OCCURRENCE OF MULTIPLE INFECTIONS IN CATTLE AT A FARM IN THE CIBIRU AREA, WEST JAVA

Kejadian Multiinfeksi pada Sapi di Peternakan daerah Cibiru, Jawa Barat

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Abstract

The health of beef cattle is a crucial aspect of livestock management. Effective health management in cattle serves to minimize potential economic losses caused by diseases that commonly affect beef cattle. These diseases may be caused by bacteria, fungi, viruses, as well as blood and gastrointestinal parasites. Multiple infections may occur, particularly when suboptimal animal conditions and environmental factors support disease transmission. This case report aims to describe the occurrence of multiple infections and to identify the causative infectious agents in a beef cattle farm located in Cibiru, West Java. The case involved a 4-year-old Limousin beef cattle that exhibited clinical signs including nasal discharge, hypersalivation, diarrhea, and tick infestation. Samples collected for diagnostic examination included nasal discharge swabs, blood, and fecal samples. Nasal swab samples were cultured on blood agar plates (BAP) and MacConkey agar (MCA), followed by primary tests including Gram staining, catalase test, coagulase test, and biochemical tests. Blood samples were examined using blood smear evaluation and differential leukocyte counts. Fecal samples were examined using native smear, flotation, sedimentation, and McMaster techniques. The results revealed a case of multiple infections, with the identification of *Staphylococcus aureus* and *Klebsiella pneumoniae*, as well as a gastrointestinal parasitic infection (coccidiosis). In addition, infestation with *Rhipicephalus* ticks contributed to blood parasitic infections, including babesiosis and anaplasmosis. These concurrent infections were associated with a compromised immune status and reduced production performance, potentially leading to economic losses for the farmer. Therefore, preventive and control measures, including proper sanitation and

biosecurity, are essential, as infected animals and contaminated environments may act as sources of disease transmission.

Keywords: Bacteria, Beef Cattle, Disease, Parasites

Abstrak

Kesehatan ternak sapi potong merupakan salah satu poin penting dalam aspek pemeliharaan ternak. Manajemen kesehatan sapi menjadi salah satu upaya dalam menekan potensi kerugian ekonomi akibat penyakit. Penyakit yang dapat menyerang sapi dapat disebabkan oleh bakteri, jamur, virus, dan parasit darah maupun parasit cerna. Kejadian multiinfeksi dapat terjadi akibat lingkungan pemeliharaan yang tidak optimal, seperti kepadatan kendang tinggi, sanitasi buruk, manajemen biosekuriti rendah, yang berkontribusi terhadap penurunan imunitas. Laporan kasus ini bertujuan untuk mendeskripsikan temuan klinis, pemeriksaan penunjang, serta agen penyebab multiinfeksi pada seekor sapi potong di peternakan Cibiru, Jawa Barat. Sapi ras Limosin berumur 4 tahun menunjukkan tanda klinis berupa anoreksia, lesu, hipersalivasi, diare, serta temuan klinis lainnya yaitu adanya infestasi caplak pada beberapa bagian tubuh sapi. Pemeriksaan penunjang dilakukan dengan cara mengoleksi sampel swab leher hidung, darah, feses, dan caplak. Sampel *swab* leleran hidung dikultur pada *media blood agar plate* (BAP) dan *MacConkey agar* (MCA) dilanjut dengan uji primer pewarnaan gram, uji katalase, uji koagulase, dan uji biokimia. Sampel darah diuji dengan metode ulas darah dan diferensial leukosit. Sampel feses diperiksa menggunakan metode uji natif, apung, sedimentasi, dan McMaster. Hasil pemeriksaan menunjukan sapi mengalami multiinfeksi dengan teridentifikasinya bakteri *Staphylococcus aureus* dan *Klebsiella pneumonia*, serta infeksi parasit cerna berupa *Eimeria* sp. Hasil pemeriksaan ulas darah menunjukkan adanya parasit *Babesia* sp. dan *Anaplasma* sp. dengan morfologi darah hipokromik dan echinocyte, serta mengalami neutrofilia, limfositopenia, dan monositosis. Tidak hanya itu, adanya infestasi caplak *Rhipicephalus* sp. berperan sebagai vektor penularan infeksi parasit darah. Kondisi multiinfeksi tersebut dapat menyebabkan penurunan sistem imun pada sapi dan mempengaruhi hasil produksi yang dapat memberikan dampak terhadap erekonomian peternak. Pencegahan dan penanganan melalui sanitasi dan biosekuriti perlu dilakukan, karena hewan yang sakit dan lingkungan dapat menjadi sumber penularan.

