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SUSPECTED PORCINE REPRODUCTIVE AND RESPIRATORY SYNDROME WITH SECONDARY STAPHYLOCOCCUS SP. INFECTION IN PIGS: A CASE STUDY

Suspek Porcine Reproductive and Respiratory Syndromedengan Infeksi Sekunder Staphylococcus sp. pada Babi: Studi Kasus

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Abstract

The pig farming industry in Indonesia plays an important role in the economy and food security. However, infections caused by the Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) and secondary infections by bacteria such as Staphylococcus sp. often threaten the health of pigs and farm productivity. This study aims to analyze PRRSV infections and secondary infections by Staphylococcus sp. in pigs through clinical, histopathological, and bacteriological approaches. The research method uses a qualitative approach, focusing on data collection in the form of signalments, anamnesis, epidemiological data, clinical signs, and laboratory examination of a sample taken from a pig farm in Gianyar, which was subsequently analyzed descriptively. The observed clinical findings in the case animal included diarrhea, malnutrition with BCS-1, stunted growth (dwarfism), anorexia, lethargy, dyspnea, paddling before death, and lesions at several body sites. Histopathological examination revealed hemorrhagic et necrotizing meningoencephalitis, tracheitis, myocarditis et edematous, hemorrhagic et necrotizing bronchopneumonia, hemorrhagic hepatitis, lymphoid depletion, hemorrhagic et necrotizing glomerulonephritis with glomerular atrophy, gastritis, enteritis with mucosal erosion, typhlitis and necrosis, and hemorrhagic colitis with mucosal erosion. Bacteriological examination identified Staphylococcus sp. pathogenic bacterium. Based on clinical analysis, anatomical pathology, histopathology, and bacteriology, the pig in question

is suspected to be infected with the PRRSV with a secondary infection by Staphylococcus sp. pathogen. Animal health management practices, especially PRRSV vaccination and proper herd management, are critical to reduce the risk of secondary infections by bacteria like Staphylococcus sp. Further research, particularly molecular Polymerase Chain Reaction (PCR) testing, is required to confirm the diagnosis.

Keywords: anatomical pathology, histopathology, Porcine Reproductive and Respiratory Syndrom, Staphylococcus aureus

Abstrak

Industri peternakan babi di Indonesia berperan penting dalam perekonomian dan ketahanan pangan. Namun, infeksi *Porcine Reproductive and Respiratory Syndrome Virus* (PRRSV) dan infeksi sekunder oleh bakteri seperti *Staphylococcus sp*. sering kali mengancam kesehatan babi dan produktivitas peternakan. Penelitian ini bertujuan untuk menganalisis infeksi PRRSV dan infeksi sekunder oleh *Staphylococcus sp.* pada babi melalui pendekatan klinis, histopatologi, dan bakteriologi. Metode Penelitian ini menggunakan metode kualitatif dengan fokus pada pengumpulan data berupa, sinyalemen, anamnesa, epidemiologis, tanda klinis dan pemeriksaan laboratorium pada satu sampel yang diambil dari salah satu peternakan babi di Gianyar untuk selanjutnya dianalisis secara deskriptif. Adapun temuan klinis yang teramati dari hewan kasus yakni diare, malnutrisi dengan BCS-1, pertumbuhan terhambat (kekerdilan), anoreksia, letargi, *dispnea*, *paddling* sebelum mati dan terdapat luka pada beberapa titik area tubuh. Hasil pemeriksaan histopatologi menunjukkan terjadi *Meningoencephalitis hemorrhagis et necroticans, Tracheitis, Myocarditis et Edematous, Bronchopneumonia hemorrhagis et necroticans, Hepatitis et hemorrhagis, Deplesi Limfoid, Glomerulonephritis hemorrhagis et necroticans* disertai *atrophi glomerulus, Gastritis, Enteriti*s disertai *erosi mukosa*, *Typhlitis et necroticans*, dan *Colitis hemorrhagis disertai erosi mukosa.* Hasil pemeriksaaan bakteriologi mengidentifikasi bakteri *Staphylococcus sp.* pathogen. Berdasarkan analisis klinis, patologi anatomi, histopatologi dan bakteriologi bahwa babi kasus diduga terinfeksi PRRSV dengan infeksi sekunder *Staphylococcus sp. pathogen.* Praktik manajemen kesehatan hewan dengan fokus pada vaksinasi PRRSV dan pengelolaan yang baik terhadap populasi ternak untuk mengurangi risiko infeksi sekunder oleh bakteri seperti Staphylococcus sp. Selanjutnya, penelitian lebih lanjut seperti uji molekuler *Polymerase Chain Reaction* (PCR) diperlukan untuk mengkonfirmasi diagnosa.

Kata kunci: histopatologi, patologi anatomi, *Porcine Reproductive and Respiratory Syndrom*, *Staphylococcus aureus*

INTRODUCTION

The swine industry plays a substantial role in supporting local economies across various regions of Indonesia and represents an important sector contributing to national food security and economic welfare. The national swine population is concentrated in several provinces, including North Sumatra, Central Java, North Sulawesi, East Nusa Tenggara, Maluku, Papua, and Bali. According to the Central Statistics Agency (2025, updated 28 February 2025), the pig population in Indonesia has reached approximately 4.1 million heads. The rapid growth of the swine industry has increased farmers' reliance on international partners for the procurement of biological products, high-quality semen, and embryos. This dependence, however, also heightens the risk of introduction and dissemination of viral infectious diseases nationwide (Suartha et al., 2013). One of the major diseases of concern is Porcine Reproductive and Respiratory Syndrome (PRRS).

Porcine Reproductive and Respiratory Syndrome (PRRS) is among the most economically devastating diseases in the swine industry and has been reported in multiple regions (Cao et al., 2014 in Ouyang et al., 2019). A study by Suartha et al. (2013) revealed that PRRSV has become endemic in pig farms in Bali. The etiological agent of PRRS is an enveloped, positive-sense RNA virus within the order *Nidovirales* and family *Arteriviridae*, which has a broad transmission potential through saliva, semen, direct contact, fomites, and even airborne routes (Lunney et al., 2016; Suartha et al., 2013). Typical clinical manifestations include reproductive failure in pregnant sows and mild to severe respiratory disease in neonates, weaned pigs, and growing pigs (Ouyang et al., 2019). Infection of PRRSV frequently serves as a key contributor to Porcine Respiratory Disease Complex (PRDC), increasing host susceptibility to secondary infections (D'Annunzio et al., 2023; Zhao et al., 2021). The term PRDC refers to a multifactorial condition involving various viral and bacterial pathogens and less commonly parasites combined with environmental, managerial, and genetic factors, ultimately leading to respiratory disease in pigs (D'Annunzio et al., 2023).

