

CASE STUDY: COLISEPTICEMIA IN A 1-MONTH-OLD PIGLET IN TARO VILLAGE, GIANYAR, BALI

Studi Kasus: Koliseptikemia pada Babi Berumur 1 Bulan di Desa Taro, Kabupaten Gianyar, Bali

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Abstract

Pig farms play an important role in the socio-cultural life of communities, but some of them are intensively managed with poor biosecurity and health management practices, as well as traditional and unhygienic husbandry practices, thereby potentially increasing the risk of health problems in livestock. Colisepticemia is a disease caused by infection with pathogenic *Escherichia coli*, which primarily affects piglets from the neonatal to post-weaning period. The subject of this case study was a one-month-old Landrace piglet from a smallholder farm in Taro Village, Tegallalang District, Gianyar Regency, Bali. This study aimed to determine the cause of death of the piglets based on epidemiological data, clinical examination, anatomical pathology, histopathology, bacteriology, and parasitology. Epidemiological data showed a total herd population of 455 pigs, with morbidity of 1.76%, mortality of 1.76%, and a case fatality rate (CFR) of 100%. Clinical signs observed in the piglet included anorexia, weakness, vomiting, yellowish-white diarrhea, and inability to stand. The piglet died after showing clinical signs for 16 days. Gross pathological examination revealed congestion in the brain, heart, lungs, liver, kidneys, and intestines, as well as hemorrhage in the trachea, lungs, and intestines.

Histopathological examination showed that almost all organs had congestion, hemorrhage, and inflammatory cell infiltration predominantly composed of neutrophils. Bacterial isolation from the lung, heart, liver, and intestines demonstrated growth of Gram-negative *Escherichia coli*. Parasitological examination using direct smear, sedimentation, and flotation methods yielded negative results for protozoan and helminth infections. Based on these findings, the piglet was diagnosed with colisepticemia.

Keywords: Colisepticemia, *Escherichia coli*, Piglet

Abstrak

Peternakan babi memiliki peran penting dalam kehidupan sosial budaya masyarakat, namun beberapa diantaranya dikelola secara intensif dengan pemahaman biosekuriti dan manajemen kesehatan yang rendah serta pola pemeliharaan tradisional dan kurang higienis, sehingga berpotensi meningkatkan risiko gangguan kesehatan pada ternak. Koliseptikemia adalah penyakit yang diakibatkan oleh infeksi bakteri *Escherichia coli* yang bersifat patogen terutama menginfeksi anak babi usia *neonatal* sampai *postweaning*. Studi kasus ini bertujuan untuk mengetahui penyebab kematian anak babi berdasarkan data epidemiologi, anamnesis, tanda klinis, pemeriksaan patologi anatomi, histopatologi, bakteriologi serta parasitologi. Data epidemiologi menunjukkan populasi ternak sebanyak 455 ekor, dengan morbiditas 1,76%, mortalitas 1,76% dan *case fatality rate* (CFR) 100%. Gejala klinis yang ditunjukkan anak babi kasus adalah anoreksia, muntah, diare putih kekuningan, lemas serta tidak mampu berdiri. Babi mati setelah 16 hari menunjukkan gejala klinis. Pemeriksaan patologi anatomi menunjukkan kongesti pada otak, jantung, paru-paru, hati, ginjal dan usus, hiperemi pada trakea, serta hemoragi pada paru-paru dan usus. Pemeriksaan histopatologi menunjukkan hampir semua organ menunjukkan adanya kongesti, hemoragi dan infiltrasi sel radang yang didominasi oleh neutrofil. Isolasi bakteri dari paru-paru, jantung, hati dan usus menunjukkan pertumbuhan bakteri Gram negatif *Escherichia coli*. Pemeriksaan parasitologi dengan metode natif, sedimentasi dan apung menunjukkan hasil negatif terhadap infeksi protozoa maupun cacing. Berdasarkan hasil pemeriksaan tersebut, anak babi kasus didiagnosis koliseptikemia.

Kata kunci: Anak babi, *Escherichia coli*, Koliseptikemia

INTRODUCTION

Pigs are monogastric livestock known for their prolific nature, meaning they can give birth to numerous offspring at each birth. Pigs grow relatively quickly, making them marketable at around six months of age. Furthermore, pigs are a meat-producing livestock species with several advantages over other livestock (Irfanto, 2020). Most Balinese raise pigs either as a primary source of income or as a side business (Ardiawan *et al.*, 2016). However, managing pig farms in Bali often faces challenges, one of which is the spread of disease-causing agents. Some common diseases affecting pigs include colisepticemia, septicemia, and other epizootic diseases (Besung, 2010).

Colisepticemia is an infectious disease caused by the presence of pathogenic *Escherichia coli*, including *Enterotoxigenic E. coli* (ETEC), which attacks pigs and causes various serious disorders, including death (Noviolita *et al.*, 2024). According to (Besung, 2010) and (Jorgensen *et al.*, 2007), colisepticemia is often found in pigs, especially newborn piglets until weaning. This disease can cause significant economic losses due to morbidity, mortality, inhibited productivity, decreased weight gain, and increased treatment and vaccination costs (De-Lorenzo *et al.*, 2018; Siahaan *et al.*, 2024; Tosi *et al.*, 2021). Various case reports of colisepticemia in piglets from the weaning to post-weaning phase have a high prevalence. According to Besung (2010), the prevalence of colisepticemia in piglets in Bali in 2005 was

26.7%, while in 2010 it increased to 42%. The bacteria that cause this disease are normal microorganisms present in the digestive tract, but can become pathogenic if the intestinal environment is disturbed, especially in perinatal piglets. This infection is usually characterized by symptoms of persistent diarrhea that leads to severe dehydration and can even lead to death. According to Sora *et al.*, (2021), in addition to digestive system symptoms, *Escherichia coli* can also cause urinary tract infections, coliform mastitis, meningitis, pneumonia, arthritis, and septicemia, which can lead to death. Colisepticemia itself often occurs in pens with poor biosecurity, thus severely harming farmers economically if not addressed promptly. Poor pen sanitation and changes in weather are factors that influence the rate of *Escherichia coli* infection. Other factors that can influence this infection rate include weak individual antibody titers (Paul, 2015).

Symptoms such as weakness and diarrhea in pigs generally indicate infection, but cannot yet be used as specific indicators for determining the type of disease. Some possible causes of these symptoms include infectious colisepticemia, viral transmissible gastroenteritis (TGE), and parasitic infections such as coccidiosis and strongyloidosis. Therefore, a more definitive diagnosis requires further examination, such as histopathological analysis, bacterial isolation and identification, and fecal examination to detect possible parasitic infections. The results of these examinations will determine the cause of piglet death and will be an important basis for determining disease management strategies on pig farms.