Kata kunci: Bakteri, Parasit, Penyakit, Sapi Potong

INTRODUCTION

Beef cattle farming has considerable potential and represents one of Indonesia's leading livestock commodities due to high consumer demand (Suryanda & Jaroji, 2024). According to Badan Pusat Statistik (2024), the beef cattle population in Indonesia has reached 11.75 million head, with total beef production of 496.25 thousand tons, while total beef consumption is estimated at 759.67 thousand tons. This high level of demand poses a major challenge in efforts to increase sustainable and efficient beef production through improved animal health management. Despite its significant economic potential, beef cattle production is also associated with risks, particularly the occurrence of animal diseases (Suryanda & Jaroji, 2024; Gustiani & Fahmi, 2022). Diseases in livestock can disrupt normal metabolic processes involved in nutrient absorption, leading to reduced animal productivity (Nuraini *et al.*, 2022). Livestock diseases are generally classified into infectious diseases and strategic infectious diseases, caused by pathogenic agents such as bacteria, viruses, fungi, and parasites (Winarsih, 2018).

Animal health is a crucial aspect of effective livestock management systems. The implementation of optimal health management plays an important role in minimizing economic

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losses due to disease and contributes to improved animal welfare, productivity, resource-use efficiency, and the sustainability of livestock enterprises. Farm management, particularly animal health and the surrounding environment, is a key factor that must be considered, as it represents a major source of disease risk (Saiful *et al.*, 2024; Nuraini *et al.*, 2022). It is not uncommon for a single animal to experience multiple disease events, resulting in coinfections. The occurrence of multi-infections arises from complex interactions among the three main components of the epidemiological triangle: host, agent, and environment. Environmental conditions and interactions with other animals within the same population greatly influence the incidence of infection (Apsari *et al.*, 2022).

According to Awaludin *et al.* (2021), one of the most frequently encountered animal health management problems is gastrointestinal parasitic infestation (helminthiasis). Gastrointestinal helminths can cause significant losses in cattle by reducing body weight and leading to poor body condition. In addition, ectoparasite infestations, such as tick infestations, serve as reservoirs for blood-borne parasitic diseases. Sigit *et al.* (2024) reported that beef cattle are among the livestock commodities most frequently affected by blood parasite infections transmitted by tick ectoparasites, resulting in clinical conditions such as anemia, emaciation, and infections that may remain latent within the host. Common blood parasites infecting cattle include *Babesia* spp., *Anaplasma* spp., and *Theileria* spp. (Dyahningrum *et al.*, 2019). Stress, compromised immune status, and environmental factors may further predispose animals to secondary infections caused by bacterial agents. Thus, multi-infections present a significant challenge for farmers and require careful consideration of contributing factors related to the environment, the causative agents, and the host.

This case report aims to identify and describe the clinical findings, supporting diagnostic examinations, and causative disease agents in a single beef cattle raised on a farm in the Cibiru area, West Java. The results of this identification are expected to provide accurate information for selecting targeted and appropriate therapeutic interventions based on the type of causative agent, as well as to support preventive efforts through the use of disinfectants that are compatible with the characteristics of the identified pathogens.

RESEARCH METHODS

Signalment and Case History

A male Limousin beef cattle, 4 years of age, with an estimated body weight of approximately 270 kg and a Body Condition Score (BCS) of 2.5/5, was presented for examination. The animal had a brown hair coat, which appeared dirty and heavily infested with ectoparasites. Based on the anamnesis, the animal had received a vaccination program and its last anthelmintic treatment approximately one year prior to examination. The owner reported a recent decline in the animal's general condition, characterized by reduced appetite and decreased body performance over the past several weeks, prompting further clinical evaluation.

Physical and Clinical Examination

Clinical examination revealed that the animal was dull, weak, and in poor nutritional status, with body growth not appropriate for its age, a BCS of 2.5/5, and signs of mild to moderate dehydration, as indicated by pale mucous membranes and a capillary refill time (CRT) exceeding 3 seconds. The rectal temperature was recorded at 39.6 °C, heart rate at 52 beats per minute, and respiratory rate was increased to 40 breaths per minute with a rapid respiratory pattern. The hair coat appeared dull and dirty, with ectoparasite infestation in the form of ticks, predominantly observed on the neck region, skin folds, and tail base. Ocular examination revealed bilateral conjunctival swelling, while other ocular structures appeared unremarkable.