Secondary infections in PRDC are commonly caused by pathogenic bacteria, including *Staphylococcus* spp. According to Paramita et al. (2020), *Staphylococcus* spp. exhibiting β-hemolytic and γ-hemolytic characteristics were identified in the respiratory tract of pigs affected by PRDC. These Gram-positive cocci function as both normal microflora and opportunistic pathogens that can be found on the skin, mucous membranes, gastrointestinal tract, and respiratory tract of pigs (Purwanti et al., 2018; Rahmawati et al., 2025). Under pathogenic conditions, they may cause severe diseases such as pneumonia, meningitis, bacteremia, gastroenteritis, osteomyelitis, and urinary tract infections (Taylor & Unakal, 2023). The severity of infection is strongly influenced by the dynamic interaction between PRRSV and *Staphylococcus* spp., which may mutually exacerbate disease progression (Pyo et al., 2025).

Although PRDC is frequently reported as a complication of PRRS, the specific pathogenic relationship between PRRSV and *Staphylococcus* spp. remains insufficiently understood, particularly in relation to multisystemic manifestations observed in field cases. Therefore, this case study aims to examine the potential role of PRRSV as the primary infectious agent while considering secondary infections by *Staphylococcus* spp., using clinical, gross pathological, histopathological, and bacteriological evaluations of organs from affected pigs. This assessment is expected to enhance understanding of disease pathogenesis and provide valuable insights for farmers and veterinary professionals in making informed decisions on swine health management, including strategies for preventing both primary and secondary infections, thereby reducing economic losses associated with PRRSV and *Staphylococcus* spp. infections.

MATERIALS AND METHODS

Case Animal

The pig in this case originated from a breeding farm owned by Mr. I Komang Adnyana, located in Banjar Patas, Taro Village, Tegallalang District, Gianyar Regency, Bali.

Anamnesis and Clinical Signs

Anamnesis was conducted through an in-depth interview with the owner to obtain information regarding management practices, disease history, previous treatments, vaccination records, and observed clinical signs.

Epidemiology

Epidemiological data were collected via direct field observation and structured interviews with the farm owner to gather key herd information, including total population, the number of

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clinically affected animals, and recorded mortality. These data were subsequently utilized to calculate critical disease impact metrics in line with standard epidemiological principles: disease distribution (morbidity rate), mortality rate, and case fatality rate (CFR). The calculations were performed using the following standard formulas: Morbidity Rate = (Number of sick animals / Number of animals at risk) × 100%; Mortality Rate = (Number of dead animals / Number of animals at risk) × 100%; and Case Fatality Rate (CFR) = (Number of dead animals / Number of sick animals) × 100%.

Gross Pathology Examination

The animal was necropsied, and each organ was collected for macroscopic evaluation of structural changes in affected or abnormal tissues. Tissue samples were immediately placed in 10% Neutral Buffered Formalin (NBF) for preservation and subsequently processed for histological preparation to enable microscopic evaluation of pathological alterations.

Histopathology Examination

Histopathological preparation was carried out at the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Udayana University. Tissue samples fixed in 10% NBF for more than 24 hours to halt biological processes and preserve tissue architecture were trimmed into 1 × 1 × 1 cm sections. Samples then underwent dehydration through a graded ethanol series (70%, 80%, 90%, 96%, and absolute 100%) to remove water, followed by clearing in xylol to eliminate residual alcohol. The tissues were subsequently impregnated with molten paraffin, embedded, and blocked to facilitate sectioning. Sectioning was performed using a microtome at a thickness of 3–4 μm, and the sections were mounted on glass slides and incubated at 50°C for 24 hours for drying. Hematoxylin Eosin (HE) staining was then conducted to visualize tissue structures, followed by mounting with coverslips using permount to protect the samples and enhance image clarity. The stained slides were examined microscopically under 100×, 400×, and 1000× magnification.

Bacterial Isolation and Identification

Bacteriological examination was performed at the Veterinary Bacteriology and Mycology Laboratory, Faculty of Veterinary Medicine, Udayana University. Bacterial culture was initiated by inoculating organ samples onto Nutrient Agar (NA) and incubating them at 37°C for 24 hours. Subculturing was then carried out on NA to obtain pure colonies, which were subjected to primary tests including Gram staining and the catalase test.

Gram staining followed the Hucker method. Fixed smears were stained with crystal violet for 1 minute, rinsed, treated with Lugol's iodine for 1 minute, rinsed again, decolorized with acetone alcohol for 30 seconds, rinsed, counterstained with safranin for 1 minute, rinsed, air dried, and examined under 1000× magnification (Lay, 1994). The catalase test was performed by transferring a bacterial colony onto a glass slide and adding 3% H_2O_2; the presence of gas bubbles (O_2) indicated a positive reaction (Tasnim, 2017).

Pure colonies were further streaked onto the selective and differential medium Mannitol Salt Agar (MSA). The test was performed by streaking NA grown colonies into MSA and incubating them at 37°C for 24 hours. Positive results were indicated by a change in medium color to yellow, while negative results showed no color change (Rahmi et al., 2015). Hemolysis testing was conducted using Blood Agar (BA). A single colony from a slant culture was streaked onto BA supplemented with 5% sheep blood, and plates were incubated at 37°C for 24–48 hours. Hemolytic activity was indicated by a clear or transparent zone around the colonies (Buxton, 2016). Additional biochemical tests including Triple Sugar Iron Agar

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(TSIA), Sulfide Indole Motility (SIM), Simmons Citrate Agar (SCA), Methyl Red Voges Proskauer, and carbohydrate fermentation (glucose) were also conducted.

Data Analysis

Data obtained from the case study were analyzed qualitatively using descriptive methods based on empirical findings and presented in tables and figures.

