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INFECTIOUS BURSAL DISEASE AND NEWCASTLE DISEASE CO-INFECTION WITH COLISEPTICEMIA IN A 15-WEEK-OLD COMMERCIAL LAYER CHICKEN

Infectious Bursal Disease dan *Newcastle Disease* Disertai dengan *Colisepticemia* pada Seekor Ayam Ras Petelur Umur 15 Minggu

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Abstract

Infectious Bursal Disease (IBD) and Newcastle Disease (ND) are major threats to the poultry industry due to their high mortality rates. These diseases often become more complex when accompanied by bacterial coinfections that further compromise the health status of affected birds. A 15-week-old laying hen exhibited clinical signs including anorexia, decreased egg production, lethargy, diarrhea characterized by watery greenish-white feces, nasal exudate, incoordination, swelling of the frontal and orbital sinuses, and torticollis. Epidemiological analysis revealed a morbidity rate of 5.7%, a mortality rate of 5.1%, and a case fatality rate of 88%. Gross pathological examination demonstrated multiorgan hemorrhage, congestion, hyperemia, petechial hemorrhages in the proventriculus, and enlargement of the bursa of Fabricius accompanied by black discoloration. Histopathological examination revealed inflammatory cell infiltration dominated by lymphocytes in nearly all organs and by heterophils in several tissues. Laboratory testing of bursal tissue using chicken embryo fibroblast cell cultures showed cytopathic effects, indicating IBD virus replication. The hemagglutination (HA) test yielded a titer of 2^5, and the hemagglutination inhibition (HI) test confirmed the

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presence of ND virus. Bacterial cultures from the intestine, liver, and lungs identified *Escherichia coli*, indicating the occurrence of colisepticemia. It can be concluded that the hen was affected by concurrent IBD and ND virus infections, which were further exacerbated by colisepticemia.

Keywords: Colisepticemia, Infectious Bursal Disease, Laying Hens, Newcastle Disease

Abstrak

Infectious Bursal Disease (IBD) dan *Newcastle Disease* (ND) merupakan ancaman utama bagi industri perunggasan karena tingkat mortalitasnya yang tinggi. Permasalahan ini sering menjadi kompleks ketika disertai oleh koinfeksi bakteri dapat yang memperparah kondisi kesehatan ayam. Seekor ayam petelur berumur 15 minggu dengan tanda klinis yang teramati meliputi anoreksia, produksi telur menurun, letargi, diare dengan konsistensi feses encer berwarna putih kehijauan, keluarnya eksudat dari hidung, inkoordinasi gerak, pembengkakan sinus frontalis dan orbitalis serta tortikolis. Hasil analisis data sidik epidemiologi diperoleh angka morbiditas 5,7%, mortalitas 5,1%, dan *Case Fatality Rate* 88%. Hasil pemeriksaan patologi anatomi menunjukkan adanya hemoragi multiorgan, kongesti, hiperemia, petekie pada proventrikulus, dan pembengkakan sekaligus perubahan warna menjadi hitam pada organ bursa fabrisius. Secara histopatologi, ditemukan infiltrasi sel radang yang didominasi oleh limfosit pada hampir seluruh organ dan heterofil pada beberapa organ. Uji laboratorium terhadap jaringan bursa fabrisius menggunakan biakan sel *chicken embryo fibroblast* menunjukkan adanya efek sitopatik pada biakan sel yang mengindikasikan replikasi virus IBD. Uji hemaglutinasi (HA) menunjukkan titer 2^5 dan terkonfirmasi positif terhadap virus ND pada uji hambatan hemaglutinasi (HI). Hasil kultur bakteri terhadap organ usus, hati, dan paru-paru menunjukkan adanya bakteri *Escherichia coli*, yang mengindikasikan terjadinya *colisepticemia.* Dapat disimpulkan bahwa ayam mengalami infeksi virus IBD dan ND yang diperberat oleh *colisepticemia.*