Examination of the respiratory system revealed abnormal lung sounds characterized by crackles (rales) on auscultation, accompanied by signs of discomfort upon thoracic compression and a positive withers pinch test, suggesting involvement of the respiratory system.

Supporting Diagnostic Examinations

Supporting diagnostic examinations were conducted through a series of laboratory tests on fecal, blood, and nasal discharge samples to identify the causative agents. Parasitological examination of fecal samples included native (direct smear), flotation, sedimentation, and McMaster techniques. Blood samples were analyzed using hematological tests, including blood smear evaluation and differential leukocyte counts. Nasal discharge samples were subjected to bacterial isolation using culture media such as blood agar plate (BAP) and MacConkey agar (MCA), followed by subculturing of single colonies onto Tryptic Soy Agar (TSA). The isolated colonies were subsequently examined microscopically and biochemically, including Gram staining, catalase and coagulase tests, as well as further biochemical assays such as Kligler Iron Agar (KIA), Motility Indole Urea (MIU), and citrate tests. Identification of the ticks collected from the animal was performed using a stereomicroscope based on their morphological characteristics.

RESULTS AND DISCUSSION

Results

Clinical examination revealed a decline in the animal's general condition, characterized by dullness, lethargy, reduced appetite, hypersalivation, diarrhea, and the presence of mucopurulent nasal discharge. Evaluation of vital signs showed an elevated body temperature accompanied by an increased respiratory rate with a rapid breathing pattern. The mucous membranes appeared pale, indicating anemia, and signs of mild to moderate dehydration were observed. Integumentary examination revealed a dull and dirty hair coat with ectoparasite infestation in the form of ticks, which were distributed across several body regions, particularly the neck, skin folds, and tail base. Respiratory system examination demonstrated abnormal lung sounds in the form of crackles (rales) on auscultation, and the animal exhibited signs of discomfort during thoracic compression and the withers pinch test. Based on these clinical findings, the animal was suspected of having a systemic disorder involving multiple infections, warranting further supporting diagnostic examinations.

Blood smear examination revealed the presence of intraerythrocytic parasites suggestive of babesiosis and anaplasmosis, accompanied by hypochromic erythrocytes and echinocyte formation. Hematological evaluation further demonstrated neutrophilia, lymphocytopenia, and monocytosis. Microscopic examination using Giemsa staining identified merozoite forms of *Babesia* spp. within erythrocytes (Figure 1A). These parasites appeared as paired, pear-shaped organisms with angular ends, although unpaired forms were occasionally observed. In addition, dark red inclusion bodies located at the periphery of erythrocytes were observed, consistent with the morphological characteristics of *Anaplasma* spp. (Figure 1B). The parasites appeared as intraerythrocytic inclusion bodies with variable numbers among red blood cells. These findings were supported by clinical manifestations including fever, anemia, and lethargy. Differential leukocyte counts performed after three days of clinical signs confirmed the presence of neutrophilia, lymphocytopenia, and monocytosis.

Bacterial culture of nasal discharge samples on MacConkey agar (MCA) and blood agar plates (BAP) revealed distinct colony morphologies. On BAP, round, golden-white colonies were observed, whereas on MCA, pink, round, mucoid colonies were identified. Gram staining of

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colonies from BAP demonstrated purple-stained, grape-like clustered cocci consistent with Gram-positive bacteria, while colonies from MCA revealed red-stained coccobacilli consistent with Gram-negative bacteria. Catalase and coagulase tests performed on BAP isolates yielded positive results, indicating *Staphylococcus aureus*. In contrast, MCA isolates were catalase-positive and coagulase-negative. Further biochemical testing of MCA isolates demonstrated Kligler Iron Agar (KIA) results showing an acid slant and acid butt with gas production and no H$_2$S, a positive citrate test, and Motility Indole Urea (MIU) test results indicating indole-negative, non-motile, and urease-positive reactions. These findings were consistent with *Klebsiella pneumoniae*.

