CLINICAL AND POST-MORTEM FINDINGS OF SAVANNA GOAT NATURALLY INFECTED WITH *Burkholderia pseudomallei*

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ABSTRACT. The case study describes the clinical and post-mortem findings of an infected goat at the farm level and deduces a confirmatory diagnosis of melioidosis through bacteriological and histopathological analysis. Melioidosis is a zoonotic disease caused by a gram-negative agent called *Burkholderia pseudomallei,* a saprophyte bacterium in deep soil that re-emerges due to heavy rainfall or soil excavation. A two-year-old female Savanna goat was observed to be weak and emaciated with a body condition score (BCS) of 1.5 out of 5 and was treated with long-acting (LA) oxytetracycline twice over 96 hours. The goat did not respond to the treatment and progressively deteriorated prior to being found dead four days post-treatment. Post-mortem findings revealed generalised whitish-yellowish nodules on the spleen, liver, and kidney, and generalised hemorrhagic spots and whitish-yellowish nodules in the lungs with whitish-yellow cream (pus) oozing upon dissection. Samples of the kidneys, lungs, and liver were taken and sent to the bacteriology and histopathology laboratories for further diagnostic work. Gram-staining revealed the organism is an intracellular, bipolar-staining, Gram-negative rod with a characteristic "safety pin" appearance, which is a distinct characteristic of *B. pseudomallei*. Histopathology observed micro-abscesses and neutrophil infiltration were observed in the lung cells. The final confirmatory diagnosis of *B. pseudomallei* was obtained through an analytical profile index (API) diagnostic kit with 99.9% accuracy. The primary cause of infection was suspected to be the reemerging of pathogens from soil excavation. The pathogen was transmitted to the naïve goats either through inhalation, contact from workers, or ingestion through contaminated water supplies. This disease may cause economic losses as well as infection in humans; thus, preventive measures are crucial to control the disease.

Keywords: Savanna goat, *Burkholderia pseudomallei*, melioidosis, bacteriological and histopathological diagnosis

INTRODUCTION

Melioidosis is a zoonotic disease caused by the agent *Burkholderia pseudomallei*, which appears as a small rod, motile, gram-negative bacterium under microscopic view and is known to be a facultative anaerobic bacillus with bipolar staining (Sprague, 2022). Melioidosis is known to be endemic in South Asia, and cases in Malaysia can be traced back to the year 1913 (Musa *et al.*, 2015). It is a saprophyte bacterium and has been previously isolated from wet soil of varying depths (25–120 cm) as well as from water sources (Sprague, 2022; Sprague & Neubauer, 2004). Cases of melioidosis tend to surge in rainy seasons, as the bacteria usually reside under the soil surface and tend to re-emerge due to rainfall (Puthucheary, 2009). The bacterium is known to be able to survive for up to 8 weeks at room temperature, 7 months in muddy waters, and in the soil for up to 30 months (Thomas & Forbes-Faulkner, 1981). The bacteria can be so resistant that they could multiply at pH 4 to 8, with a minimal humidity of 10-15% and a temperature of 4 – 42 °C. In addition to seasonal factors, importation and migration of animals have also been identified as one of the causes of the emerging disease (Sprague &

Neubauer, 2004). It was reported in a survey that the risk of melioidosis increased on farms that had nearby bush fire activity, a mix of various species of animals (jungle fowl, cattle, deer, cats, dogs, et cetera), and a history of water logging or flooding in the area (Musa *et al.*, 2015).

The most common route of infection is through inhalation, though there has also been percutaneous infection (Soffler *et al.*, 2014). Sprague (2022) reported various clinical signs from acute to chronic conditions that include fever, anorexia, swollen glands, mastitis, aortic aneurysms, severe coughing, respiratory distress, mucopurulent nasal discharge, circling, incoordination, blindness, nystagmus, and spasms and lameness observed in ruminants, mainly sheep, goats, and cattle. Signs of fever could be seen as early as day 2, with a temperature as high as 41 °C, but tend to slowly decrease to normal temperatures (Soffler *et al.,* 2014). Other post-mortem reports of melioidosis cases in goats observed were subcutaneous 2–4 mm, cream-coloured abscesses, prescapular lymph node abscesses, and abscesses in the lungs, spleen, and kidney. In the lungs, the abscesses were surrounded by a dark red rim of consolidated parenchyma and 3–4 mm of protruding nodules above the renal surface (Soffler *et al.*, 2014). Three types of infections have been identified as acute, chronic, and latent. Acute cases are presented with fulminant septicemia, and death is observed within days post-exposure, while latent cases, on the other hand, may be asymptomatic and remain in the host for years (Lowe *et al.*, 2002). The characteristic features of this disease affect not only the lungs, spleen, and liver through the formation of multiple abscesses but also the subcutis and regional lymph nodes (Omar, 1963). Early microscopic lesions of the infection (Days 2–7) would observe lymphangiectasia and lymphoedema, perivascular fibrin deposition, and microscopic haemorrhages in the dermis and panniculus. The later point of the infection tends to observe multifocal and coalescing pyogranulomas, necrotic epidermis clefted from the underlying dermis, and granulomas (Soffler *et al.*, 2014). Besides that, lesions can also be seen on the testes and scrotal sac, and microscopic lesions have been observed in the brainstem and spinal cord (Fatimah *et al.*, 1984; Omar, 1963).