RESULTS AND DISCUSSION

Results

Signalement, Anamnesis, and Clinical Signs

The case animal was a 9 day old male piglet of mixed Landrace Yorkshire breed originating from a breeding farm owned by Mr. I Komang Adnyana, located in Banjar Patas, Taro Village, Tegallalang District, Gianyar Regency, Bali. According to the owner, the piglet experienced continuous diarrhea, reduced appetite, and stunted growth. The animal was also reported to have been weak since birth. Similar clinical signs were observed in 30 other piglets, among which seven died within the past week. All 31 piglets, including the case animal, originated from five sows, each with a history of reproductive problems, including repeated abortions and weak or stillborn piglets.

No specific treatment had been administered for the clinical signs; management focused only on oxytocin administration in sows and vitamin supplementation as needed. The piglets were fed through natural suckling, while the sows received NP56P feed produced by PT Charoen Pokphand Indonesia Tbk. Drinking water was supplied ad libitum from the local public water company. The case animal had not yet been vaccinated because vaccinations were scheduled post-weaning; all sows, however, had been vaccinated against Foot-and-Mouth Disease (FMD) and Hog Cholera. Previous disease history on the farm included reproductive failure in sows, respiratory and gastrointestinal disorders in pre- and post-weaning piglets, FMD, colibacillosis, and streptococcosis. Several other pig farms were located within a 500-meter radius of the premises.

Clinically, the piglet showed diarrhea, malnutrition with BCS 1, growth retardation, anorexia, lethargy, dyspnea, pre-mortem paddling, and skin lesions on several body regions (tarsal, carpal, pinna, and upper lip).

Epidemiological Profile

The farm operated an open semi-slatted housing system with relatively high stocking density, consisting of 700 pigs: 20 boars, 180 sows, and 500 piglets. Feed provided was NP56P, and water was supplied ad libitum from PDAM. Environmental factors such as continuous rainfall, fluctuating temperatures, and increased humidity likely contributed to higher susceptibility to infection. Additionally, Porcine Reproductive and Respiratory Syndrome (PRRS) cases had recently increased in Bali, raising concern among pig farmers regarding impacts on herd productivity and health. The presence of several pig farms within a 500-meter radius may further facilitate disease transmission. Epidemiological calculations showed a morbidity rate of 4.28%, mortality of 1.14%, and a case fatality rate of 26.67%.

Gross and Histopathological Findings

Gross pathological examination revealed extensive abnormalities across multiple organs, consistent with a systemic or multi-organ pathological process. The brain, trachea, lungs, heart, liver, spleen, stomach, and intestines showed marked discoloration ranging from red to dark, along with hemorrhage, swelling, and necrosis (Figure 1).

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Histopathology demonstrated lesions including hemorrhagic and necrotizing meningoencephalitis; tracheitis; edematous myocarditis; hemorrhagic and necrotizing bronchopneumonia; and hemorrhagic hepatitis. Additional changes included lymphoid depletion, hemorrhagic and necrotizing glomerulonephritis with glomerular atrophy, gastritis, enteritis with mucosal erosion, necrotizing typhlitis, and hemorrhagic colitis with mucosal erosion (Figure 2-3). All findings are summarized in Table 1.

Bacterial Isolation and Identification

Isolation and identification began with culturing brain and lung tissues on Nutrient Agar (NA), yielding morphologically distinct colonies. Brain isolates (BO1 and BO2) appeared white and white-yellowish, respectively, while the lung isolate (BP) produced white colonies. All were round, convex, with smooth edges, measuring <1–2 mm.

Gram staining revealed purple, clustered cocci consistent with *Staphylococcus* spp. Catalase testing was positive, indicated by gas bubble formation. Subculture on Mannitol Salt Agar (MSA) showed no color change for BO1 and BP, whereas BO2 produced yellow colonies, suggesting mannitol fermentation. Hemolysis testing on Blood Agar revealed β-hemolysis in all isolates.

Additional biochemical tests including Triple Sugar Iron Agar (TSIA), Sulfide Indole Motility (SIM), Simmons Citrate Agar (SCA), Methyl Red Voges Proskauer (MRVP), and carbohydrate fermentation are presented in Tables 2–3 and Figure 4.

Discussion

This study describes important findings related to Porcine Reproductive and Respiratory Syndrome (PRRS), particularly PRRSV infection followed by secondary pathogenic Staphylococcus sp. infection. The results demonstrate that PRRSV with secondary bacterial involvement can induce widespread systemic damage in pigs. Epidemiological data from the affected herd showed morbidity of 4.28%, mortality of 1.14%, and a case fatality rate of 26.67%, indicating relatively low outbreak severity. In Bali, PRRSV has long been endemic in pig farms (Suartha et al., 2013). This aligns with Donoso and Jarvis (2022), who reported that endemic strains often display lower virulence, leading to outbreaks characterized by comparatively mild morbidity and mortality. This reduced severity is likely attributable to viral adaptation to the local pig population and improved immune responses in persistently infected pigs, which together lessen disease severity and mortality (Lunney et al., 2016; Ouyang et al., 2019). During persistent infection, viral replication declines to levels at which PRRSV is no longer detectable in the blood or lungs, and pigs may cease showing overt clinical signs (Lunney et al., 2016).

PRRSV transmission dynamics in pig farms are shaped by multiple interacting factors, including herd management practices (such as vaccination) and environmental conditions such as pig density, proximity to other farms, and climate (Franzo et al., 2021). In this case, the pigs had not been vaccinated against PRRSV, which increased their risk of infection, given that vaccination is a primary tool for PRRSV control (Chae, 2021). The farm used an open-raised platform system with relatively high stocking density. High-density housing increases the risk of PRRSV outbreaks by facilitating viral spread through direct contact, bodily secretions, and stress-induced immune suppression (Jara et al., 2021 in Franzo et al., 2021). The farm also experienced fluctuating weather characterized by heavy rainfall followed by heat, and several nearby farms kept pigs as well. Climate contributes significantly to infection pressure, as temperature and climate variability influence viral migration and persistence by affecting host susceptibility and environmental stability of the virus. This is consistent with findings showing

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that PRRSV spread between locations is associated with low temperature, low relative humidity, and reduced sunlight exposure (Pitkin et al., 2009; Hermann et al., 2007; Dee et al., 2009 in Franzo et al., 2021). The presence of multiple pig farms in close proximity further supports the possibility of inter-farm PRRSV transmission (Jara et al., 2021).