RESEARCH METHODS

Animal Case

The case animal with protocol number 140/N/25 is a 1-month-old male piglet, originating from a community farm in Taro Village, Tegallalang District, Gianyar Regency, Bali, which was taken on Thursday, May 15, 2025. The pig then died on May 17, 2025.

Anamnesis and Epidemiological Screening

Anamnesis was conducted by interviewing barn staff to collect data such as livestock age, total population, number of individuals showing symptoms of illness, mortality rate, previous disease history and treatment, and the management system implemented. Meanwhile, observations were conducted to evaluate the environmental conditions of the barn, including sanitation levels and the completeness and effectiveness of available biosecurity measures.

Epidemiological investigations were then conducted. Morbidity, mortality, and case fatality rates (CFR) were calculated. The formula for calculating these figures is as follows:

$$\text{Morbidity} = \frac{\text{Number of Sick Animals}}{\text{AmountPopulation}} \times 100\%$$

$$\text{Mortality} = \frac{\text{Number of Dead Animals}}{\text{AmountPopulation}} \times 100\%$$

$$\text{Case Fatality Rate} = \frac{\text{Number of Dead Animals}}{\text{Number of AnimalsSick}} \times 100\%$$

Anatomical Pathology and Histopathology Examination

The necropsy procedure was carried out at the Veterinary Pathology Laboratory, Faculty of Veterinary Medicine, Udayana University to observe anatomical pathological changes. First, the fur was wetted, then the animal's body was incised ventrally to view and document all organs

showing changes, which were then described, then 1x1x1 cm organ samples were then fixed in 10% Neutral Buffered Formaldehyde (NBF) reagent. This reagent is the most commonly used for fixation of histological specimens (Virgiawan *et al.*, 2024).

Furthermore, the preparation of histopathology preparations was also carried out in the same laboratory based on the Kiernan (2015) method, starting with dehydration of the sample using graded alcohol solutions, namely 70%, 80%, 90%, 96% alcohol for ± 2 hours, followed by a clearing process using Toulena I and II to remove residual alcohol. The tissue was then inserted into liquid paraffin, printed into blocks and cut thinly (3-4 μm) using a microtome, then the tissue was floated in a water bath at 46°C before being attached to a glass object, dried and stained with Hematoxylin-Eosin (HE). The final stage was mounting the preparation with a cover glass and entellan, before finally being observed under a microscope for histopathology examination.

Isolation and Identification of Bacteria

Bacteriological examinations were conducted at the Veterinary Bacteriology and Mycology Laboratory, Faculty of Veterinary Medicine, Udayana University. Samples were taken from the lungs, heart, liver, and intestines of necropsied piglets. Bacterial isolation was performed by swabbing the inside of each organ sample with sterile tissue, then smearing the sterile tissue onto the surface of Nutrient Agar (NA) media using the streak line method. The media was then incubated at 37°C for 24 hours (Kurniawan *et al.*, 2023). Bacterial colonies that successfully grew separately on Nutrient Agar (NA) media were analyzed descriptively by observing colony morphology. Bacteria suspected of being *Escherichia coli* were examined microscopically using Gram staining and a catalase test was performed.

The Gram staining stage begins by cleaning the glass object using 70% alcohol. One bacterial colony on a slide is homogenized with PBS on the glass object to form a thin layer, then fixed on a spirit lamp. The preparation is given 2% crystal violet for 60 seconds, washed with distilled water, then given iodine for 60 seconds, washed, then diluted with 95% alcohol solution for 15 seconds and washed again. Next, given safranin for 60 seconds, washed, then dried, the preparation is dropped with immersion oil before being observed under a microscope at 1000x magnification. Next, the catalase test is carried out by dropping H₂O₂ solution (3% hydrogen peroxide) onto the bacterial colony that has been taken using a sterile slide and placed on the glass object, then observed for the presence or absence of oxygen bubbles.

Furthermore, bacterial colony samples in the heart, liver, lungs, and intestines that were previously taken from Nutrient Agar (NA) media were then inoculated on Selective Differential MacConkey Agar (MCA) media using the streak line method to obtain separate colonies, then incubated at 37°C for 18-24 hours. After the samples were isolated on Selective Differential MacConkey Agar (MCA) media, they were then inoculated on Eosin Methylene Blue Agar (EMBA) media using sterile slide using the streak line scratching technique, then incubated again at 37°C for 24 hours. After incubation, visual observation of the characteristics of the colonies was carried out before continuing with biochemical tests (Putri *et al.*, 2023).

Samples of lung, heart, liver, and intestine organs were cultured on a common medium, namely Nutrient Agar (NA). Incubation was carried out at 37 °C for 24 hours. Colony growth on the media was observed macroscopically to see the shape, color, elevation, edge, and diameter of the colony. Next, a single colony was taken from the NA media using a sterile slide and then smeared on MacConkey Agar (MCA) and Eosin Methylene Blue (EMBA) media with the streak line method, incubation at 37°C for 24 hours. Next, primary tests were carried out in the form of catalase tests and gram staining. Biochemical tests were also carried out such as Triple Sugar iron Agar (TSIA) Simmons Citrate Agar (SCA), Sulfide Indole Motility (SIM), Methyl Red

(MR), Voges-Proskauer (VP), followed by sugar tests, namely the glucose test.

Qualitative Stool Examination

Fecal samples were taken from the intestines of the case animals and placed in urine containers containing 10% Neutral Buffered Formalin (NBF) solution. The samples were examined in the Parasitology Laboratory, Faculty of Veterinary Medicine, Udayana University. Qualitative fecal examinations were conducted using direct native tests, sedimentation concentration tests, and floatation concentration tests.

The native method involves taking a matchstick-sized piece of feces, placing it on a glass slide, adding 1-2 drops of distilled water, and homogenizing it. The coarse fibers are then removed, and the slide is covered with a cover glass. The slide is then observed microscopically at 100x and 400x magnification.

The sedimentation method involves taking a matchstick-sized piece of feces, mixing it with 10% distilled water, and then homogenizing it. The mixture is then filtered and collected in a centrifuge tube, followed by centrifugation at 1,500 rpm for 5 minutes. The supernatant is discarded, and the sediment is collected, placed on a glass slide, and covered with a coverslip. Identification is performed under a microscope at 100x and 400x magnification.

