemiological analysis was performed by calculating the morbidity, mortality, and case fatality rate (CFR) in the chicken population on the farm. Morbidity describes the percentage of sick animals in the entire population, while mortality shows the percentage of animals that died from disease in the total population. The case fatality rate (CFR) is the percentage of animals that died compared to the number of sick animals.

Data Collection Methods

This manuscript is a case report. Data were obtained through post-mortem examination of animal samples found dead without prior intervention. Data collection included macroscopic observation, lesion documentation, and tissue sampling for laboratory analysis according to standard procedures. The case animals were broiler chickens that died 7 h after being taken from the farm and underwent necropsy at the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Udayana University. During necropsy, anatomical pathology observations were made, and samples were collected for examination by the Virology, Bacteriology, Parasitology, and Histopathology Laboratories.

Anatomical Pathology and Histopathology Examination

The necropsy procedure for the chicken cases was carried out at the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Udayana University, to observe anatomical and pathological changes. The feathers of the dead animals were moistened, and the ventral part of the body was incised to expose all organs. Observations and photographs were then taken of the organs that underwent anatomical pathological changes. The results of the images of each sample were described in terms of the changes that occurred. Each organ sample was then cut into 1 × 1 × 1 cm pieces and fixed in a 10% Neutral Buffered Formaldehyde (NBF) solution. Histopathological preparations were made at the Veterinary Pathology Laboratory of the Faculty of Veterinary Medicine, Udayana University. The preparation of histopathological specimens based on the Kiernan method (2015) involved placing the sample in a tissue processor, followed by dehydration using alcohol with varying concentrations, namely 70%, 80%, 90%, and 96% alcohol for approximately 2 h each. This was followed by a clearing process, which involved the removal of alcohol residues by soaking the sample in xylol. The tissue is then placed in liquid paraffin and molded into a block (paraffin block) and followed by a cutting process, which is the cutting of tissue using a microtome with a thickness of 3-4 µm. The tissue was then floated in a water bath at 46^{o}C. The tissue was placed on an object glass, dried on filter paper, and stained with hematoxylin and eosin (HE). After hematoxylin and eosin (HE) staining, the mounting process was performed, covering the preparation with a

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cover glass with Entellan. The prepared specimens were observed under a microscope for histopathological examination.

Virus Isolation and Identification

Inoculum Preparation

The samples used were organs that had undergone pathological anatomical changes, and the inoculum procedure was performed under aseptic conditions. The organs used were the brain, trachea, heart, lungs, proventriculus, pancreas, and intestine. All samples were ground to make a 10% suspension in sterile phosphate-buffered saline (PBS) solution. The samples were then centrifuged at 2,500 rpm for 10-15 minutes. The supernatant was collected and transferred to a new sterile Eppendorf tube, and 0.1 ml each of penicillin and streptomycin antibiotics were added. The mixture was vortexed and incubated at 37°C for 30 min.

Inoculation of Embryonic Chicken Eggs

Inoculation was performed on 9-day-old embryonic chicken eggs. Before inoculation, the eggs were candled to ensure that the embryos were still alive and to mark the air sac area with a pencil. The eggshell was disinfected with 70% alcohol and then pierced with a syringe between the air sac and embryo. The inoculum fluid was injected at a rate of 0.2 ml per egg into the allantoic cavity. The hole in the eggshell was sealed with nail polish and labelled, and the eggs were incubated in an incubator at 37°C. Observations will be made daily, and harvesting will be performed on the 3rd day after inoculation.

Harvesting Embryonic Chicken Eggs After Inoculation

Observations were made by candling each inoculated egg to determine whether the embryo was dead. Embryonic chicken eggs were harvested on the third day after their inoculation. Eggs with dead embryos were removed from the incubator and placed in a refrigerator for a few hours. The purpose of this study was to reduce bleeding when opening eggshells. The eggshell was then opened by cutting the egg in the air sac area, which had been marked beforehand. Next, the allantoic fluid was collected using a micropipette and stored in a sterile Eppendorf tube.

Preparation of 1% Erythrocyte Suspension

A 1% erythrocyte suspension was prepared according to the modified OIE (2012) procedure using the following technique: 3 mL of chicken blood was collected from the brachial vein using a 3 mL disposable syringe. Chicken blood was collected in a sterile tube containing 2.5 mL alselver anticoagulant. The chicken red blood cells were washed by adding 5 mL of PBS (pH 7.2) to the tube containing the blood solution and mixed slowly to avoid damaging the red blood cells. The blood sample was then centrifuged at 2500 rpm for 10 min. Blood was then separated from the buffy coat and supernatant, leaving only the red blood cell sediment in the tube. The next step involved washing the red blood cells again by adding PBS to 2/3 of the tube and homogenizing it. The blood washing process was repeated thrice using the same method. The concentration of the red blood cell sediment is then measured by centrifuging it using a microhematocrit tube. The red blood cells were measured for Packed Cell Volume (PCV), then diluted with PBS to a concentration of 1%, and were ready for use in the HA/HI test (Kencana et al., 2016).