Historical information from this breeding farm included reproductive failure in sows, such as repeated abortions and the delivery of weak or stillborn piglets, along with preweaning and postweaning pigs that exhibited poor growth, respiratory distress, and digestive disturbances. These signs are characteristic of PRRS, which causes reproductive failure in pregnant sows and mild to severe respiratory disease in neonates, weaners, and growing pigs (Ouyang et al., 2019). Clinical examination of the case pigs revealed diarrhea, malnutrition with a body condition score of 1, stunted growth, anorexia, lethargy, dyspnea, pre-mortem paddling, and skin lesions on the tarsal, carpal, pinna, and upper lip regions. These signs are consistent with previous reports (Chen et al., 2024; Allan & Ellis, 2000 in Suartha et al., 2013; Vetmedica, 2025). The piglets in this case were also born weak, suggesting in utero infection. This corresponds with findings by Terpstra et al. (1991); Mengeling et al. (1994); Park et al. (1996); Cheon & Chae (2000); and Ladig et al. (2015) in Lunney et al. (2016), which state that PRRSV infection in late-gestation sows frequently results in transplacental fetal infection and the birth of congenitally infected, weak piglets. Clinical severity often increases in the presence of co-infections with viral or bacterial pathogens (Franzo et al., 2021). Lunney et al. (2016) also described how PRRSV-induced immunomodulation promotes secondary bacterial infection or PRDC, as immunosuppression allows opportunistic pathogens to cause more severe and chronic disease.

Among secondary bacteria involved in PRDC, Staphylococcus sp. is a potential pathogen. Bacterial isolation and identification revealed colonies from the brain (BO1 and BO2) that were white and pale yellow, respectively, and white convex colonies from the lungs (BP), all measuring <1–2 mm with smooth margins. Gram staining showed Gram-positive cocci arranged in grape-like clusters, consistent with Staphylococcus morphology. Gram-positive bacteria retain the crystal violet–iodine complex due to their thick peptidoglycan layer (Suardana et al., 2021), as the low lipid content and dense cell wall prevent dye removal during decolorization (Bisen, 2014).

Microscopic examination and a positive catalase reaction confirmed the identity of Staphylococcus sp. The presence of oxygen bubbles indicated enzymatic detoxification of hydrogen peroxide (Hidayat & Alhadi, 2012). Further biochemical testing using Mannitol Salt Agar (MSA) produced positive results for BO2, indicated by a yellow color change, confirming mannitol fermentation consistent with *S. aureus*. Negative results for BO1 and BP suggested *S. epidermidis* (no color change) or *S. saprophyticus* (localized yellow–pink discoloration), following criteria described by Abdilah & Kurniawan (2022). Hemolysis testing showed beta-hemolysis, characterized by complete lysis of red blood cells and clear zones around colonies (Buxton, 2016).

Staphylococcus aureus is the most virulent Staphylococcal species. It forms white to golden colonies and typically produces dual hemolysis zones on sheep blood agar, with complete inner hemolysis caused by beta-hemolysin and partial outer hemolysis caused by alpha-hemolysin (Frana & Hau, 2019). This pathogen can cause a wide range of diseases, from chronic biofilm-associated infections to life-threatening systemic conditions such as bacteremia, pneumonia in piglets, and osteomyelitis (Chen et al., 2017). It may also lead to mastitis, vaginitis, metritis, and umbilical abscesses (Frana & Hau, 2019). Host susceptibility increases with age, immune dysfunction, stress, and dysbiosis caused by excessive antimicrobial use (Zimmerman et al., 2019).

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Skin lesions observed in this case (tarsal, carpal, pinna, and upper lip) were likely entry points for *S. aureus*, consistent with statements by Frana and Hau (2019) that compromised skin and mucosa increase infection risk. Some *S. aureus* strains produce exfoliative toxins that damage tissues, and systemic invasion may lead to bacteremia and life-threatening neonatal sepsis, which can cause stunting in piglets (Frana & Hau, 2019), matching the clinical findings in this case. In severe cases, bacteremia may progress to infections of the heart valves, liver, kidneys, spleen, and other internal organs (Frana & Hau, 2019).

Gross and histopathological examinations revealed significant changes across nearly all major organs, reflecting severe damage caused by combined viral and bacterial infection. In the brain, congestion, hemorrhage, and swelling were observed, with histopathology confirming hemorrhagic and necrotizing meningoencephalitis characterized by congestion, hemorrhage, inflammatory infiltration, necrosis, demyelination, and gliosis. These results are consistent with Tian et al. (2007), who reported PRRSV-associated central nervous system involvement. *S. aureus* may also contribute to brain pathology. Astrup et al. (2013) documented meningitis and microabscesses in pigs with systemic bacterial infection, commonly associated with conditions such as endocarditis.

Respiratory lesions included reddening of the trachea and diffuse pulmonary hemorrhage with pale foci, consistent with PRRSV-induced interstitial pneumonia described by IOWA (2025), ranging from multifocal to diffuse patterns with mottled lungs. Histopathology confirmed hemorrhagic and necrotizing bronchopneumonia, with bronchiolar exudate, alveolar septal thickening and necrosis, and pulmonary congestion. These findings align with Chen et al. (2024) and Suartha et al. (2013). PRRSV replicates in the respiratory tract and disseminates systemically, making viremia central to respiratory disease development (Franzo et al., 2021). Secondary *S. aureus* infection likely exacerbated pulmonary pathology, as shown in studies by Soerensen et al. (2005), who observed *S. aureus*-associated bronchopneumonia.

Cardiac examination revealed diffuse redness, and histopathology demonstrated myocarditis with congestion, hyperemia, edema, and inflammatory infiltration, consistent with Tian et al. (2007). The liver displayed dark brown discoloration at the margins, hemorrhage, and swelling. Histopathology confirmed hemorrhagic hepatitis with portal and hepatic vascular congestion, hepatocellular necrosis, and lymphocytic and neutrophilic infiltration, similar to findings by Tian et al. (2007).

Splenic lesions consisted of red-brown discoloration and enlargement, with histopathology showing lymphoid depletion. Similar observations were reported by Chen et al. (2024). Lymphoid depletion correlates positively with viral load in infected tissues (Darwich & Mateu, 2012; Meng, 2013 in Shi et al., 2021). Renal examination revealed cortical redness and necrosis. Histopathology showed hemorrhagic and necrotizing glomerulonephritis with glomerular atrophy, congestion, inflammatory infiltration, and tubular necrosis, consistent with previous reports of interstitial nephritis and hemorrhage by Suartha et al. (2013).