Kata kunci: Ayam Petelur, *Colisepticemia, Infectious Bursal Disease*, *Newcastle Disease*

INTRODUCTION

Layer chickens are one of the most important poultry commodities, contributing significantly to the supply of animal protein through egg production as a nutritious and affordable food source. Egg production plays a major role in poultry-derived protein consumption in Indonesia, making the layer farming industry economically important. The productivity of layer chickens is strongly influenced by management practices, including feed quality, housing environment, biosecurity implementation, and effective disease control (Wahyuni, 2022). However, the layer industry continues to face various challenges, particularly infectious diseases that can lead to reduced production performance, impaired growth, increased morbidity and mortality, and substantial economic losses (Susanti *et al.*, 2021). One of the major diseases affecting layer chickens is Infectious Bursal Disease (IBD), also known as Gumboro disease. This disease is particularly important because of its immunosuppressive nature, in which IBD virus (IBDV) targets immune cells in the bursa of Fabricius and other lymphoid organs, resulting in severe impairment of the chicken's immune system (Wibowo *et al.*, 2017).

In the present case, a 15-week-old commercial layer chicken in the grower phase, approaching the onset of lay, exhibited weakness, anorexia, reduced activity, and a diminished response to environmental stimuli. The observed clinical signs included diarrhea with watery greenish-white feces, mucoid nasal discharge, swelling of the frontal and orbital sinuses, impaired motor coordination, and torticollis. In addition, the bird showed a decline in physiological condition, as evidenced by reduced production performance. The combination of these clinical signs

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suggested the involvement of multiple organ systems, particularly the respiratory, gastrointestinal, and nervous systems. The clinical manifestations observed in this case may be associated with several infectious poultry diseases. Respiratory signs, including nasal discharge and swelling of the frontal and orbital sinuses, may occur in Infectious Coryza, Chronic Respiratory Disease (CRD), Infectious Bronchitis, Newcastle Disease (ND,) and Avian Influenza (AI) (Liu *et al.*, 2025). Diarrhea characterized by watery greenish-white feces is frequently associated with viscerotropic velogenic ND, salmonellosis, and colibacillosis due to gastrointestinal involvement (Suardana & Putra, 2016; Kabir, 2010). Meanwhile, neurological signs such as incoordination and torticollis are commonly reported in cases of neurotropic ND and AI (Hewajuli & Dharmayanti, 2015; Gayanti *et al.*, 2024). The concurrent occurrence of respiratory, enteric, and neurological signs suggested the possibility of a multisystemic infection or coinfection involving multiple pathogens, which may have exacerbated the clinical condition of the bird. Given the wide range of potential etiologies associated with the observed clinical manifestations, a definitive diagnosis could not be established based solely on clinical examination. Therefore, a comprehensive investigation incorporating clinical, pathological, microbiological, and laboratory examinations was required to accurately identify the etiological agents involved.

RESEARCH METHODS

Case Animal

The case animal (protocol number 243/N/26) was a 15-week-old layer chicken originating from a commercial farm in Mangesta Village, Penebel District, Tabanan Regency, Bali. The bird died on February 26, 2026, and a necropsy was performed on the same day at the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Udayana University.

Anamnesis and Epidemiological Investigation

Epidemiological data were collected directly through interviews with the farmer regarding disease history on the farm, vaccination status, and treatments administered to affected birds. Sick chickens were separated from healthy birds as a disease control measure to prevent transmission. Epidemiological parameters were determined based on calculations of morbidity, mortality, and CFR (Nurmayani *et al.*, 2023).

Gross Pathology and Histopathological Examination

Gross pathological changes were examined in the case animal, and organ samples exhibiting lesions were collected. The samples were fixed in 10% neutral buffered formalin and processed for histopathological examination using routine Hematoxylin and Eosin (H&E) staining. Histopathological slide preparation was performed according to the protocol of the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Udayana University (Sewoyo *et al.*, 2025).