Hemagglutination Assay (HA)

The hemagglutination assay (HA) is a method used to detect the ability of viral antigens to agglutinate red blood cells, such as Avian Influenza. The hemagglutination assay (HA) was performed using a microtitre technique by adding 0.025 mL of PBS to each well of the

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microplate using a micropipette. Next, 0.025 mL of the virus antigen suspension was added to the first and second wells. Serial dilutions in multiples of 2 were performed up to the 11th well and discarded in the 12th well. Subsequently, 0.025 mL of PBS and 1% poultry red blood cells were added to each well of the microplate, filtered, and shaken using a microplate shaker for approximately 30 s. The microplate was left at room temperature for one hour and observed every 15 min. The test result was considered positive if crystals formed at the bottom of the microplate wells as a result of the hemagglutination reaction with 1% poultry red blood cells. The HA titre was determined from the highest antigen dilution that could agglutinate the red blood cells. The sample was then diluted to 4 hemagglutination (HA) units for the rapid HI test (Kencana et al., 2016).

Hemagglutination Inhibition (HI) Test

The hemagglutination inhibition (HI) test is a serological test that uses serum antibodies against ND and AI viruses. In this test, antibodies bind to viral antigens, preventing the virus from agglutinating red blood cells. This causes the red blood cells to settle at the bottom of the microplate well and form a precipitate (Zhao et al., 2020). In the hemagglutination inhibition (HI) test, the viral antigen titre was first diluted to 4 HA units. The test was performed by adding 0.025 mL of PBS to wells 1-4, the addition of serum containing ND antibodies to the first well and AI antibody serum to the second well. Next, 0.025 mL of 4 HA antigen was added to wells 1-3, then shaken on a microplate shaker for 30 s, and incubated at room temperature for 10 min. Next, 0.05 mL of 1% red blood cell suspension was added to wells 1-4 and shaken again for 30 s, followed by incubation for 15-30 minutes. The test result was considered positive if there was a red blood cell sediment at the bottom of the well, indicating that the antibodies could bind to the antigen, allowing the red blood cells to settle freely.

Bacteriological Examination

Bacterial isolation and identification were performed at the Veterinary Bacteriology and Mycology Laboratory, Faculty of Veterinary Medicine, Udayana University, to identify bacterial pathogens that may be involved in broiler chicken infections. The organ samples used for bacterial isolation included the heart, lungs, and intestines of infected chickens. These organs were selected because they are often the primary sites of bacterial infection in chickens, which can provide important information regarding the underlying cause of the disease. (Gholami et al., 2021). Organ samples were cultured on a general medium, namely, Nutrient Agar (NA). Culturing was performed by swabbing the sterile ossa of the organ specimen and then swabbing it on the NA medium using the streak-line method. Incubation was carried out at 37 °C for 24 h. Colony growth on the medium was observed macroscopically to determine the shape, colour, elevation, edges, and diameter of the colony. Next, subculture on NA medium using the same method and incubate for 24 h. Then, a single colony from the NA medium was taken using sterile swabs and swabbed on MacConkey Agar (MCA) medium using the streak line method and incubated at 37°C for 24 h. Next, primary tests, such as the catalase test and Gram staining, are performed to determine the shape and colour of the colony. Gram staining of bacteria involves several materials, including crystal violet, Lugol's iodine solution, alcohol, and safranin. Biochemical tests are also performed, such as Triple Sugar Iron Agar (TSIA), Simmons Citrate Agar (SCA), Sulfide Indole Motility (SIM), Methyl Red (MR), and Voges-Proskauer (VP), followed by sugar tests, namely the glucose test.

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Parasitological Examination

Fecal Examination

Faecal examination is performed qualitatively to detect the presence of intestinal parasites in broiler chickens. This examination uses three main methods: the native, sedimentation concentration, and flotation concentration methods. These three methods are designed to identify parasites based on the morphology, size, and specific gravity of parasite eggs or oocysts found in faeces (Foreyt et al., 2018).

Data Analysis

Anamnesis data, epidemiology, clinical signs, anatomical pathology, histopathology, and laboratory test results were presented in narrative form, tables, figures, and analysed descriptively and qualitatively.