The flotation method involves taking a sample of feces the size of a candlenut seed ($\pm 3g$) and mixing it with 10% distilled water, then homogenizing it. The mixture is then filtered and collected in a centrifuge tube and centrifuged at 1500 rpm for 2-3 minutes. The supernatant is discarded and saturated salt is added to the sediment until the tube fills $\frac{3}{4}$ of the volume. Centrifuge again at 1500 rpm for 2-3 minutes. Open the tube and add saturated salt solution until the surface becomes convex, leaving it for 3 minutes to allow the worm eggs or oocysts to float to the surface. The cover glass is then touched to the convex surface of the flotation fluid and attached to the object glass. Examine under a microscope at 100x and 400x magnification, then identify the specimen.

Data analysis

Epidemiological data, anatomical pathological changes, histopathological changes, bacterial isolation, and parasite examination were analyzed descriptively and qualitatively and presented in the form of tables and figures.

RESULTS AND DISCUSSION

Anamnesis Results and Epidemiological Data

The case pig is a one-month-old Landrace pig and has been weaned. Clinical symptoms found in the case animal include anorexia, diarrhea with yellowish-white feces accompanied by vomiting, reddish skin, swollen joints, and loss of balance, as seen in Figure 1. The piglet has not been given antibiotics or vaccinated. No treatment has been administered to the case pig since it first showed signs of illness. Adult pigs have been vaccinated against Hog Cholera twice a year.

The pig farming system uses an open house system with concentrated feed. The pig population on the farm is 455, consisting of 155 adult pigs and 300 piglets. According to the farmer, at least one to two piglets develop illness with similar symptoms each week and die. One piglet, which showed symptoms for 14 days, was taken as a case and died two days later. A necropsy was performed at the Pathology Laboratory of the Faculty of Veterinary Medicine, Udayana University. Based on epidemiological calculations, the morbidity and mortality rates were 1.76% each within one month, while the case fatality rate (CFR) reached 100%.

Anatomical Pathology Examination Results

After a necropsy, the results of the anatomical pathology examination at the Veterinary Pathology Laboratory showed changes in the brain, lungs, trachea, heart, liver, spleen, stomach and intestines which can be seen in (Table 2).

Histopathological Examination Results

The results of histopathological examinations carried out at the Veterinary Pathology Laboratory showed that the piglets experienced histopathological changes in various organs including the brain, trachea, lungs, heart, liver, spleen, stomach and intestines which can be seen in (Figure 2-9).

Bacteriological Examination

The results of bacteriological examination include the stages of culture and identification of bacteria on Nutrient Agar (NA) media. Colonies appear round and grayish white, Gram staining shows rod-shaped cells and is pink (Gram negative), Catalase test shows the presence of bubbles, on MacConkey Agar (MCA) media the colonies are pink, Eosin Methylene Blue Agar (EMBA) colonies show a metallic green sheen, Triple Sugar Iron Agar (TSIA) shows a yellow color with gas without black sediment, Sulphide Indole Motility (SIM) shows a red ring on the surface (indole positive), the media becomes cloudy (motile), and there is no black sediment (H₂S negative), Methyl Red (MR) test shows a red color (positive), Voges-Proskauer (VP) test shows no color change (negative), Simmons Citrate Agar (SCA) remains green (citrate negative). The sugar test produces a yellow color change and gas formation. The results of the bacteriological test can be seen in (Table 3) and in (Figures 10-12) which show infection due to *Escherichia coli* bacteria.

Parasitology Examination

The results of parasite examinations at the Veterinary Parasitology Laboratory by conducting qualitative fecal examinations including native, sedimentation, and floating methods showed negative results for the presence of protozoa, worms, and worm eggs.

Discussion

Colisepticemia is a disease caused by infection with the pathogenic *Escherichia coli* bacteria and often occurs in newborn piglets until post-weaning. This bacteria is a normal flora found in the digestive organs of animals in controlled amounts, but *Escherichia coli* can turn into a pathogen if there are changes in the supportive environment and a decrease in the immune system in the host (Brooks *et al.*, 2004). This statement is in line with research by Lyutskanov (2011) who stated that colisepticemia in young pigs can be caused by low levels of antibodies that should be obtained from colostrum or mother's milk, which plays an important role in inhibiting bacterial proliferation in the intestinal lumen of newborn piglets.

According to Sora VM *et al.* (2021), *Escherichia coli* can be divided into two types based on their ability to cause infection, namely Intestinal Pathogenic *Escherichia coli* (IPEC) in the gastrointestinal system and Extraintestinal Pathogenic *Escherichia coli* (ExPEC) outside the gastrointestinal system. ExPEC is further divided into Neonatal meningitis *Escherichia coli* (NMEC), Sepsis-associated *Escherichia coli* (SEPEC), and Avian pathogenic *Escherichia coli* (APEC). Pathogenic *Escherichia coli* bacteria in the gastrointestinal system can also be grouped into invasive and non-invasive *Escherichia coli*. Invasive *Escherichia coli* bacteria cause infections by invading cells, so they are also called Enteroinvasive *Escherichia coli* (EIEC). Meanwhile, non-invasive *Escherichia coli* bacteria are further divided into Enteropathogenic *Escherichia coli* (EPEC) and Enterotoxigenic *Escherichia coli* (ETEC). Enteropathogenic bacteria can be grouped into two

groups of pathogenic bacteria, namely Enteropathogenic *Escherichia coli* (EPEC) and Enterohemorrhagic *Escherichia coli* (EHEC). The Enterotoxigenic *Escherichia coli* (ETEC) group is a strain of pathogenic bacteria that can produce one or more exotoxins that are bound in the digestive tract, either heat-stable toxins or heat-labile toxins. These toxins stimulate the intestines to hypersecrete fluid, resulting in diarrhea (Berata *et al.*, 2014). According to Kim *et al.*, (2022) Enterotoxigenic *Escherichia coli* is the most common pathogenic strain that causes diarrhea and enteritis in piglets.

Based on clinical signs In pigs, cases show symptoms of diarrhea with yellowish-white feces and an inability to stand. Barros *et al.* (2023) stated that in pre-weaning pigs experiencing colisepticemia, the typical symptom that appears is white diarrhea, this can occur due to the mother's milk not being digested properly and being excreted through the feces. D This diarrhea is caused by pathogenic *Escherichia coli* that attaches and colonizes small intestinal enterocytes via fimbriae, accompanied by the release of toxins that disrupt the homeostatic balance of intestinal epithelial cells. This disruption triggers increased fluid secretion that exceeds the intestinal absorptive capacity, resulting in clinical symptoms of diarrhea (Kim *et al.*, 2022). Continuous fluid and electrolyte loss due to untreated diarrhea can weaken the body, ultimately leading to loss of balance and leading to death.