Fecal examination using direct smear, flotation, sedimentation, and McMaster techniques revealed the presence of oocysts. Quantification of eggs per gram of feces (EPG) using the McMaster method yielded a count of 1,000 oocysts per gram. Morphological evaluation of the oocysts was consistent with unsporulated oocysts of *Eimeria* spp., indicating coccidiosis as a protozoal infection.

Discussion

The detection of blood parasites on blood smear examination was associated with the presence of ticks on the animal's body. According to Perveen *et al.* (2021), ticks such as *Boophilus* spp., *Dermacentor* spp., *Rhipicephalus* spp., *Ixodes* spp., *Hyalomma* spp., and *Ornithodoros* spp. act as biological vectors of babesiosis and anaplasmosis, transmitting these pathogens through their saliva, which contains toxic substances. These parasites infect red blood cells and cause damage to erythrocyte morphology. This finding is consistent with the observation of hypochromic erythrocytes and echinocytes, reflecting anemia resulting from parasitic infection. Hypochromic erythrocytes indicate reduced hemoglobin content, causing the cells to appear pale, whereas echinocytes represent deformed red blood cells with small, spiculated projections on their surface (Okahara *et al.*, 2024).

Neutrophilia observed in this case resulted from the recognition of parasitic agents as foreign pathogens by the immune system, which subsequently triggered the release of proinflammatory cytokines and chemokines. These mediators promote neutrophil recruitment to the site of infection (Dhanamjayam *et al.*, 2024). Monocytosis plays a role in the phagocytosis of erythrocytes damaged by lysis due to *Babesia* and *Anaplasma* infections (Hussein *et al.*, 2007; Abdullah *et al.*, 2019). Massive erythrocyte destruction caused by parasitic invasion leads to an increased accumulation of red blood cell debris that must be cleared by phagocytic cells, particularly monocytes. During the acute phase of babesiosis and anaplasmosis, lymphocytes migrate to lymphoid organs such as the spleen, where immune cell activation and proliferation occur, resulting in lymphocytopenia (Sajid *et al.*, 2023; Dhanamjayam *et al.*, 2024). In cases of coinfection with *Babesia* and *Anaplasma*, systemic inflammatory responses tend to be more severe (Casa *et al.*, 2023; Razanske *et al.*, 2019).

Infection with *Babesia* spp. causes intravascular hemolysis through several mechanisms, including erythrocyte damage due to intracellular parasite replication, production of anti-erythrocyte antibodies, and macrophage activation that enhances erythrophagocytosis (Dhanamjayam *et al.*, 2024). In addition, *Anaplasma* spp. infection exacerbates anemia by accelerating erythrocyte destruction. These bacteria replicate within erythrocytes via binary fission and release infectious forms that invade other red blood cells. Infected erythrocytes are subsequently phagocytosed by the reticuloendothelial system, thereby increasing the rate of erythrocyte loss (Constable, 2017).

Nasal discharge swab culture results indicated the presence of *Staphylococcus aureus* and *Klebsiella pneumoniae*. On blood agar plates (BAP), beta-hemolysis characterized by complete

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hemolytic zones and alpha-hemolysis characterized by partial hemolytic zones were observed. *Staphylococcus aureus* produces hemolysins that damage red blood cells, particularly their cytoplasmic membranes (Nawar *et al.*, 2021). Hemolytic zones form as a result of bacterial exotoxins with cytotoxic and lytic effects on host cells (Nasaj *et al.*, 2020; Pakshir *et al.*, 2017). On MacConkey agar (MCA), smooth, pink, mucoid colonies were observed. These findings were suggestive of *Klebsiella* spp., which characteristically produce large mucoid colonies due to their ability to synthesize capsular polysaccharides (CPS) (Munther *et al.*, 2017).

Gram staining of isolates from BAP revealed purple-stained, clustered cocci consistent with Gram-positive bacteria, whereas isolates from MCA showed red-stained coccobacilli consistent with Gram-negative bacteria. Differences in Gram staining characteristics are attributed to variations in cell wall structure, with Gram-positive bacteria possessing a thicker peptidoglycan layer than Gram-negative bacteria (Yanto *et al.*, 2021). The ability to retain crystal violet dye is related to this thick peptidoglycan layer, which becomes dehydrated during alcohol treatment, causing cell wall pores to close and trap the dye within the cell (Paray *et al.*, 2023; Yanto *et al.*, 2021).