RESULTS AND DISCUSSION

Results

Epidemiological Data

According to the results of an interview with one of the farm guards, the total chicken population was 900 chickens aged 25 d. The feed provided was concentrated, and drinking water was obtained from a well. Sick chickens or those showing clinical signs were not separated from other healthy chickens. The number of sick animals reached 300 in the last week, and 205 chickens died during the same period. According to the interview with the farm keeper, all chickens were vaccinated at the time of hatching, but it is not known what vaccine was given, and the livestock were not administered booster shots. The chickens were given to the author on 29 June 2025 in a weak condition, and the chickens died 7 h after being taken from the farm. Based on these data, the morbidity rate was 33.3%, the mortality rate was 22.7%, and the case fatality rate (CFR) was 68.3%.

Anatomical Pathology Examination

Anatomical pathology examination revealed multiorgan haemorrhage. The brain showed hyperaemia and congestion; the heart was swollen at the apex and showed haemorrhage and congestion; the lungs were swollen and showed haemorrhage in both lobes; the proventriculus showed petechiae; the pancreas was swollen and congested; and the intestines showed congestion and haemorrhage. The anatomical pathology of the chicken is shown in Figure 1.

Histopathological Examination

Histopathological examination of the affected chickens revealed haemorrhage, congestion, and inflammation, as indicated by lymphocyte inflammatory cell infiltration in multiple organs. Histopathological examination with haematoxylin-eosin (HE) staining showed that the brain had encephalitis, the lungs had bronchopneumonia, the heart had pericarditis and myocarditis, the proventriculus had necrotic proventriculitis, the pancreas had pancreatitis, and the intestines had enteritis. The histopathology of the chickens in this case is shown in Figure 2.

Isolation and Identification of the Virus

The results of inoculation in embryonated chicken eggs showed that the embryos died on the third day post-inoculation. The embryos exhibited haemorrhages throughout their bodies (Figure 3). The HA test using the microtitre technique yielded positive results with a titre of 26, as indicated by the agglutination of red blood cells (Figure 4). The HI test results showed

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that the chickens were infected with Avian Influenza, as seen in the second well, where antibody binding with the antigen caused the red blood cells to settle completely (Figure 5).

Isolation and Identification of Bacteria

The results of bacterial isolation and identification showed that the chickens were infected with *Staphylococcus spp*. in the lungs and Escherichia coli in the intestines. The results of bacterial identification on general media, selective media, primary catalase test, Gram staining, biochemical test, and glucose test are shown in Table 1 and Figure 6.

Fecal Examination

The results of the parasitological examination of the faeces of the case animal using the qualitative faecal examination method (Table 2) showed positive results for *Eimeria spp*. in the flotation method examination presented in Figure 7.

Discussion

The broiler chicken farm used a semi-intensive rearing system with a total population of 900 chickens. Based on interviews and field observations, 300 chickens showed clinical symptoms consistent with respiratory and digestive tract disorders, such as anorexia, greenish-white diarrhoea, and swollen eyes. Of this number, 205 chickens died in the last week. Based on this data, three main parameters were calculated in the epidemiological evaluation of infectious diseases, namely, the morbidity rate of 33.3%. This value indicates the proportion of infected chickens in the entire population during the incident period. The mortality rate was 22.7%. This reflects the total mortality rate in the population due to the disease. The case fatality rate (CFR) was 68.3%. This high CFR indicates that the infection is highly virulent or deadly, identifying the possible involvement of aggressive pathogens such as the Avian Influenza virus (Hosseini et al., 2017; Lee et al., 2021). This is in line with the research of Rehman et al. (2023) which states that in broiler chickens, infection with the Avian Influenza virus, especially the highly pathogenic H5N1 strain (HPAI), can cause high mortality rates and significant economic impacts in the livestock sector.

The pathological anatomical changes observed in the chickens in this case indicate changes in several organs, reflecting the characteristics of systemic infection. The changes found include congestion and hyperaemia in the brain, which affect the mechanism of oxygen and glucose transport to the brain (Sanjiwani et al., 2024), whereas haemorrhage and swelling were found in the lungs, which can disrupt the respiratory process. The anatomical pathological changes found in the heart included congestion and haemorrhage, as well as changes in the apex of the heart. The damage observed in the digestive tract included congestion, haemorrhage, and petechiae, as well as a change in the colour of the spleen to dark red, which is a characteristic change in the AI virus. According to (Damayanti et al., 2004), chickens infected with the Avian Influenza virus will show bleeding in almost all organs. This is because Avian Influenza virus infection, which has a high level of pathogenicity, can be multisystemic.