The pathological anatomical changes seen in the pig cases were the small intestine and large intestine which appeared to experience changes in mucosal color, congestion and distension due to the presence of gas. According to Pereira *et al.*, (2016) although pathological lesions in colisepticemia are not specific, the real changes that can be observed are in the intestinal organs that appear distended. The results of histopathological examinations showed changes in the small intestine in the form of infiltration of inflammatory cells of neutrophils and lymphocytes in the lamina propria and depletion of Peyer's patches. Meanwhile, the large intestine showed erosion of the intestinal mucosa, inflammatory cell infiltration is a manifestation of the body's immune response to pathogens and tissue damage, which aims to eliminate foreign agents while processing tissue regeneration. In *Escherichia coli* bacterial infections, the acute inflammatory reaction is generally characterized by the accumulation of neutrophils at the site of infection which can be observed histopathologically. The number of inflammatory cells that enter the tissue depends on the duration of infection and the number of invading bacteria. The more severe the infection, the inflammatory response will increase, characterized by the accumulation of neutrophils that invade the tissue (Noviolita *et al.*, 2024).

Other pathological anatomical changes found included hyperemia of the tracheal mucosa in the form of red lines, as well as focal necrosis in the form of miliary abscesses in the lungs upon incision. Furthermore, congestion was seen in several organs, such as blood vessels in the brain, lung lobes, and the left side of the heart, and a dark red discoloration of the liver. This hemorrhage occurs due to *Escherichia coli* toxins that cause endothelial damage, resulting in blood leaking from the blood vessels (Meha *et al.*, 2016; Noviolita *et al.*, 2024). Histopathological examination also showed hemorrhage in the trachea and lungs, with hemorrhage in the lobes, and hemorrhage and edema in the intermyocardium in the heart. The liver showed portal vein and sinusoid congestion, while the kidneys experienced cortical congestion. The pathogenic bacteria *Escherichia coli* that have colonized various organs along with their toxins can cause inflammation, damage the epithelium, cause congestion, hemorrhage, necrosis, and edema, as well as damage the intestinal barrier and reduce the body's immune function (Paul, 2015; He *et al.*, 2022).

To strengthen the diagnosis, bacteriological laboratory examinations are carried out. According to Pudjiatmoko *et al.*, (2014), establishing a diagnosis of colisepticemia is absolutely done

through the isolation and identification of *Escherichia coli* bacteria as the cause of the disease. Samples of the liver, heart, lungs, and intestines in piglets showed positive results for *Escherichia coli* infection. *Escherichia coli* colonies grown on Nutrient Agar media are characterized by a round shape, a smooth and convex surface, flat edges, a milky white color, and a size of approximately 1–3 mm. These findings align with those described by Al-Ayubi *et al.* (2022) regarding the morphology of *E. coli* colonies on Nutrient Agar media. Gram staining is performed to determine the morphology of bacteria and to differentiate gram-positive and gram-negative bacteria based on the characteristics of their cell walls. *Escherichia coli* bacteria are included in the Gram-negative bacteria group, pink in color because they are seen from their inability to maintain the purple color of Crystal Violet due to the loss of the lipid layer of the cell wall by alcohol and absorb safranin dye, resulting in a red appearance under a microscope (Rahayu & Gumilar, 2016). The results of microscopic observations support these characteristics, where the bacteria appear rod-shaped (bacilli), red in color, and arranged singly. The catalase test results also showed positive results, indicated by the bacterial isolates that were dripped with H₂O₂ producing bubbles. According to Lindawati and Suardana (2016), the catalase test is intended to determine the group of bacteria that can break down H₂O₂ into oxygen where the bubbles formed are the result of the release of oxygen gas. This is because bacteria are able to produce the catalase enzyme (Rajput *et al.*, 2014). On MacConkey Agar (MCA) media, observation of the morphology and color of the colony is the initial key to identification, the results of observation of the colony are round, pink, *Escherichia coli* itself is a Gram-negative lactose-fermenting bacteria. where pink colonies indicate bacteria ferment lactose and produce acid, while clear colonies indicate non-lactose-fermenting bacteria (Anisa *et al.*, 2023). Acid causes the color of the pH indicator to change to red when the pH drops below 6.8 (Ginting *et al.*, 2018). Meanwhile, bacterial isolation on Eosin Methylene Blue Agar (EMBA) media showed typical *Escherichia coli* colony growth, characterized by a round, shiny shape, flat edges, a convex surface, a size of 1–3 mm, and a metallic green color. This metallic green color is a characteristic indicator of *Escherichia coli* bacterial growth (Alifia & Aji, 2021).

To further identify the bacteria, a series of biochemical tests were performed. These tests aim to observe certain reactions that indicate the typical characteristics of the bacteria, which can be influenced by the type of media and environmental conditions. Some of the tests used include Triple Sugar Iron Agar (TSIA), Sulphide Indole Motility (SIM), Methyl Red (MR) Test, Voges-Proskauer (VP) Test, Simmons's Citrate Agar (SCA) and the sugar test (Ummamie *et al.*, 2017). In TSIA media, a color change was seen in the TSIA media from red to yellow on the slant and butt, as well as the media being lifted, indicating gas formation. No H₂S production was found. According to Mahon *et al.*, (2015), these changes indicate that *Escherichia coli* bacteria are able to ferment sucrose, glucose and lactose and produce gas. The SIM test results showed no H₂S, but motility and Indole were positive. Positive motility is seen from the turbidity around the puncture point, while a positive Indole test indicates that *Escherichia coli* bacteria have the enzyme tryptophanase which can break down the amino acid tryptophan into indole. The SCA result is negative, meaning the bacteria cannot use citrate as a carbon source (Dadheech *et al.*, 2016). The MR-VP test shows a positive reaction in the Methyl Red test and a negative reaction in the Voges-Proskauer test. This indicates that these bacteria are able to ferment proteases into organic acids, and the bacteria cannot produce acetoin from glucose fermentation. The sugar fermentation test also shows a positive result, confirming that the bacteria are able to ferment carbohydrates (Bastian *et al.*, 2021). Based on all these test results, the biochemical reaction patterns that appear match the typical characteristics of *Escherichia coli*, so this isolate can be identified as *Escherichia coli*.