The digestive tract exhibited marked pathological changes, with reddish to black discoloration of the serosa and mucosa. Histopathology confirmed gastritis and enteritis with mucosal erosion and infiltration of macrophages, lymphocytes, and neutrophils. These changes align with Tian et al. (2007), who found intestinal ulceration in PRRSV-infected pigs. Secondary *S. aureus* likely worsened these lesions, as enterotoxin-producing strains can contribute to enteritis (Taylor et al., 1982 in Frana & Hau, 2019).

The interaction between PRRSV and secondary *Staphylococcus aureus* infection underscores the need for a deeper understanding of the pathogenesis of systemic disease in pigs. As the primary immunosuppressive agent, PRRSV compromises host defense mechanisms, enabling

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opportunistic bacteria to invade vital organs. Nevertheless, this study has limitations. Although clinical, pathological, and bacteriological findings strongly support a PRRSV-associated secondary bacterial infection, the absence of molecular methods such as polymerase chain reaction (PCR) for specific detection of PRRSV and *S. aureus* reduces diagnostic precision. Additionally, this investigation is based on a single case, which may not represent the broader population of infected pigs.

CONCLUSION AND RECOMMENDATIONS

Conclusion

Based on the results of this study, which included signalment, clinical history, clinical signs, epidemiological data, gross pathology, histopathology, and bacteriological findings, the affected pig was suspected to be infected with Porcine Reproductive and Respiratory Syndrome Virus (PRRSV) accompanied by a secondary infection with pathogenic *Staphylococcus* spp. This co-infection resulted in multisystemic lesions involving the brain, trachea, lungs, heart, liver, spleen, kidneys, and gastrointestinal tract.

Recommendations

Improved animal health management with an emphasis on PRRSV vaccination and appropriate husbandry practices is strongly recommended to reduce the risk of secondary bacterial infections such as *Staphylococcus* spp. In addition, further investigations using molecular methods, particularly Polymerase Chain Reaction (PCR), are needed to confirm the diagnosis.

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Table

Table 1. Gross and Histopathological Findings in the Case Animal

Organ	Gross Pathology	Histopathology
Brain	Congestion, diffuse reddening, and swelling of the cerebrum	Hemorrhagic and necrotizing meningoencephalitis
Trachea	Reddening of the serosa and between tracheal rings	Tracheitis
Lungs	Multifocal reddening, uneven discoloration to dark red-brown, and interstitial pneumonia	Hemorrhagic and necrotizing bronchopneumonia
Heart	Generalized reddening of the myocardium	Myocarditis et edematous
Liver	Dark brown discoloration along the margins with swelling	Hepatitis et hemorrhagis
Spleen	Red-brown discoloration with rounded, swollen edges	Lymphoid depletion
Kidneys	Reddening and cortical necrosis	Hemorrhagic and necrotizing glomerulonephritis with glomerular atrophy
Stomach	Red to black discoloration of the serosa and mucosa	Gastritis
Small Intestine	Distension and red to black discoloration in several segments	Enteritis with mucosal erosion
Cecum	Red to black discoloration	Typhlitis et necroticans
Large Intestine	Distension and red to black discoloration in several segments	Hemorrhagic colitis with mucosal erosion

Table 2. Morphological Characteristics of Bacterial Colonies Isolated from the Case Animal

Isolate Code	Color	Shape	Margin	Diameter	Surface
BO1	White	Round	Smooth	<1–2 mm	Convex
BO2	Yellowish-white	Round	Smooth	<1–2 mm	Convex
BP	White	Round	Smooth	<1–2 mm	Convex

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Table 3. Results of Bacterial Isolation and Identification in the Case Animal

Test	Result	Description
Gram Stain	Gram positive	Purple, coccoid cells arranged in grape-like clusters
Catalase	Positive (+)	Bubble formation upon addition of 3% H_2O_2
Mannitol Salt Agar (MSA)		
• BO1	Negative (–)	No color change
• BO2	Positive (+)	Medium turns yellow
• BP	Negative (–)	No color change
Hemolysis Test		
• BO1	Beta-hemolysis	Clear zone surrounding colonies on Blood Agar
• BO2	Beta-hemolysis	Clear zone surrounding colonies
• BP	Beta-hemolysis	Clear zone surrounding colonies
Triple Sugar Iron Agar (TSIA)		
• Butt	Positive (+)	Color change from red to yellow
• Slant	Positive (+)	Color change from red to yellow
• H_2S Production	Negative (–)	No blackening of the medium
• Gas Production	Negative (–)	No lifting or cracking of the medium
Sulfide Indole Motility (SIM)		
• Sulfide (H_2S)	Negative (–)	No H_2S formation
• Indole	Negative (–)	No red ring after Kovac's reagent
• Motility	Non-motile	Stab line remains clear
Simmons Citrate Agar (SCA)	Negative (–)	No color change from green to blue
Methyl Red–Voges Proskauer (MR-VP)		
• Methyl Red	Positive (+)	Medium turns red after MR reagent
• Voges–Proskauer	Negative (–)	No color change after VP reagent
Carbohydrate Fermentation (Glucose)	Positive (+)	Gas formation in Durham tube; medium changes from blue to yellow