Detection of IBDV Using Chicken Embryo Fibroblast (CEF) Cell Culture

IBDV isolation was performed using a cell culture method by inoculating clinical specimens prepared from homogenized bursal tissue. CEF cells are commonly used for the isolation and propagation of IBDV (Librani *et al.*, 2019). Inoculation was carried out in vitro by introducing the viral suspension into the culture medium. The isolation procedure began with the removal of the growth medium from the cell culture, followed by inoculation of the IBDV suspension onto the cell monolayer and incubation for 60 minutes. Subsequently, maintenance medium supplemented with 5% fetal bovine serum (FBS) was added, and the culture was incubated at 37°C under approximately 5% CO_2. The cultures were monitored daily for the development of cytopathic effects.

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HA and HI Tests

The HA and HI tests were performed to identify viruses capable of agglutinating red blood cells, particularly NDV and AI viruses. Viral identification began with inoculum preparation, followed by inoculation into 9-day-old embryonated chicken eggs obtained from a poultry farm in Gubug Village, Tabanan District, Tabanan Regency, Bali. Allantoic fluid was harvested through the allantoic cavity and subsequently subjected to HA and HI testing. All procedures were conducted according to the methods described by Sari *et al.* (2024).

Bacterial Isolation and Identification

Samples collected for bacterial isolation included the intestine, liver, and lungs. Bacterial isolation was performed using Nutrient Agar (NA), MacConkey Agar (MCA), and Eosin Methylene Blue Agar (EMBA). Primary identification included Gram staining to determine bacterial morphology and staining characteristics, as well as the catalase test to assess catalase enzyme production. Additional biochemical tests included Triple Sugar Iron Agar (TSIA), Simmons Citrate Agar (SCA), Sulfide Indole Motility (SIM), Methyl Red (MR), Voges-Proskauer (VP), and carbohydrate fermentation tests using glucose (Hariyanto, 2018).

Parasitological Examination

Parasitological examination was conducted on fecal samples from the case animal to determine the presence or absence of helminth eggs and protozoan infections. Samples were examined qualitatively using direct smear, sedimentation, and flotation techniques with saturated salt solution (Zajac *et al.*, 2021).

Data Analysis

Data obtained from the examinations were analyzed descriptively and qualitatively based on anamnesis, epidemiological findings, clinical observations, gross pathology, histopathology, and laboratory results. The findings were presented in the form of narratives, tables, and figures to support case interpretation.

RESULTS AND DISCUSSION

Results

Epidemiological Investigation

A total population of 9,000 layer chickens with an average age of 15 weeks was maintained in battery cages. The number of clinically affected birds was 518, and 457 birds died. Based on these data, the morbidity rate was 5.7%, the mortality rate was 5.1%, and the CFR was 88%. The flock had been vaccinated against ND, AI, IBD, fowl pox, and coryza, and anthelmintic treatment had been administered. The birds received commercial feed and drinking water supplied by the municipal water system (PDAM). Clinical signs observed included anorexia, decreased egg production, and lethargy. Additional findings included diarrhea with watery greenish-white feces, nasal exudate, incoordination, swelling of the frontal and orbital sinuses, and torticollis, which appeared one day before death.

Gross Pathological Examination

Before necropsy, the case animal showed anorexia, lethargy, decreased egg production, diarrhea with watery greenish-white feces, nasal exudate, incoordination, swelling of the frontal and orbital sinuses, and torticollis. Gross examination revealed lesions in multiple organs. The brain showed hemorrhage and hyperemia; the trachea showed hemorrhage; the lungs showed hemorrhage and dark discoloration; the heart was congested; the liver had a cream-colored discoloration; the spleen was enlarged (splenomegaly) and darkened; the

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kidneys showed hemorrhage; the esophagus showed hemorrhage; the proventriculus showed hemorrhage and petechial lesions; the ventriculus had a grayish-black discoloration; the pancreas showed hemorrhage; the intestines showed hemorrhage; and the bursa of Fabricius was enlarged and blackened.