The results of anatomical pathology, histopathology, and bacterial culture examinations in the

pigs in the case study were mutually supportive. Pathological anatomical changes in the brain, spleen, heart, trachea, lungs, liver, kidneys, stomach, and intestines indicated inflammation. Bacterial isolation and identification revealed *Escherichia coli*, and histopathology confirmed signs of inflammation and inflammatory cell infiltration, dominated by neutrophils. These findings support the diagnosis that the piglets in the case study had cholesepticemia.

The differential diagnosis of colisepticemia is often confused with Transmissible Gastroenteritis (TGE) and coccidiosis or entamobiosis (Morin *et al.*, 1983). The results of the anamnesis, epidemiology and clinical symptoms of Transmissible Gastroenteritis (TGE) are not enough to lead to a diagnosis, because Transmissible Gastroenteritis (TGE) is caused by a virus that has a high morbidity and mortality rate of 100% (Underdahl *et al.*, 1975). The results of bacterial culture showed positive for short-rod gram-negative bacteria, namely *Escherichia coli*. The results of parasite examination showed negative results for the presence of protozoa and worms. Based on these results, the differential diagnosis was eliminated and the pig case was definitively diagnosed with Colisepticemia.

CONCLUSION AND SUGGESTION

Conclusion

Based on findings from clinical examination, anatomical pathology, histopathology, and laboratory examinations, it can be concluded that the case of a one-month-old Landrace piglet in Taro Village with protocol number 140/N/25 was diagnosed with colisepticemia. The findings included diarrhea, organ congestion, neutrophil-dominant inflammatory cell infiltration, and isolation of gram-negative bacteria that matched the characteristics of pathogenic *Escherichia coli* that can cause colisepticemia, resulting in sepsis exacerbated by dehydration, leading to multi-organ failure and ultimately death.

Suggestion

Farmers are advised to tighten biosecurity practices and maintain clean pens. An unclean pen environment can be a breeding ground for various disease-causing agents. Providing colostrum to piglets after birth is also crucial, as it contains natural antibodies that boost immunity and protect them from various diseases during their early life. Furthermore, further study and research on livestock health management, particularly related to disease prevention and increased productivity, is recommended to enrich the development of scientific knowledge in the field of animal husbandry.

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Tables

Table 1. Results of anatomical pathology examination

| Organ | Pathological Changes |
|-----------------|---|
| Brain | There is congestion in the meninges and cerebrum of the brain |
| Trachea | The presence of hyperemia in the tracheal cartilage |
| Esophagus | Looks normal |
| Lungs | The existence of kcongestion and hemorrhage in multilobed areas and the presence of focal necrosis (miliary abscesses) during incision. |
| Heart | The existence of kcongestion at the left apex |
| Heart | There is congestion in the central veins |
| Kidney | Changes in kidney color Al turned a little pale |
| Spleen | Color changes in the spleen |
| Stomach | The presence of hyperemia and changes in the gastric mucosa |
| Small intestine | There is congestion, hyperemia, and changes in color of the small intestinal mucosa and accompanied by the presence of gas. |

Table 2. Results of *Escherichia coli* Bacteria Identification

| Bacteriology & Identity Testing | Results | Interpretation |
|---|--|--|
| <i>Nutrient Agar</i> (NA) | The colony morphology is round, convex elevation, smooth surface, flat edges, milky white in color and measuring $\pm 1-3$ mm. | Bacteria were successfully isolated |
| <i>Mac Conkey Agar</i> (MCA) | Shows pink colonies. | Lactose fermenting bacterial isolates. |
| <i>Eosin Methylene Blue Agar</i> (EMBA) | The bacterial colonies are metallic green. | Bacteria ferment lactose vigorously and produce acid. |
| Gram staining | Red, rod-shaped, single bacteria. | Bacteria are included in the category of gram-negative bacteria. |
| Catalase Test | There are bubbles (+) | The formation of gas bubbles due to the breakdown of H ₂ O ₂ by the catalase enzyme. |
| <i>Triple Sugar Iron Agar</i> (TSIA) | <i>Acid slant</i> (+), <i>Acid butt</i> (+) Gas (+), H ₂ S (-) | Bacteria ferment glucose, lactose and/or sucrose, producing gas and not H ₂ S. |
| <i>Sulphide Indole Motility</i> (SIM) | <i>Sulphide</i> (-), <i>Indole</i> (+), <i>Motility</i> (+) | Does not produce H ₂ S, produces indole, is motile (moves). |

| | | |
|-----------------------------------|--|--|
| Methyl Red (MR) Test | the media color turns red (+) | Bacteria have the ability to utilize glucose by producing stable (strong) acids. |
| Voges-Proskauer (VP) test | There is no color change on the media (-) | Bacteria do not produce acetoin. |
| <i>Simmons Citrate Agar</i> (SCA) | There are no changes to the media (-) | Cannot utilize citrate as the sole carbon source. |
| Glucose Test | There is a change in the color of the media to clear (+) | Capable of fermenting glucose, lactose, sucrose, maltose and mannitol. |

Figures



Figure 1. Clinical condition of the case pig. Description: (A) Vomiting (B) Diarrhea with yellowish-white feces

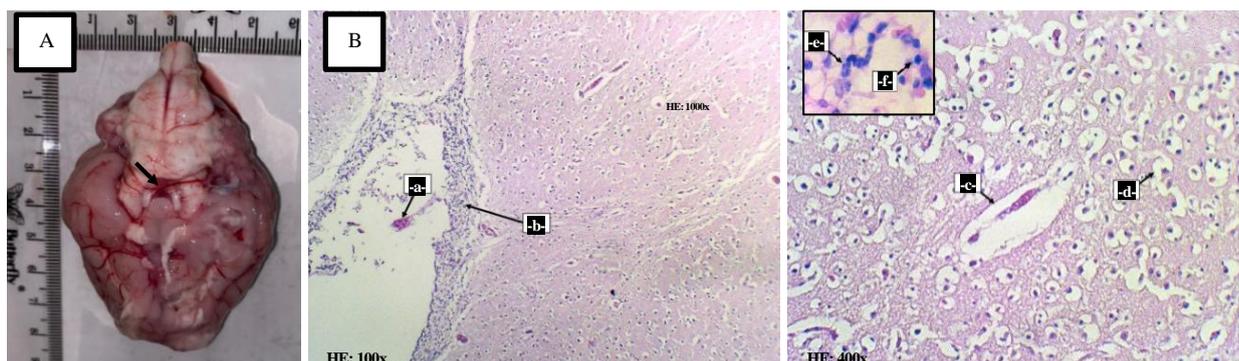


Figure 2. Brain. (2A) Congestion in the cerebrum, (2B) Meningitis. Captions: (a) Congestion of the meninges, (b) Infiltration of inflammatory cells in the meninges, (c) Congestion and perivascular edema (d) Demyelination, (e) neutrophils, (B) lymphocytes