Histopathological examination of chicken organs in this study showed that almost all organs underwent changes, such as mononuclear inflammatory cell infiltration. According to Nofantri et al. (2017), this systemic viral infection damages various vital organs, such as the brain, lungs, intestines, and other organs. Histopathological examination of the brains of the affected chickens revealed congestion, demyelination of neurones, and lymphocyte inflammatory cell infiltration. The heart showed lymphocytic inflammatory cell infiltration in the pericardium and myocardium. In the lungs, lymphocyte inflammatory cell infiltration was observed in the peribronchial and interbronchial septa, as well as congestion. Lymphocyte inflammatory cell infiltration and congestion were observed in the pancreas. Spleen observations revealed

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congestion, white pulp necrosis, and lymphoid cell depletion. This is in line with the statement by Sánchez-González et al. (2020) that HPAI variants can cause tissue damage in lymphoid organs, as this causes a decrease in the body's ability to produce and maintain lymphocytes. Observations of the proventriculus and intestine also revealed similar findings, including congestion, lymphocyte inflammatory cell infiltration, erosion of the mucosa, and necrosis of the tissue. The findings of lymphocyte infiltration in the heart and extensive haemorrhage in the lungs of the chickens in this case directly reflect the characteristic pathogenesis mechanism of Highly Pathogenic Avian Influenza (HPAI) H5N1. The HPAI virus has a polybasic cleavage site on the haemagglutinin (HA) protein, which allows the virus to be activated by various tissue proteases, enabling systemic replication in various organs of poultry (Luczo et al., 2015). Viral replication in blood vessel endothelial cells causes vascular damage and increased capillary permeability, which ultimately leads to multiorgan haemorrhage, including extensive haemorrhage in the lung tissue, as seen in this case. In addition, the H5N1 virus is known to have a high affinity for myocardial cells, and its replication in the heart triggers an inflammatory response in the form of non-suppurative myocarditis, characterised by lymphocyte infiltration in the pericardium and myocardium (Damayanti et al., 2004). Excessive inflammatory reactions can cause necrosis of the intestinal mucosa cells. Histopathologically, all tissues of organs in animals infected with the Avian Influenza virus show non-suppurative inflammation, indicating that inflammatory cells are dominated by single-nucleated cells (lymphoid cells), which are typically caused by infectious agents such as viruses (Gayanti et al., 2024).

Virus isolation was performed using embryonated chicken eggs, which are widely used for virus isolation. The isolation results showed that the embryos died on the third day after inoculation, and the allantoic fluid was harvested immediately. HPAI virus infection can cause embryo death within 48-72 hours, whereas LPAI viruses often do not cause embryo death (Wibowo et al., 2016). Macroscopic observation revealed that the embryos appeared stunted and haemorrhagic throughout the body. Furthermore, the harvested embryonic chicken egg fluid was used as an antigen. The tests conducted in this study to confirm the diagnosis of avian influenza (AI) virus were the hemagglutination assay (HA) microtitre technique and hemagglutination inhibition (HI) assay. The microtitre HA test yielded positive results, indicated by the absence of red blood cell deposits at the bottom of the microplate wells, because the avian influenza virus has haemagglutinin protein that can agglutinate red blood cells and prevent them from settling. The HA virus antigen titre was 26. Meanwhile, the HI test yielded a positive result for the avian influenza virus, indicated by the presence of red blood cell sedimentation at the bottom of the microplate wells. This can occur because serum antibodies bind to viral antigens and agglutinate red blood cells, allowing them to settle freely at the bottom of the microplate wells.

Bacteriological testing of chickens was performed because of the immunosuppressive effects often associated with Avian Influenza virus infection, which can facilitate opportunistic bacterial infections in poultry. Bacterial isolation identified two distinct types of bacterial colonies. The first colony exhibited gram-positive cocci morphology, arranged in grape-like clusters, characteristic of *Staphylococcus spp.* The second bacterial colony was Escherichia coli, which displayed gram-negative bacilli morphology. Both bacteria are part of the normal flora and exhibit opportunistic behaviours. These findings align with those of Oladokun and (Oladokun & Sharif, 2024) concerning normal poultry lungs (lower respiratory tract/LRT), who identified several relatively dominant bacterial genera in young chickens, including Staphylococcus.

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The results of chicken faecal examination in the parasitology laboratory revealed unsporulated *Eimeria spp.* oocysts (Figure 7). Coccidiosis is a parasitic disease caused by protozoa of the genus *Eimeria spp*. of the phylum Apicomplexa (Ekawasti & Martindah, 2019). Seven species of Eimeria infect chickens: *E. tenella, E. maxima, E. acervulina, E. mitis, E. brunetti, E. praecox, and E. necratix* (Rumapea et al., 2023). *Eimeria tenella* is the species that most commonly infects broiler chickens. The prevalence of *Eimeria spp*. infection is more common in chickens older than 2 weeks of age. *Eimeria spp.* oocysts are oval in shape and have walls consisting of one or two transparent layers. Unsporulated oocysts contain one sporoblast, whereas sporulated oocysts consist of four sporocysts containing two sporozoites (Ekawasti & Martindah, 2019). According to Simamora et al. (2017), *Eimeria spp*. infections often affect chickens older than two weeks but rarely affect chickens younger than two weeks. This finding is consistent with that of a 25-day-old chicken. This is because chickens younger than two weeks do not produce much trypsin and bile salts; therefore, the process of releasing sporozoites from the oocysts does not occur.