Histopathological Examination

Histopathological examination demonstrated inflammatory infiltration dominated by lymphocytes throughout multiple organs. Polymorphonuclear inflammatory cells were observed in the heart, lungs, liver, proventriculus, and large intestine. Additional histopathological changes included vasculitis and perivascular edema in the brain; hemorrhage in the brain, trachea, heart, lungs, liver, proventriculus, small intestine, large intestine, and bursa of Fabricius; and necrosis in the spleen, proventriculus, and small intestine.

Virological Examination

Virus isolation in cell culture using bursal tissue specimens produced cytopathic effects, indicating viral replication. Inoculation into embryonated chicken eggs resulted in embryo death at 3 days post inoculation; the embryos appeared stunted and hemorrhagic. The HA test performed using the microtiter technique was positive with a titer of 2^5, as evidenced by red blood cell agglutination. The HI test was positive for NDV infection, indicated by inhibition of hemagglutination with red blood cell sedimentation. HI testing for AI virus was negative.

Pemeriksaan Bakteriologi dan Parasitologi

Bacterial isolation and identification from the intestine, liver, and lungs confirmed infection with Escherichia coli. Colonies that grew on NA also grew on MCA and EMBA. Colony morphology was described as circular, convex, with relatively smooth margins and a diameter of approximately 1–3 mm; colonies were milky white on NA, pink on MCA, and metallic green on EMBA. Gram staining showed pink, rod-shaped bacteria, and the catalase test was positive, as indicated by bubble formation. TSIA results showed an acid slant and acid butt with gas production and no H_2S production. SIM results were indole positive and motility positive, with no H_2S production. Additional biochemical results were MR positive, VP negative, SCA negative, and glucose fermentation positive. Fecal examination by direct smear, sedimentation, and flotation methods was negative for parasitic infection

Discussion

The provisional diagnosis in this case was ND, based on the anamnesis, clinical signs, epidemiological findings, and gross pathological examination, with AI, IBD, and colibacillosis considered as differential diagnoses. Epidemiological analysis revealed a morbidity rate of 5.7%, a mortality rate of 5.1%, and a CFR of 88%. NDV infection has a substantial impact on the poultry industry because of its high morbidity and mortality rates (Ilham and Yusdja, 2010). The clinical signs observed in the case bird, including anorexia, reduced egg production, diarrhea with watery greenish-white feces, nasal exudate, incoordination, swelling of the frontal and orbital sinuses, and torticollis, are characteristic manifestations of ND, which rapidly and extensively affects the respiratory system (Swayne *et al.*, 2000).

Gross pathological examination revealed lesions in multiple organs, reflecting the systemic nature of the infection. The bursa of Fabricius was enlarged and hemorrhagic, consistent with the findings reported by Damairia *et al.* (2023) in chickens confirmed to be infected with IBD. Additional lesions included cerebral hemorrhage accompanied by hyperemia, hemorrhage in the trachea and lungs, petechial hemorrhages in the proventriculus, and intestinal hemorrhage. Splenomegaly observed in the case bird may be attributed to increased splenic activity in

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response to concurrent IBD and ND infections, resulting in stimulation of lymphocytes within lymphoid organs to produce antibodies against the infectious agents (Etriwati *et al.*, 2018).