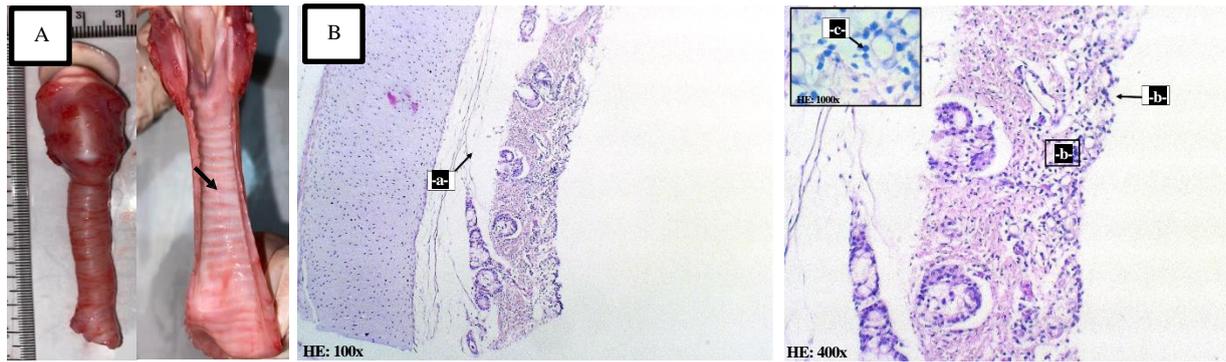


Figure 3. Trachea. (3A) Experiencing hyperemia in the tracheal cartilage, (3B) Tracheitis. Description: (a) Submucosal edema, (b) Mucosal erosion, (c) Infiltration of lymphocyte inflammatory cells.

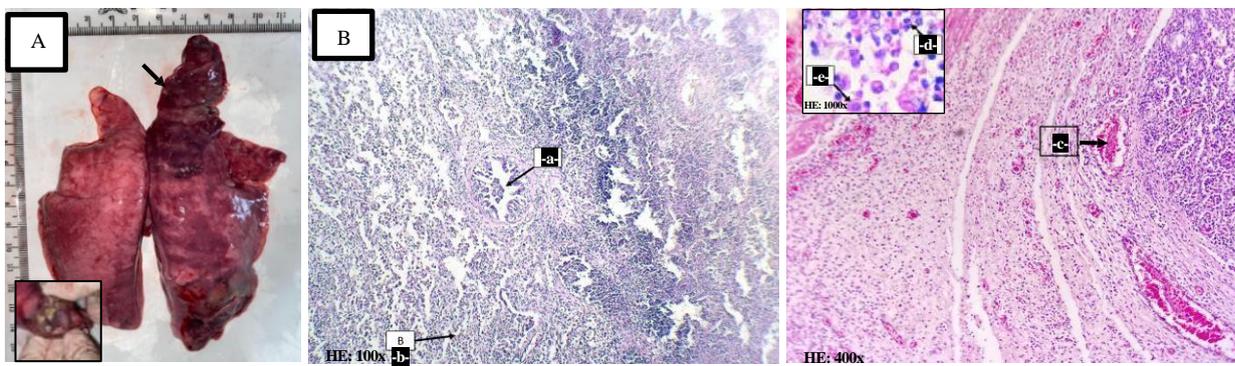


Figure 4. Lungs. (4A) Experiencing congestion and hemorrhage in multilobes and the presence of focal necrosis (miliary abscesses) when incised, (4B) Bronchopneumonia. Description: (a) Exudate in the lumen of the bronchioles, (b) inflammatory cell infiltration in the alveoli, (c) congestion, (d) neutrophils, (e) macrophages

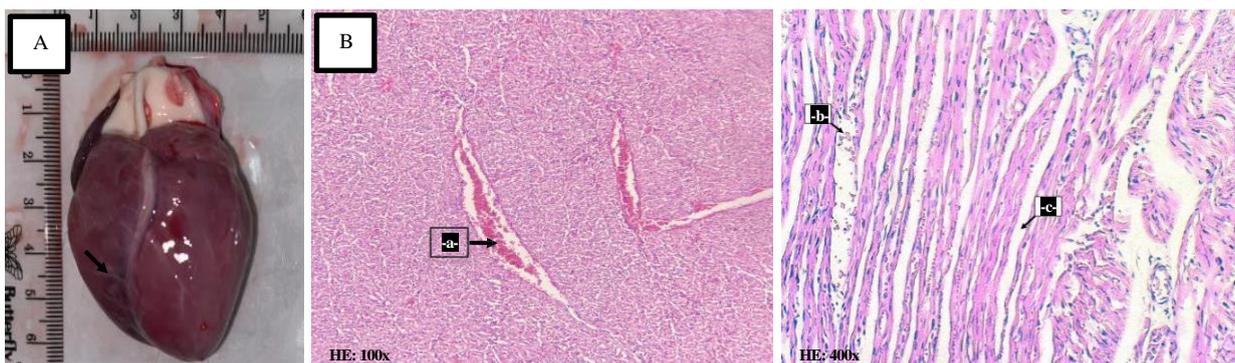


Figure 5. Heart. (5A) Experiencing congestion at the left apex, (5B) Hemorrhage and edema of the intermyocardium. Information: (a) Congestion, (b) Hemorrhage, (c) Intermiocardial edema.

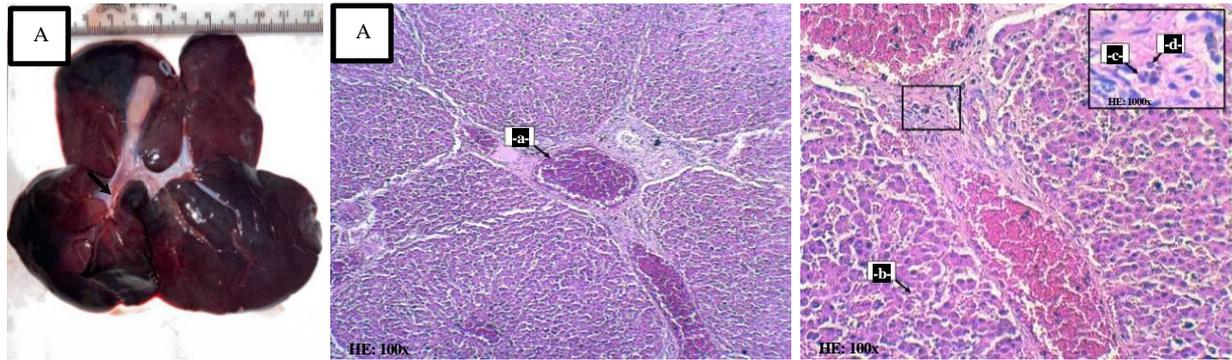


Figure 6. Liver. (6A) Experiencing congestion in the central vein, (6B) Hepatitis. Caption: (a) Portal vein congestion, (b) Sinusoid congestion, Inflammatory cell infiltration, (c) Neutrophils, (d) Lymphocytes in the periportal area.