In this instance, coinfection with HPAI H5N1 and *Eimeria spp*. exacerbated the condition of chickens through mucosal damage, excessive inflammation, and immunosuppression. Infection by *Eimeria spp*. compromises the intestinal epithelium and impairs nutrient absorption, thereby weakening the host immune response and facilitating the systemic dissemination of the virus (Kusumawardani et al., 2020). Conversely, coccidiosis induces the release of proinflammatory cytokines, which, when combined with the intense inflammatory response elicited by HPAI H5N1, culminate in a hyperinflammatory state that accelerates organ damage (Krammer et al., 2025). Additionally, *Eimeria spp*. infection diminishes lymphocyte populations in lymphoid organs, contributing to immunosuppression and increasing susceptibility to secondary bacterial infections, such as Escherichia coli and *Staphylococcus spp.* (Rumapea et al., 2023). Consequently, coccidiosis is a significant predisposing factor that exacerbates the pathogenesis of HPAI H5N1 and increases mortality.

CONCLUSION AND RECOMMENDATIONS

Conclusion

Based on the comprehensive analysis of medical history, epidemiological investigation, clinical symptoms, anatomical pathology, histopathology, viral and bacterial identification, and faecal examination, it was concluded that the chicken identified by protocol number 157/N/25 was diagnosed with an Avian Influenza H5N1 strain infection, concomitant with coccidiosis.

Recommendations

Avian Influenza can be effectively controlled through vaccination and subsequent booster doses. Additionally, maintaining optimal coop sanitation, implementing stringent biosecurity measures, and ensuring meticulous management are essential. It is imperative to consider the physical condition of chickens prior to vaccination to ensure an optimal immune response. The administration of anticoccidials, as advised by veterinarians, is recommended to prevent coccidiosis transmission.

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Table

Table 1. Results of bacterial isolation and identification

Isolation/ identification media	Results
NA	The colonies appear round, small, white, dense, and convex.
MCA	The presence of pink colonies
Gram staining	There were purple, round (coccus) bacteria colonies that were stacked on top of each other. There were also pink, rod-shaped (bacillus) bacteria colonies.
Catalase test	Positive (+) for bubbles
TSIA	The slanted field (+) shows a color change, the vertical field (+) shows a color change, gas (+) shows bubbles, H2S (-) the medium does not turn black.
SIM	Indol (+) red ring formation on the surface, motility (-) no turbidity at the puncture site, sulfide (-) no change in medium color to black
MR	Positive (+) Indicated by a color change in the medium to red after adding methyl red reagent.
VP	Negative (-) Characterized by the absence of turbidity in the bacterial culture medium.
SCA	Negative (-) indicates no change in color.
Glucose test	Positive (+) Characterized by the formation of bubbles and color change

Table 2. Results of animal fecal examination

Methods	Results
Native	Negative (-)
Sedimentation	Negative (-)
Floation	Positive (+) *Eimeria spp.*

Figure

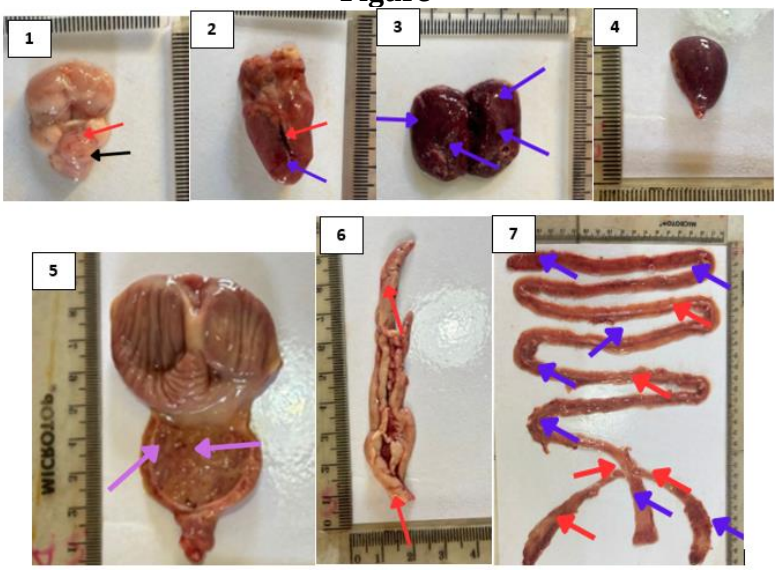

Figure 1. Anatomical pathology of chicken organs: The brain (1) shows hyperemia (black arrow) and congestion (red arrow); the heart (2) shows congestion (red arrow), hemorrhage (blue arrow), and a blunt cardiac apex; the lungs (3) show swelling and hemorrhage (blue