Catalase tests yielded positive results for isolates grown on both BAP and MCA. The combination of Gram-positive cocci morphology and catalase positivity further supported the identification of *Staphylococcus* spp. for BAP isolates. Catalase positivity in Gram-negative bacteria is also characteristic of *Klebsiella* spp., as reported by Salauddin *et al.* (2019), who noted bubble or gas formation during catalase testing due to enzyme activity. Catalase-producing bacteria degrade hydrogen peroxide (H_2O_2) into water and oxygen (Yanto *et al.*, 2021), serving as a defense mechanism against oxidative stress (Yuan *et al.*, 2021).

Coagulase testing of BAP isolates yielded positive results, indicated by clot formation in the test tube, confirming the identification of *Staphylococcus aureus*. In contrast, *Klebsiella pneumoniae* does not produce the coagulase enzyme. A positive coagulase test is characterized by clot formation, whereas a negative result shows no clot formation (Hayati *et al.*, 2019). Biochemical testing of MCA isolates demonstrated characteristic reactions, including Kligler Iron Agar (KIA) results showing an acid slant and acid butt with gas production and no H_2S, Motility Indole Urea (MIU) results indicating indole-negative, non-motile, and urease-positive reactions, and a positive citrate test indicated by a blue color change. These biochemical characteristics are consistent with *Klebsiella pneumoniae*.

Staphylococcus aureus is considered part of the normal microbiota of animals and humans, inhabiting both skin and mucosal surfaces (Misic *et al.*, 2015). However, it can act as an opportunistic pathogen due to its ability to produce toxins and invade host tissues (Karimela *et al.*, 2017). According to Cheng *et al.* (2018), infections caused by *Klebsiella pneumoniae* commonly occur in animals with immunosuppression and chronic pulmonary disease. When correlated with differential leukocyte findings, the presence of an active inflammatory response in this case reflects the host's attempt to combat acute bacterial infection caused by *K. pneumoniae* (Wood, 2024). *Klebsiella pneumoniae* is recognized as an important causative agent of pneumonia (Wareth *et al.*, 2021) and employs multiple virulence factors to evade host immune responses, including K-capsular antigens, adherence factors, O-lipopolysaccharide (LPS), and siderophores, all of which contribute to pathogenicity and immune evasion (Zhu *et al.*, 2021).

Fecal examination revealed the presence of unsporulated *Eimeria* spp. oocysts, indicating a moderate level of infection. Moderate infections typically range from 500 to 5,000 oocysts per gram of feces, whereas severe infections are characterized by counts exceeding 5,000 oocysts per gram (Shatyaayyupranathasari *et al.*, 2021). This finding is consistent with the observed

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clinical signs of diarrhea in the affected animal, indicating gastrointestinal tract involvement. Gastrointestinal infections caused by protozoa, particularly *Eimeria* spp., are commonly referred to as coccidiosis (Kurniawan *et al.*, 2025).

Based on these findings, the diagnosis in this case was a multi-infection involving blood parasite infestation caused by bacterial (*Anaplasma* spp.) and protozoal (*Babesia* spp.) agents, respiratory bacterial infection (*Staphylococcus aureus* and *Klebsiella pneumoniae*), and gastrointestinal parasitic infection (*Eimeria* spp.). Clinical signs such as dyspnea accompanied by nasal discharge and hypersalivation, along with the presence of opportunistic bacterial pathogens, indicate secondary infection associated with a compromised immune status. This interpretation is supported by Cheng *et al.* (2018), who reported that such infections are common in animals with reduced immune competence.

CONCLUSION AND SUGGESTIONS

Conclusion

A Limousin beef cattle raised on a farm in the Cibiru area, West Java, presenting with clinical signs of dyspnea, nasal discharge, hypersalivation, lethargy, diarrhea, fever, and tick infestation, was diagnosed with a multi-infection involving blood parasites (*Babesia* spp. and *Anaplasma* spp.), gastrointestinal parasites (*Eimeria* spp.), and respiratory bacterial infections caused by *Staphylococcus aureus* and *Klebsiella pneumoniae*.

Suggestions

Multi-infections in livestock generally arise from complex interactions among infectious agents, host susceptibility, and environmental factors that facilitate pathogen transmission. Therefore, the implementation of strict biosecurity measures is crucial to maintain housing hygiene and to interrupt pathogen transmission cycles. In addition, early and accurate identification of causative agents is essential to ensure appropriate and targeted therapeutic interventions.