Histopathological examination demonstrated lesions in nearly all organs evaluated. Widespread lymphocytic infiltration was observed in several vital organs, including the brain, trachea, heart, lungs, liver, proventriculus, intestines, and bursa of Fabricius, suggesting a viral etiology. In addition to lymphocytic infiltration, polymorphonuclear inflammatory cells were detected in the heart, lungs, liver, proventriculus, kidneys, and large intestine, indicating concurrent bacterial infection (Putri *et al.*, 2025). IBDV infection is known to induce lesions in the bursa of Fabricius, including hemorrhage, vasculitis, and lymphoid follicle depletion (Damairia *et al.*, 2023; Wahyuwardani *et al.*, 2011), whereas NDV infection commonly causes vasculitis and perivascular edema in the brain (Nofantri *et al.*, 2017). These pathological alterations were also identified in the present case. In the respiratory tract, the trachea exhibited inflammation and hemorrhage, while the lungs showed inflammation, congestion, hyperemia, and hemorrhage associated with viral replication (Putra *et al.*, 2012). Necrosis observed in the spleen and small intestine may have resulted from an excessive inflammatory response and may also be associated with Chick Lethal Toxin (CLT) produced by *Escherichia coli*. Following absorption into the bloodstream, this toxin can damage endothelial cells, leading to tissue injury (Pranatha *et al.*, 2018; Wardani *et al.*, 2024).

Virus isolation using homogenized bursal tissue cultured in Minimum Essential Medium (MEM) demonstrated cytopathic effects characterized by cell aggregation, cell rounding, and perinuclear granulation, indicating active replication of IBDV in the cultured cells (Rekha *et al.*, 2014). IBD is an acute and highly contagious viral disease that primarily affects chickens younger than four months of age. The disease is caused by a virus belonging to the family *Birnaviridae* and the genus *Avibirnavirus*. The primary target cells of IBDV are immune cells within the bursa of Fabricius and other lymphoid organs; therefore, infection can result in severe immunosuppression (Wibowo *et al.*, 2017). Two serotypes of IBDV have been identified, namely serotype 1 and serotype 2. Serotype 1 is pathogenic in poultry, whereas serotype 2 can induce an antibody response but generally does not produce clinical disease in chickens or turkeys (Putri *et al.*, 2024).

IBDV infection generally occurs via the oral route through contaminated feed, after which the virus enters and replicates within the intestinal tract. Common clinical manifestations include anorexia, lethargy, drooping wings, diarrhea, and fecal staining around the cloaca. In young chickens lacking maternal antibodies, clinical signs typically appear within 48 hours post-infection and become more severe between 56 and 72 hours. In vaccinated chickens, clinical signs generally become apparent approximately three days after infection, and death may occur two to three days after the onset of clinical disease. Birds that survive often experience growth retardation and are predisposed to secondary infections, including ND and colibacillosis. To establish and maintain strong and stable immunity against IBDV throughout the production period, repeated vaccination is recommended, including administration of a live IBD vaccine at 30–33 days of age, followed by inactivated vaccines at 85 and 120 days of age (Wahyuwardani *et al.*, 2011).

NDV infection is caused by *Avian orthoavulavirus 1* (AOAV-1), which belongs to the genus *Orthoavulavirus*, subfamily *Avulavirinae*, and family *Paramyxoviridae* (Adi *et al.*, 2019; Joao *et al.*, 2022). NDV is classified into three pathotypes based on virulence: velogenic (highly virulent), mesogenic (moderately virulent), and lentogenic (low virulence) (Joao *et al.*, 2022). Based on clinical manifestations and pathological lesions, ND is further categorized into five pathotypes: viscerotropic velogenic, which causes acute fatal infection characterized primarily by hemorrhagic intestinal lesions; neurotropic velogenic, which results in high mortality

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preceded by respiratory and neurological disturbances; mesogenic, which causes acute respiratory disease with low mortality; lentogenic or respiratory, which causes mild or inapparent respiratory signs; and asymptomatic, which does not produce clinical signs (Hewajuli & Dharmayanti, 2015). NDV causes respiratory disease because it preferentially replicates within ciliated epithelial cells lining the respiratory tract (Costa *et al.*, 2012). Conjunctival swelling and hemorrhage are also frequently observed in chickens affected by ND (Angreini *et al.*, 2023). NDV is capable of replicating in the mucosal epithelium of the digestive tract and damaging intestinal villi, thereby impairing nutrient and water absorption, resulting in watery diarrhea. The green discoloration of the feces is associated with impaired liver and pancreatic function, whereas the white coloration is attributed to excessive uric acid excretion secondary to dehydration and renal dysfunction (Suardana & Putra, 2016). Furthermore, NDV possesses immunosuppressive properties that may increase susceptibility to secondary *E. coli* infection.