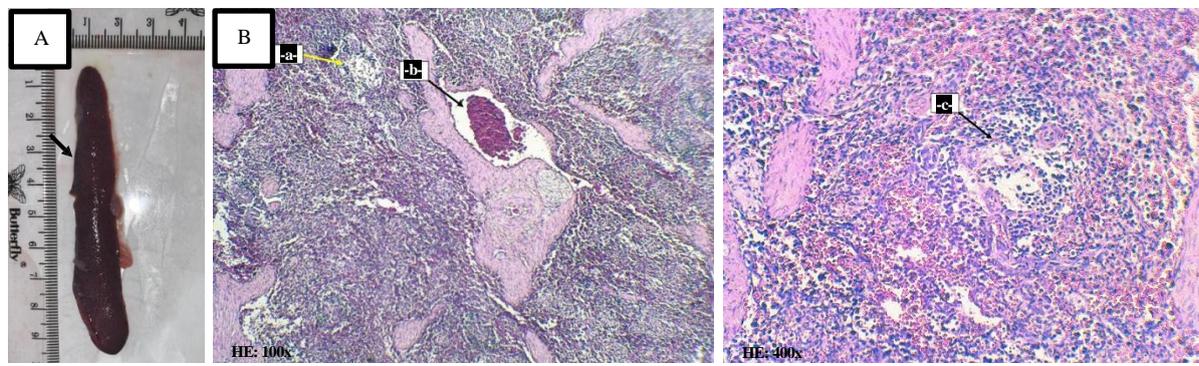


Figure 7. Spleen. (7A) Undergoing color changes, (7B) Depletion of splenic lymphoid cells. Caption: (a) Depletion of lymphoid cells, (b) Hyperemia in the trabecular arteries, (c) Depletion of lymphoid cells.

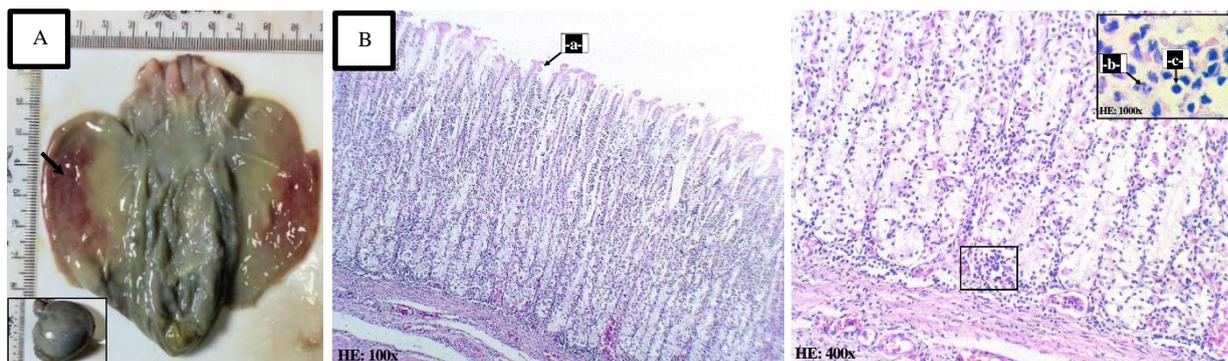


Figure 8. Stomach. (8A) Hyperemia and discoloration of the gastric mucosa, (8B) Gastritis. Description: (a) Desquamation of the mucosal epithelium, (b) Neutrophils, (c) Lymphocytes in the mucosa.

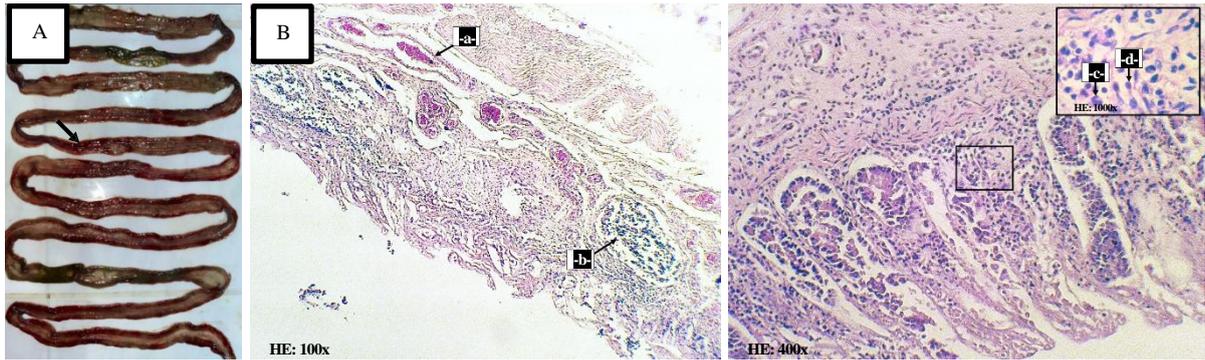


Figure 9. (9A) Experiencing congestion, hyperemia, and color changes in the small intestine, (9B) Enteritis. Caption: (a) Congestion, (b) Depletion of Peyer's patches (9C), (c) Neutrophils, (d) Lymphocytes in the lamina propria.