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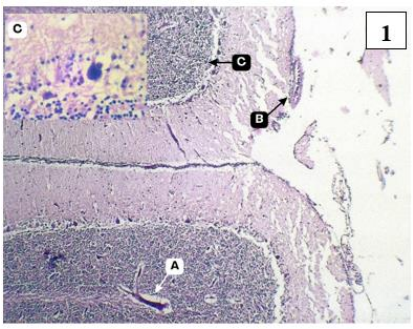
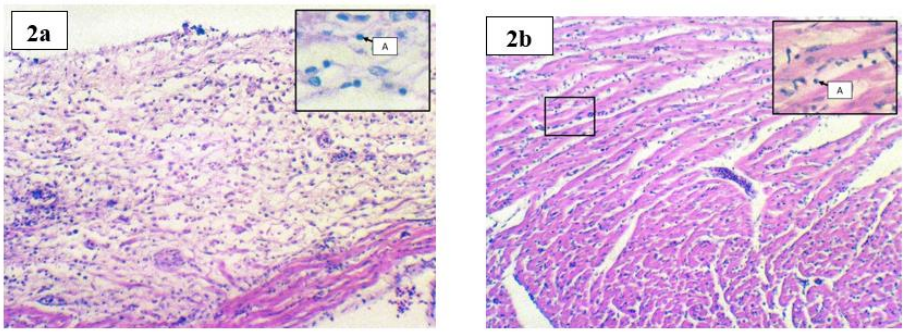
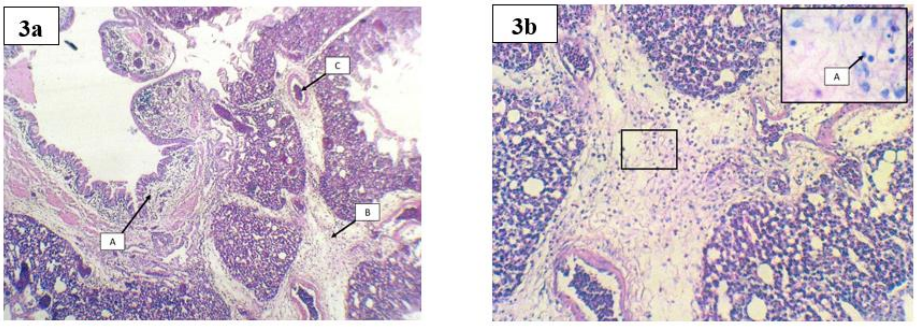
arrow); Spleen (4) shows a change in color to dark red; Proventriculus (5) shows petechiae (purple arrow); Pancreas (6) shows swelling and congestion (red arrow); Intestines (7) show congestion (red arrow) and hemorrhage (blue arrow).