In addition to viral infection, bacteriological examination confirmed that the case bird was positive for *E. coli* infection, with the organism isolated from extraintestinal organs, including the lungs and liver. Although *E. coli* is a normal component of the intestinal microbiota, it acts as an opportunistic pathogen and may cause colisepticemia, particularly when associated with strains of Avian Pathogenic *Escherichia coli* (APEC) (De Carli *et al.*, 2015). Immunosuppression induced by other pathogens may facilitate secondary invasion by *E. coli*. Colisepticemia caused by *E. coli* is characterized clinically by emaciation, dull feathers, anorexia, depression, diarrhea, and soiling or matting of feathers around the cloaca (Hastarinda, 2016). Transmission may occur through contaminated feed and drinking water, with subsequent spread to other birds through fecal-oral or aerosol routes (Wardani *et al.*, 2024).

The pathogenesis begins when contaminated dust particles are inhaled or contaminated feed is ingested, allowing *E. coli* to adhere to the intestinal mucosa and the epithelial villi of the respiratory tract. The bacteria subsequently damage intestinal and respiratory tissues through the production of enterotoxins. These toxins contribute to intestinal lesions, including villous rupture, tracheal hemorrhage, and pulmonary congestion (Cheeseman, 2007), all of which were observed in the present case. The toxins can also disrupt the intestinal mucosal barrier, enabling *E. coli* to enter the bloodstream and cause bacteremia. Once in circulation, the bacteria proliferate by resisting phagocytosis and bactericidal activity through the production of capsules and colicins. Phagocytic activity may result in bacterial lysis, releasing endotoxins (lipopolysaccharides) into the bloodstream. These endotoxins are subsequently distributed throughout the body and may reach the myocardium, pericardium, liver, and other organs, resulting in fibrinous exudation in the heart and liver (Mahari, 2014). Polymorphonuclear inflammatory cell infiltration was also observed in the kidneys, indicating acute bacterial infection and inflammation. This finding is consistent with the role of the kidneys as one of the primary organs involved in filtering bacterial toxins (Ananda *et al.*, 2023). Dutta *et al.* (2013) reported that kidneys affected by *E. coli* infection commonly exhibit congestion and hemorrhage, lesions that were likewise identified in the present case. Predisposing factors for colisepticemia include inadequate sanitation and disinfection practices, poor housing conditions, insufficient equipment management, the presence of immunosuppressive diseases, high stocking density, excessive dust, elevated ammonia concentrations, and fecal accumulation (Ananda *et al.*, 2023).

In the present case, the bird was affected by a multipathogen infection involving IBDV, NDV, and systemic *E. coli* infection. Several factors may have contributed to the occurrence of this multiple infection: (1) vaccination failure, potentially resulting from the absence of booster vaccination against IBDV in layer chickens (Wahyuwardani *et al.*, 2011), improper vaccine

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handling, or inadequate vaccine storage (Shekaro, 2015); (2) the development of colisepticemia secondary to the immunosuppressive effects of IBDV and NDV infection; (3) respiratory and gastrointestinal lesions caused by NDV that facilitated the entry of *E. coli* into the bloodstream; (4) inadequate biosecurity measures; (5) suboptimal sanitation and disinfection practices; (6) high stocking density, excessive dust, elevated ammonia levels, and fecal accumulation; (7) insufficient isolation and segregation of sick birds; and (8) environmental stress associated with seasonal weather fluctuations during the rainy season.