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Figure

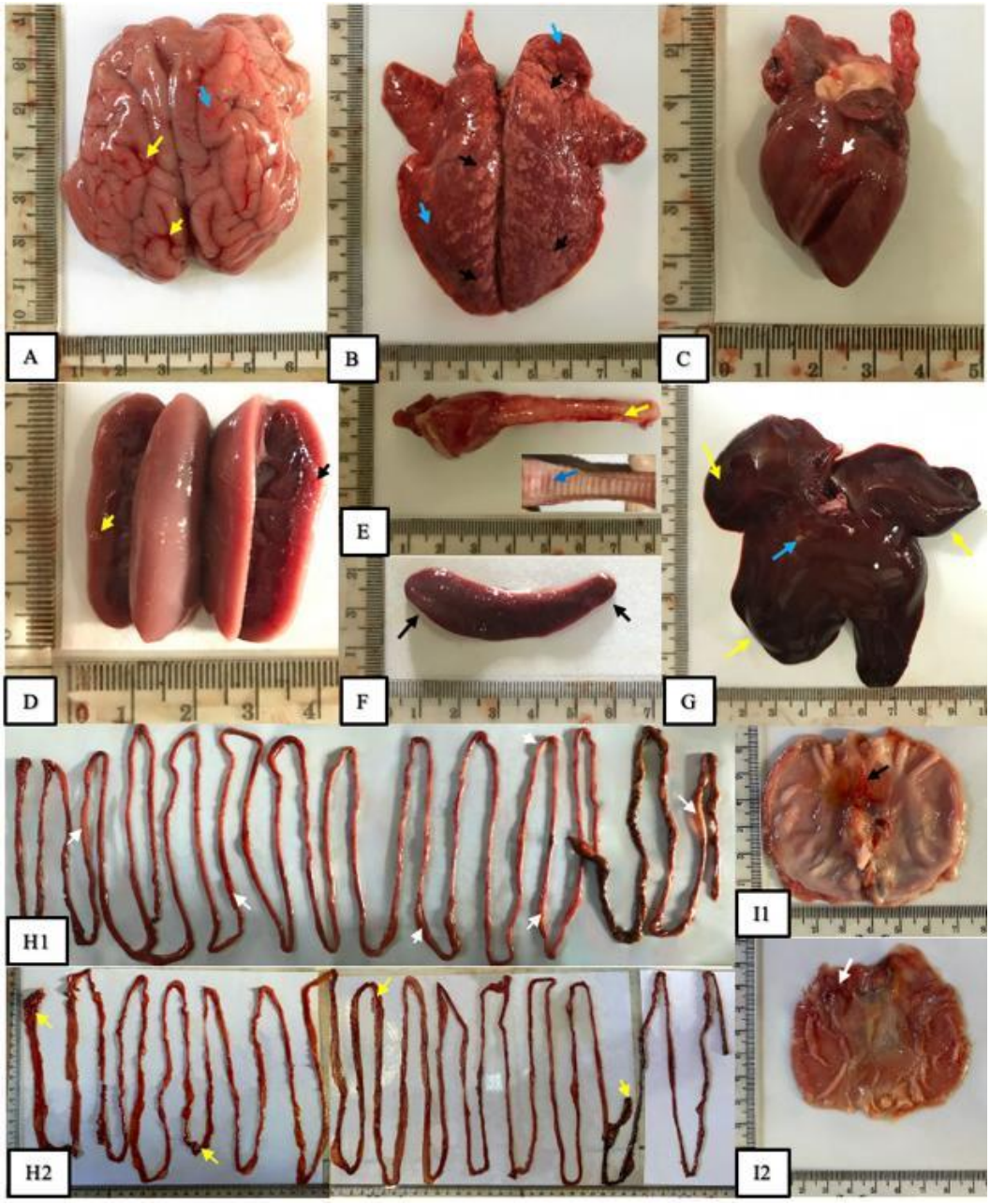

Figure 1. Gross Pathological Findings in the Case Animal. (A) Brain: diffuse reddening (blue arrow) and congestion (yellow arrow); (B) Lungs: generalized reddening of all lobes (blue arrow) and interstitial pneumonia (black arrow); (C) Heart: multifocal reddening (white arrow); (D) Kidneys: cortical reddening (black arrow) and necrosis (yellow arrow); (E) Trachea: serosal reddening (yellow arrow) and discoloration between the hyaline cartilage rings (blue arrow); (F) Spleen: reddish-brown discoloration with swelling (black arrow); (G) Liver: dark brown discoloration along the margins with swelling (yellow arrow) and multifocal necrosis (blue arrow); (H1) Intestinal Serosa: distension (white arrow); (H2) Intestinal Mucosa: reddish to black discoloration (yellow arrow); (I1) Gastric Serosa: reddening (black arrow); (I2) Gastric Mucosa: reddish to black discoloration (white arrow). Arrow legend: blue = pb; yellow = pk; black = ph; white = pp.

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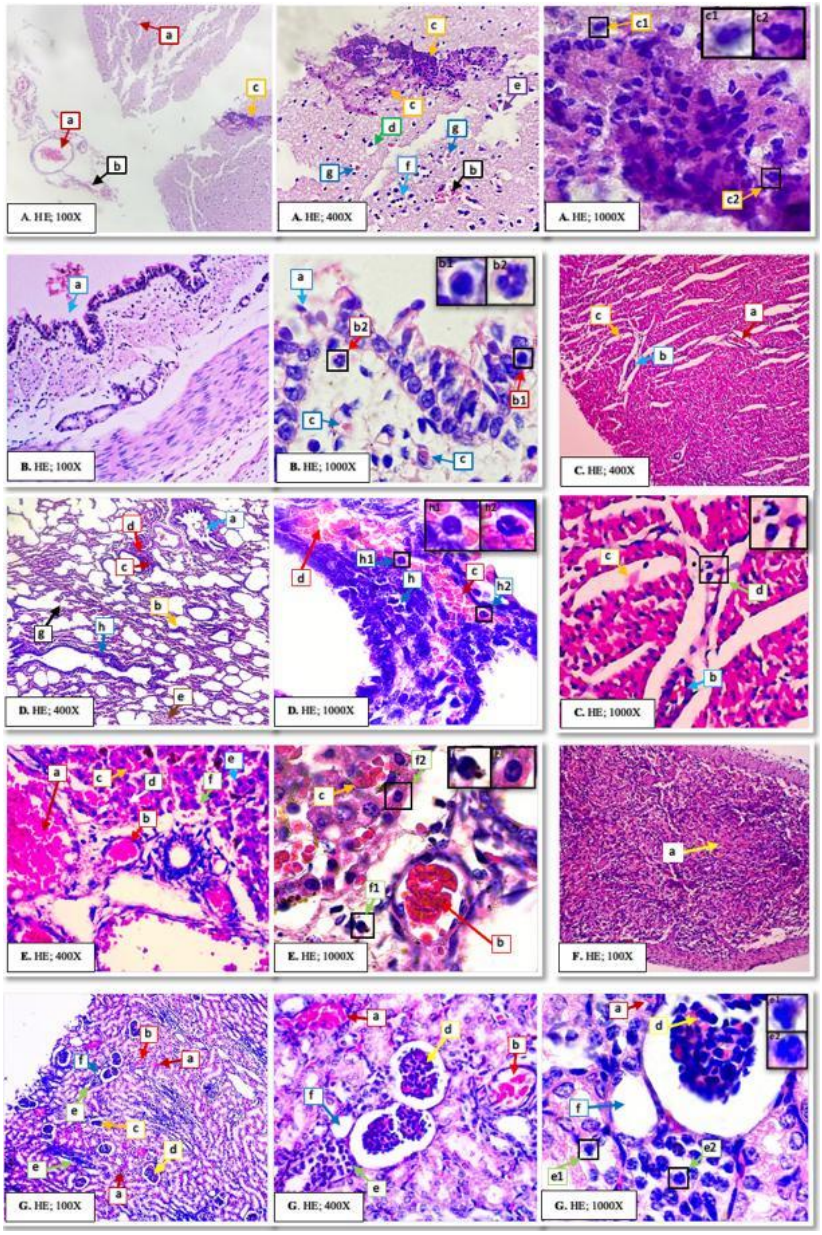