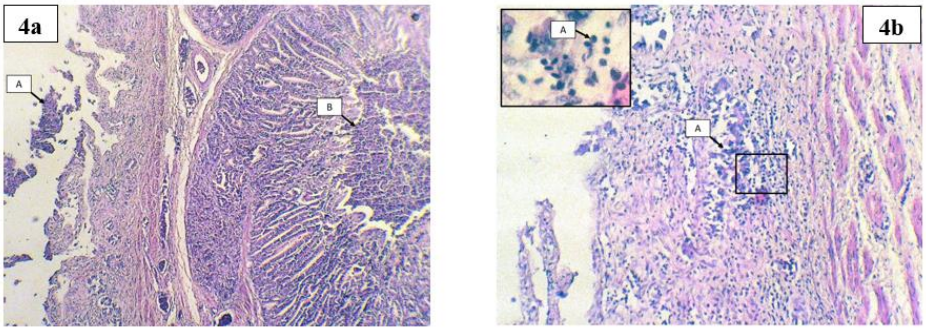
Brain



Heart



Lung



Proventriculus

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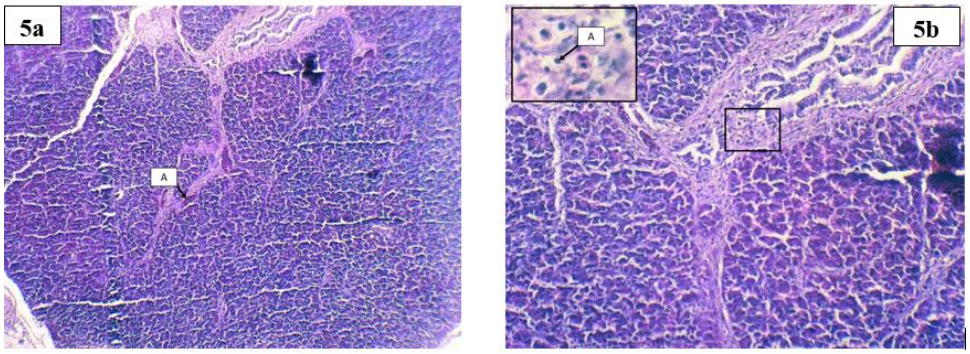
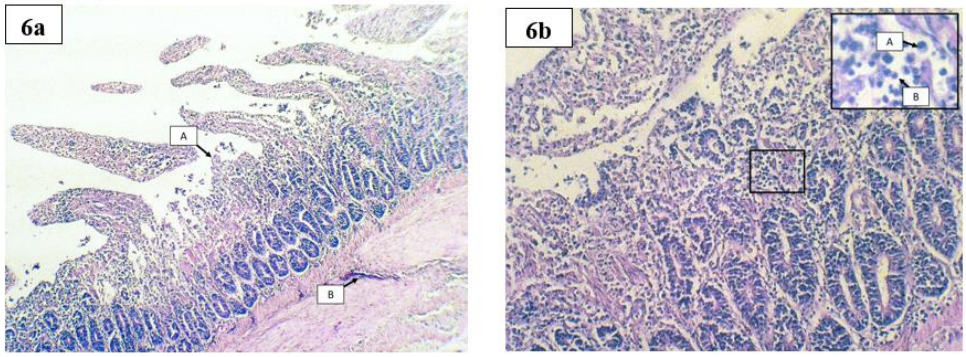
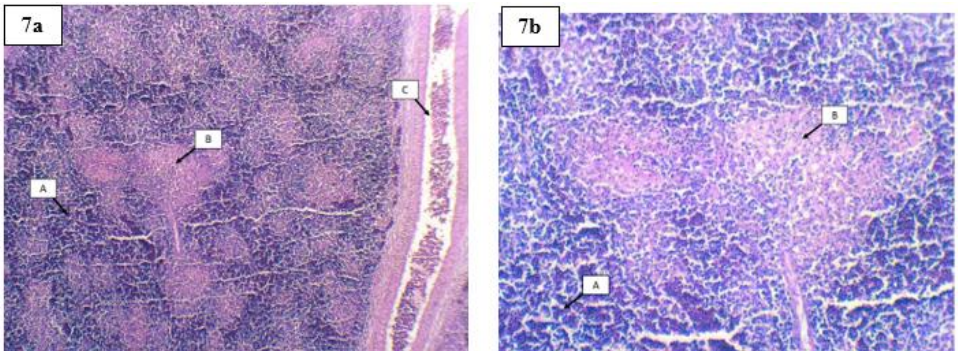


Pancreas



Gut



Spleen

Figure 2. Histopathology of chicken organs in cases of brain (1) (A) Demyelination (100x), (B&C) Infiltration of mononuclear inflammatory cells (100x & 1000x); Heart (2a) (A) Infiltration of lymphocyte inflammatory cells in the pericardium (400x & 1000x), (2b) (A) Infiltration of lymphocyte inflammatory cells in the myocardium (400x & 1000x); Lungs (3a) Infiltration of inflammatory cells in (A) the peribronchial area and (B) the interbronchial septum. (C) Congestion. (100x), (3b) (A) Infiltration of inflammatory lymphocytes in the interbronchial septa (400x & 1000x); Proventriculus (4a) (A) Mucosal erosion (B) Necrosis in the glands (100x), (4b) (A) Infiltration of inflammatory lymphocytes in the necrotic area (400x & 1000x); Pancreas (5a) (A) Congestion (100x), (5b) (A) Infiltration of inflammatory lymphocytes (400x & 1000x); Intestine (6a) (A) Villi erosion (B) Congestion (100x), (6b) Inflammatory cell infiltration (A) macrophages and (B) lymphocytes in the lamina propria (400x & 1000x); Spleen (7a) Depletion of lymphoid cells (B) Necrosis of white pulp (C) Congestion (100x), (7b) (A) Depletion of lymphoid cells (B) necrosis of white pulp (400x).

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Figure 3. The results of the harvest of embryonated chicken eggs on the third day after virus inoculation show embryos that have died and exhibit stunting and hemorrhaging.