CONCLUSION AND SUGGESTIONS

Conclusion

The mortality observed in this layer chicken was attributed to a complex infection involving IBD, ND, and secondary colisepticemia. The combined effects of these infectious agents contributed to the high CFR of 88%.

Suggestions

Enhanced biosecurity measures, implementation of scheduled vaccination programs, and early disease detection through routine monitoring are recommended to prevent similar outbreaks. Furthermore, farmer education regarding the prompt and appropriate management of diseased birds is essential to reduce mortality rates and minimize economic losses associated with infectious diseases.

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Figures

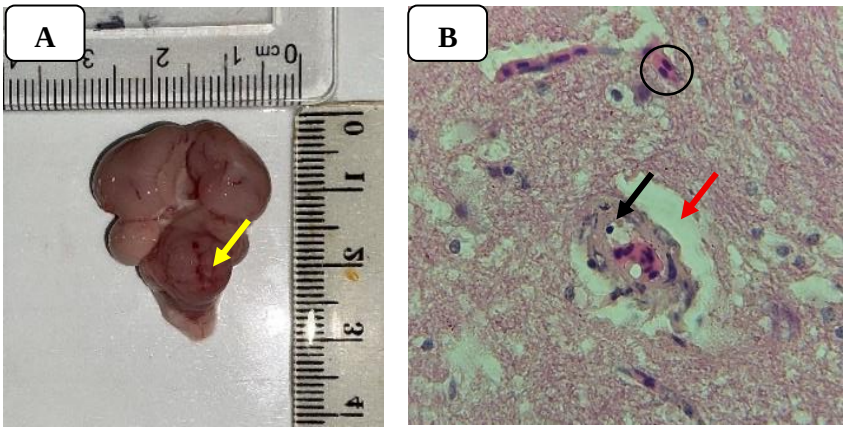

Figure 1. (A) Brain showing hyperemia. (B) Non-suppurative encephalitis, characterized by vasculitis (black arrow) and perivascular edema (red arrow).

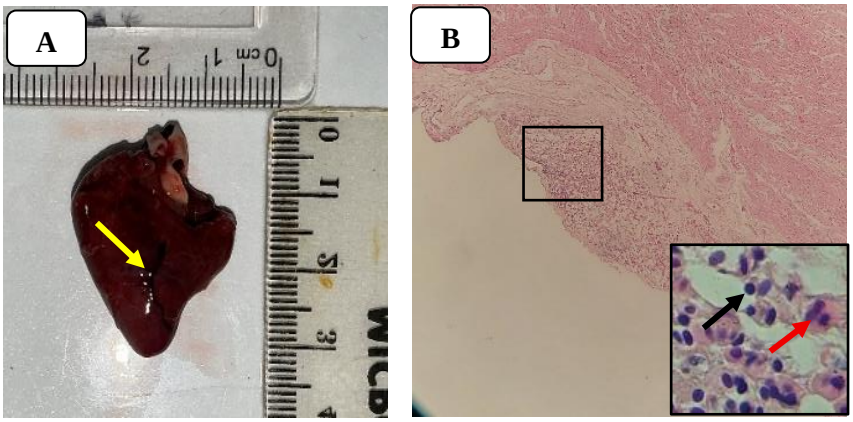

Figure 2. (A) Heart showing congestion and dark red to black discoloration. (B) Hemorrhagic epicarditis, characterized by epicarditis (black box), lymphocytic infiltration (black arrow), and hemorrhage (red arrow).