Figure 2. Histopathological Findings in the Affected Animal. (A) Brain (hemorrhagic and necrotizing meningoencephalitis): (a) congestion of meningeal blood vessels, (b) hemorrhage, (c) inflammatory cell infiltration (c1 = lymphocytes, c2 = neutrophils), (d) demyelination, (e) necrosis, (f) gliosis, and (g) inclusion bodies. (B) Trachea (tracheitis): (a) mucosal erosion, (b) inflammatory cell infiltration in the submucosa (b1 = lymphocytes, b2 = neutrophils), and (c) inclusion bodies. (C) Heart (myocarditis with intermyocardial edema): (a) congestion, (b) hyperemia, (c) intermyocardial edema, and (d) inflammatory cell infiltration. (D) Lungs (hemorrhagic and necrotizing bronchopneumonia): (a) exudate within the bronchiolar lumen, (b) thickening of the alveolar septa, (c) hemorrhage, (d) congestion, (e) hyperemia, (f) emphysema, (g) necrosis of the alveolar septa, and (h) inflammatory cell infiltration (h1 = lymphocytes, h2 = neutrophils). (E) Liver (hepatitis with hemorrhage): (a) congestion of the portal vein, (b) congestion of the hepatic artery, (c) hemorrhage, (d) karyolysis, (e) necrosis, and (f) inflammatory cell infiltration (f1 = lymphocytes, f2 = neutrophils). (F) Spleen (splenitis): (a) depletion of lymphoid cells. (G) Kidneys (hemorrhagic and necrotizing glomerulonephritis with glomerular atrophy): (a) intertubular renal hemorrhage, (b)

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congestion, (c) glomerular atrophy, (d) inflammatory cell infiltration within the glomeruli, (e) inflammatory cell infiltration in the renal intertubular region (e1 = lymphocytes, e2 = neutrophils), and (f) tubular necrosis.