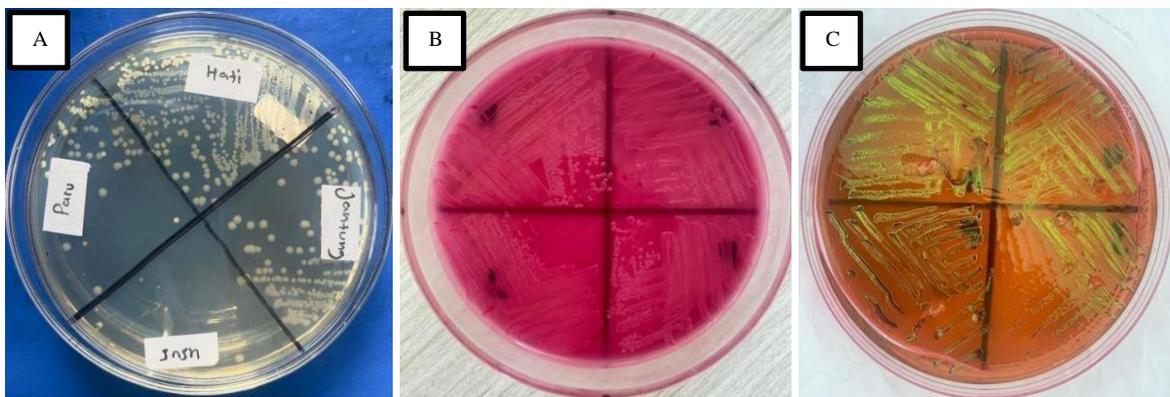


Figure 10. Isolation and identification of bacteria. Caption: (A) Nutrient Agar, (b) MacConkey Agar, (c) Eosin Methylene Blue Agar (EMBA), (H) Liver, (J) Heart, (P) Lung, (U) Intestine

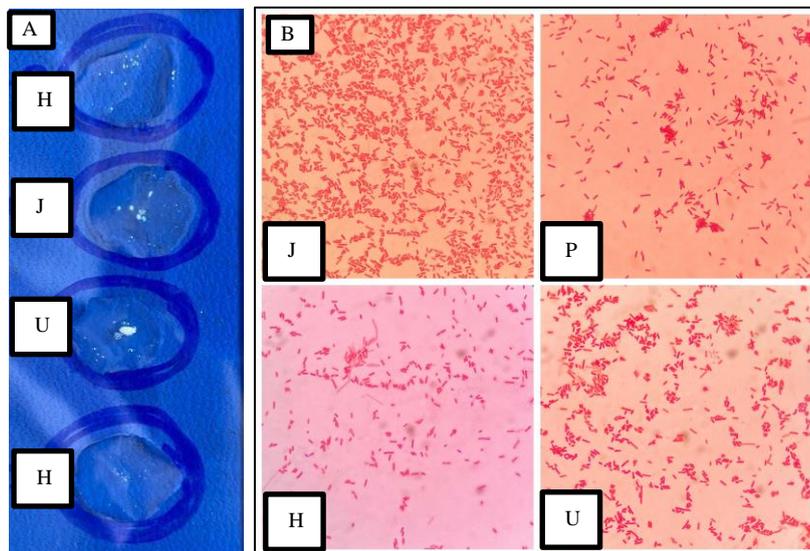


Figure 11. (A) Positive catalase test results are indicated by the presence of bubbles, (B) Gram staining results show red colonies indicating gram-negative bacteria with a rod shape. Information (J) Heart, (P) Lungs, (H) Liver, (D) Intestines

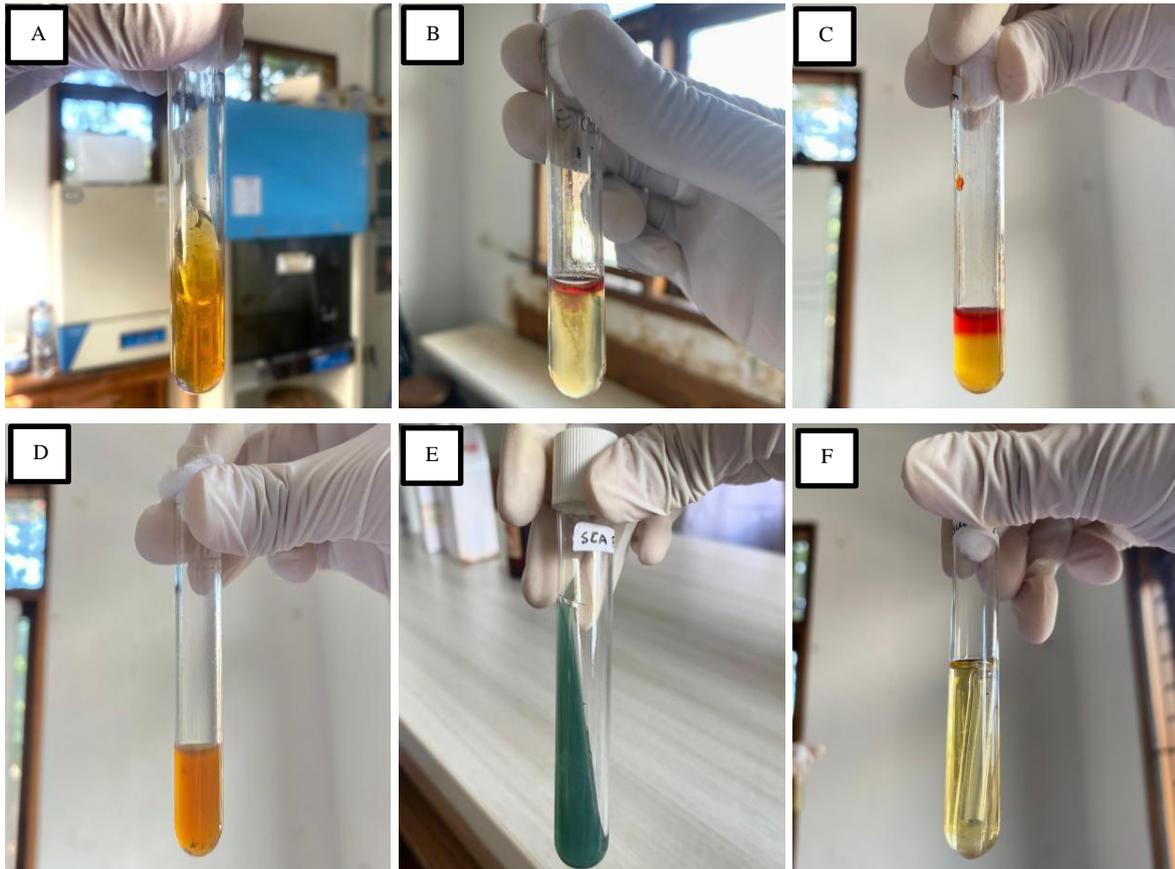


Figure 12. Biochemical test results. Description: (A) Triple Sugar Iron Agar (TSIA) test results, (B) Sulphide Indole Motility (SIM) test results, (C) Methyl Red (MR) test results, (D) Voges-Proskauer (VP) test results, (E) Simmons Citrate Agar (SCA) test results, (F) Glucose test results.