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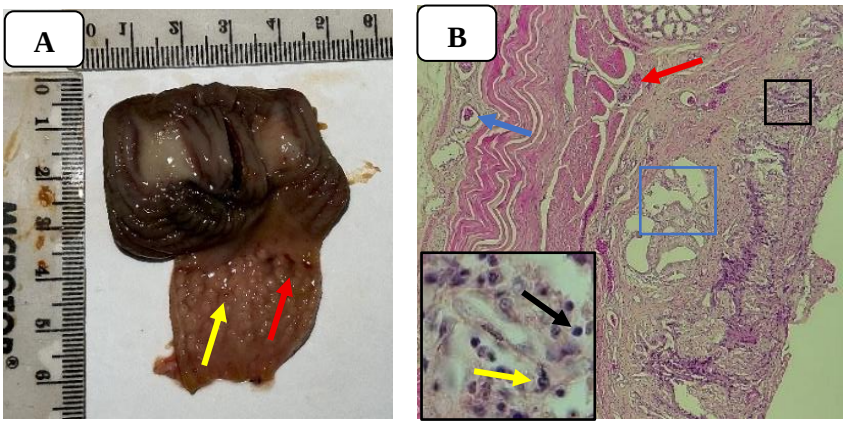

Figure 3. (A) Proventriculus showing petechial lesions and hemorrhage. (B) Congestive, hemorrhagic, and necrotizing proventriculitis, characterized by lymphocytic infiltration (black arrow), polymorphonuclear inflammatory cell infiltration (yellow arrow), congestion (blue arrow), and hemorrhage (red arrow).

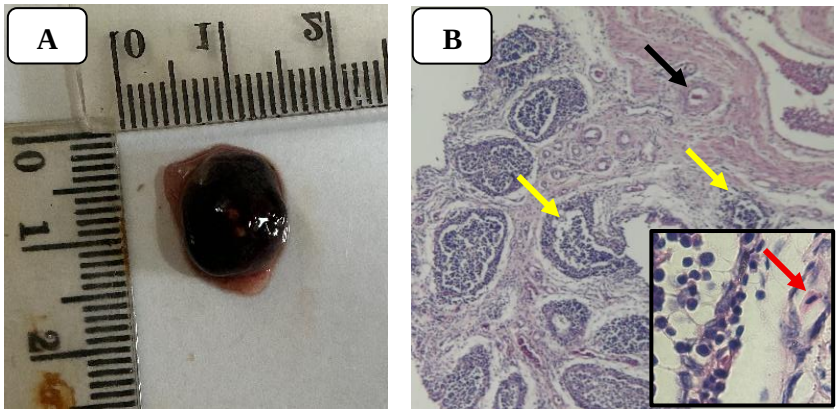

Figure 4. (A) Bursa of Fabricius showing enlargement and black discoloration. (B) Hemorrhagic bursitis, characterized by hemorrhage (red arrow), vasculitis (black arrow), and lymphoid follicle depletion (yellow arrow).

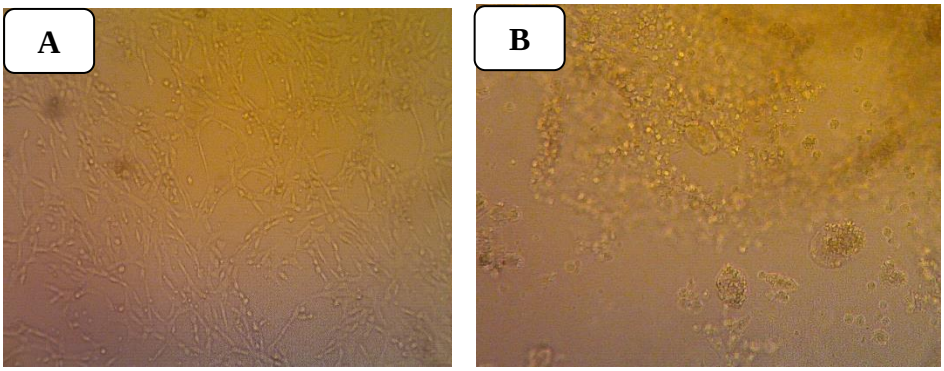

Figure 5. Results of virus isolation in first-passage cell culture: (A) normal cell culture control and (B) cytopathic effects characterized by cell rounding and cytoplasmic granulation.

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