Figure 4. Hemagglutination assay (HA) results

: Positive control/ antigen
: HA Titer 2^6
: Negative control/ blood

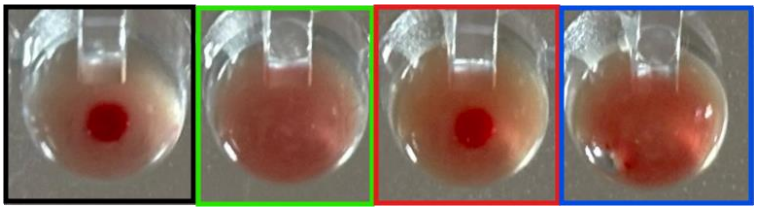

Figure 5. Hemagglutination inhibition (HI) test results

: Red blood cell control
: Antigen control
: AI positive, hemagglutination inhibition occurs (there is red blood cell precipitation)
: ND negative, no hemagglutination inhibition (presence of red blood cell agglutination)

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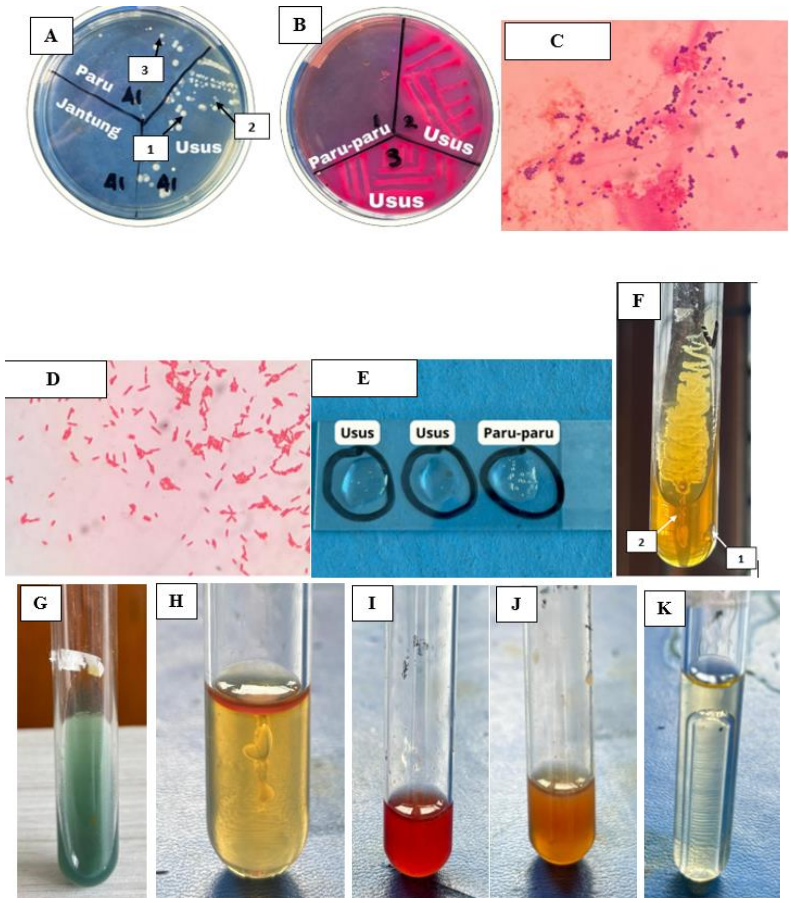

Figure 6. Results of bacterial isolation and identification from chicken organs (A) Results of bacterial culture on general NA media (1) small round white bacterial colonies in the intestine, (2) clustered round transparent white colonies in the intestine, (3) dense, convex round white bacterial colonies in the lungs; (B) Results of bacterial culture on selective MacConkey Agar (MCA) medium; (C) Gram staining results of colonies from the lungs; (D) Gram staining results of bacterial colonies from the intestines; (E) Catalase test results; (F) TSIA test results; (G) SCA test results; (H) SIM test results; (I) MR test results; (J) VP test results; (K) glucose test results.

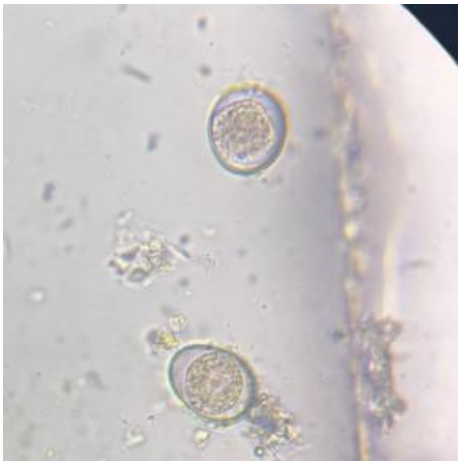

Figure 7. The results of the examination of the animal's feces revealed protozoa *Eimeria spp.*

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