ACKNOWLEDGMENTS

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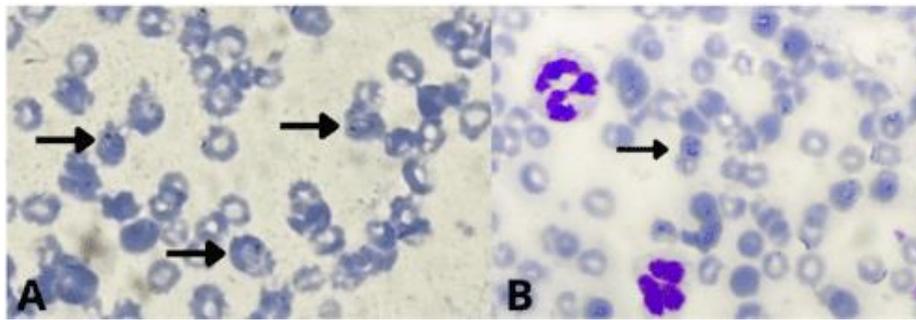
Figures

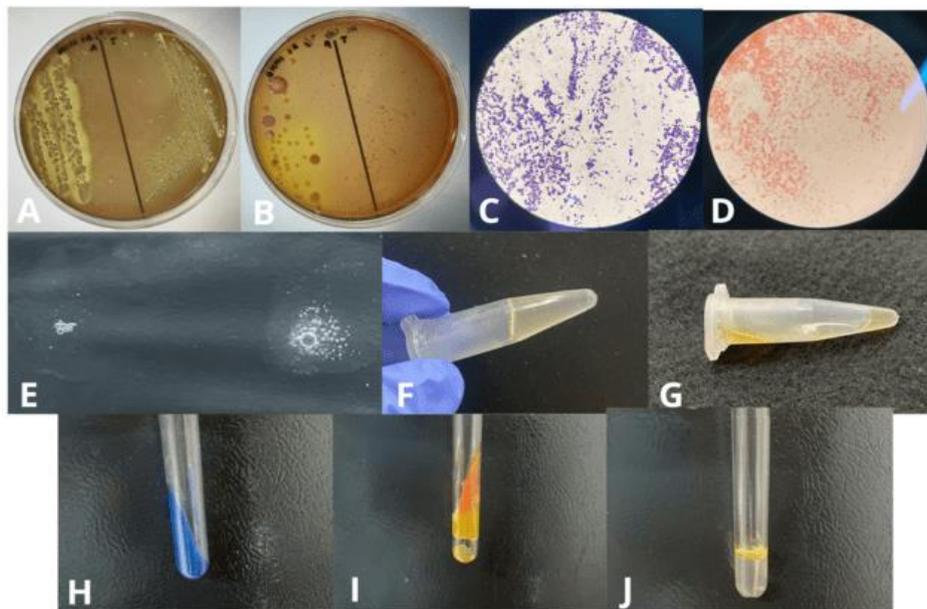Figure 1. (A) Detection of *Anaplasma* sp. (B) Detection of *Babesia* sp.

Figure 2. (A) BAP: beta-hemolytic colonies. (B) MCA: smooth pink (mucoid) colonies. (C) Gram staining: purple clustered cocci. (D) Gram staining: red coccobacilli. (E) Catalase-positive reaction on BAP and MCA. (F) Coagulase-positive reaction on BAP. (G) Coagulase-negative reaction on MCA. (H) Citrate-positive test. (I) TSIA: acid slant and acid butt, gas production, non-H_2S. (J) MIU: non-motile, indole-negative, and urease-positive.
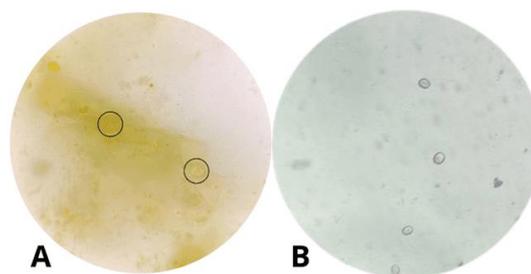

Figure 3. Detection of Eimeria sp. oocysts. (A) Direct smear. (B) Flotation test.