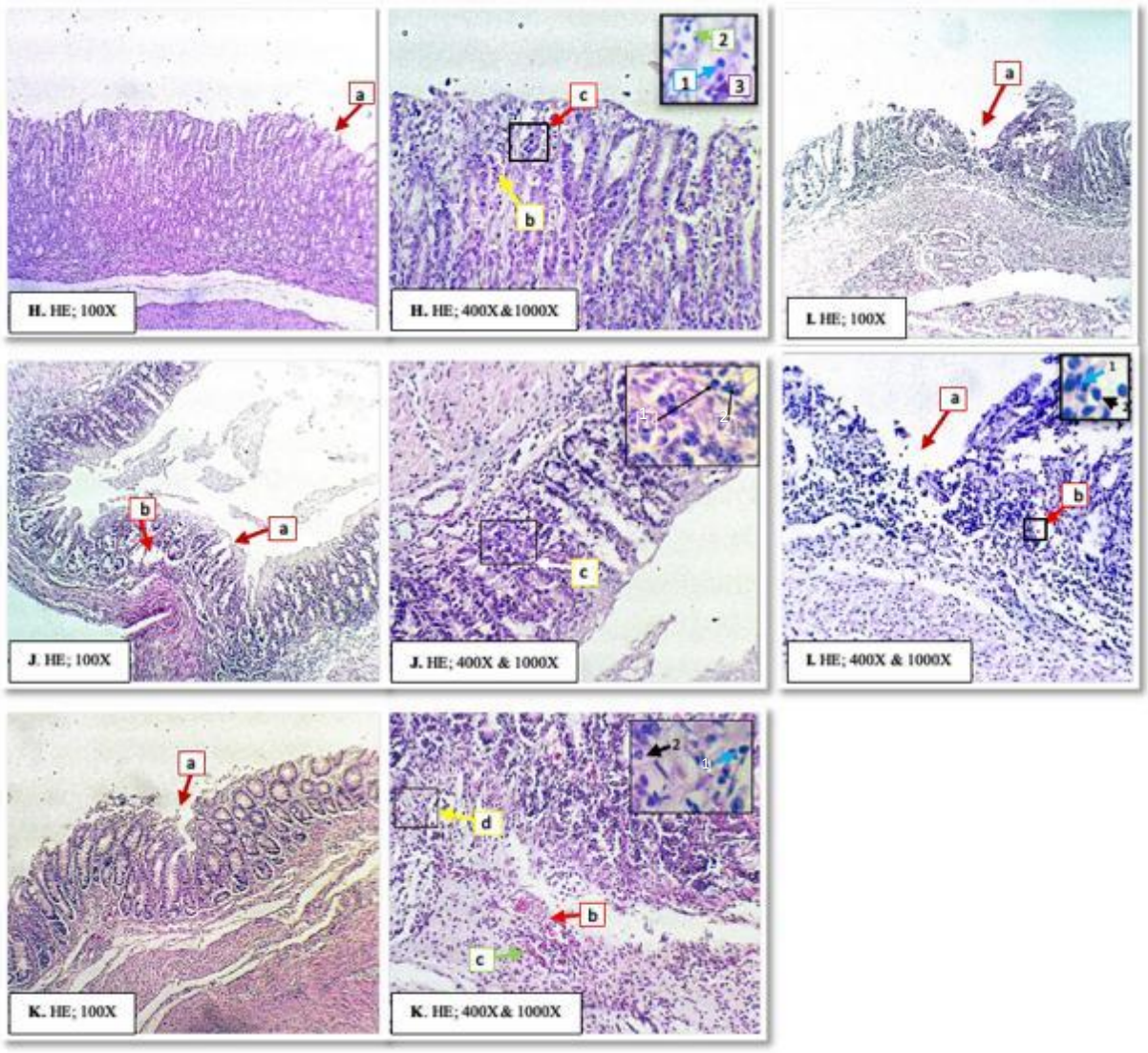

Figure 3. Histopathological Findings in the Affected Animal. (H) Stomach (gastritis): (a) mucosal erosion, (b) congestion, and (c) inflammatory cell infiltration (c1 = macrophages, c2 = lymphocytes, c3 = neutrophils). (I) Small intestine (enteritis with mucosal erosion): (a) villous erosion and (b) inflammatory cell infiltration in the lamina propria (b1 = lymphocytes, b2 = neutrophils). (J) Cecum (necrotizing typhlitis): (a) desquamation of villous epithelium, (b) necrosis of the crypts of Lieberkühn, and (c) inflammatory cell infiltration in the lamina propria (c1 = lymphocytes, c2 = neutrophils). (K) Large intestine (hemorrhagic colitis with mucosal erosion): (a) villous erosion, (b) congestion, (c) hemorrhage, and (d) inflammatory cell infiltration in the lamina propria (d1 = lymphocytes, d2 = neutrophils).

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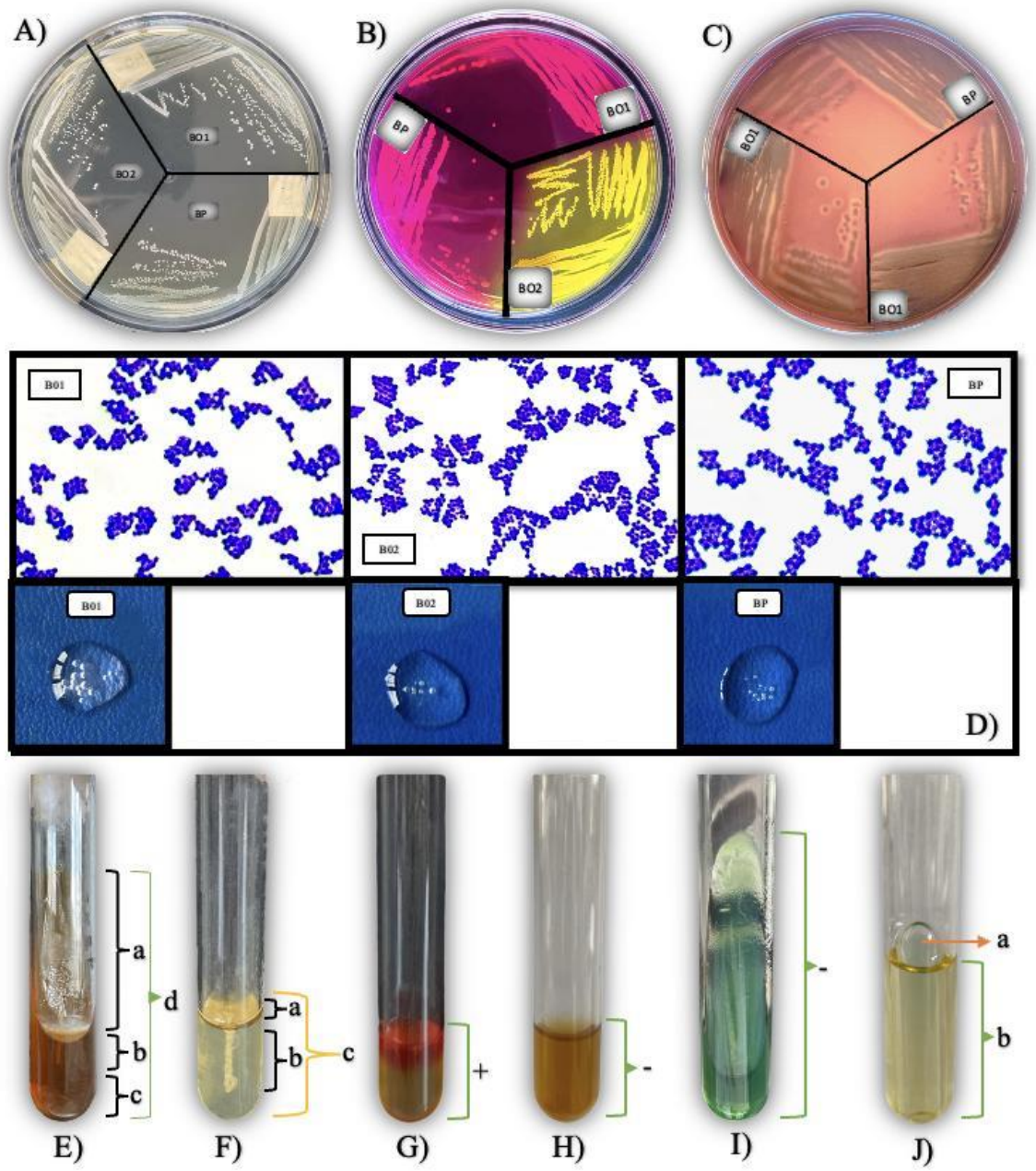

Figure 4. Bacterial Isolation and Identification Results. Bacterial colonies grown on Nutrient Agar showed white colonies for BO1, white-yellowish colonies for BO2, and white colonies for BP. On Mannitol Salt Agar, BO1 remained red (negative), BO2 turned yellow (positive), and BP remained red (negative). Blood Agar Plate demonstrated β-hemolysis for BO1, BO2, and BP. Gram staining and catalase testing indicated that all isolates (BO1, BO2, and BP) were Gram-positive and catalase-positive. In Triple Sugar Iron Agar, the isolates exhibited an acid slant (positive), an acid butt (positive), no gas production (negative), and no H_2S formation (negative). The Sulfide Indole Motility test showed indole-negative results, non-motile characteristics, and no H_2S production. The Methyl Red test produced a red color change (positive), while the Voges Proskauer and Simmons Citrate Agar tests showed no reaction (negative). In the carbohydrate fermentation test using glucose, gas bubble formation was observed (positive) along with a clear color change (positive).

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