Although cases of melioidosis in goats and sheep are not common in Malaysia, several cases have been reported. For example, a collection of reported cases from 2000 to 2009 taken from the Department of Veterinary Services (DVS), Putrajaya, and the Veterinary Research Institute (VRI), Ipoh, revealed a total of 8,068 goat and 1,874 sheep cases were reported in Peninsular Malaysia. The cases of Melioidosis are not breed specific as the data consist of various breeds of goats, including local and imported breeds such as Katjang, Boer, Anglo-Nubian, Jamnapari, Kalahari, Charmoise, Saanen, Alpine, and other crossbreds. Breeds of sheep included Dorper, Santa, Marina, Damara, Merino, Barb, Malin, Meat Master, and crossbreds of some of the above-listed breeds. The majority of these small ruminants (66.7%) were kept on a raised, slatted floor, whereby the majority were kept in semi-intensive (50%) and intensive systems (35%) (Musa *et al.*, 2015). In a separate study, seroprevalence of melioidosis cases from 2012 to 2016, a total of 78,500 goats and 54,130 sheep samples were tested in the Serology Laboratory of the Veterinary Research Institute (VRI), where 331 (0.42%) and 487 (0.90%) positive cases were found, respectively. According to the report, Perak recorded the highest number of cases (109) in Malaysia, followed by Sabah (85), Pahang (31), Sarawak (19), Kedah (16), Perlis (14), and Kelantan (14), while other states reported less than 10 cases (Masrin *et al.*, 2018). Reported cases

of melioidosis in humans are even lower but fatal with a total of 67 reported human cases in Malaysia from 1975 to 2015, with a mortality rate as high as 43% (Kingsley *et al.*, 2016). Although Melioidosis is known to be zoonosis, animal-to-human transmission has been rare with only one case of sheep-to-human transmission has been recorded (Nathan *et al.*, 2018).

The DVS Malaysia conducted a complement fixation test based on the WOAH (OIE) protocol to diagnose melioidosis, classifying an animal as positive when the antibody titre is 1:8 or higher (Masrin *et al.*, 2018; OIE Manual, 2016). Gram staining, bacterial isolation and identification, an API test kit, and histopathology are methods for further confirmatory testing. The current case study will discuss these test results in further detail. Melioidosis has an economic impact on the small ruminant industry, as animal productivity tends to drop due to emaciation and condemned carcasses (Brett & Woods, 2000; Hambali *et al.*, 2018). This case report details the history, clinical, pathological, and bacteriological findings leading to a confirmative diagnosis of chronic melioidosis, and concludes with an antibiotic sensitivity test.

CASE PRESENTATION

In October 2018, the Malaysian Agricultural Research and Development Institute (MARDI) in Kluang, Johor, Malaysia, imported 200 purebred female Savanna goats and 200 purebred female Boer goats, including 12 males from both breeds, at the age of 12 months from South Africa. The goats were brought in by air cargo and transited in Singapore before being brought in to Malaysia by land through the state of Johor. They were kept in multi-layered wooden crates throughout the journey, bedded with hay, and given sufficient food and water during the course of the journey. No mortality was reported upon arrival. The goats were vaccinated according to protocols set by the DVS. Five months later, in January 2019, prior to this case, one female Savanna goat was found dead, and upon post-mortem examination, generalised yellowish pustules and pus were found in many organs. Several other mortalities were observed in Boer goats; however, melioidosis was not deemed a differential diagnosis as no pus or pustules were found. Post-mortem examination of these goats indicated pasteurellosis due to haemorrhaging of the apical lobes of the lung.

During the time the case was observed, construction of two newly raised goat houses was being carried out approximately 100 metres away from the pen that housed this goat. Goats were kept intensively in a raised house. Daily forages were harvested approximately 15 km away and delivered to each pen house. Imported goats were kept separately from existing goats as quarantine measures and remained isolated and segregated according to breed. Water sources, however, were shared from the same lake and pumped into individual houses, where filters were placed to prevent mud from mixing into drinking water. The filters were occasionally cleaned to maintain a visibly clear water source supplied to all goats. No further treatments were conducted at the water source. Prior to the importation of Savanna and Boer goats, screening for foot and mouth disease (FMD), melioidosis, and caseous lymphadenitis (CLA) was negative, and clearance permits for importation were obtained. However, after the arrival in January 2019, another series of total herd screenings were conducted on all existing goats in MARDI Kluang due to the high mortality rates up to 3-4% a month mainly observed in the imported Boers. The screening revealed one positive case of melioidosis in the Boer herd. The positive case was subjected to culling. CLA has

been endemic on the farm; a few positive cases of CLA were observed through screening, but no positive cases of FMD were seen.

In February, a two-year-old female Savanna goat was observed to be weak and emaciated. Clinical examinations revealed a body condition score (BCS) of 1.5 to 2 (Figure 1) out of 5, while other vital parameters were within the normal range. Thus, differential diagnoses of chronic melioidosis, chronic pasteurellosis, and tuberculosis were listed based on the history and previous post-mortem findings. The goat was treated with oxytetracycline every 2 days and given sufficient green grass and concentrate. Molasses was given to stimulate appetite. However, body conditions did not improve leading to eventual mortality after four days. Upon death, a post-mortem examination was conducted and revealed generalised whitish-yellowish nodules on the spleen, liver, and kidney (Figure 1). Generalised hemorrhagic spots and whitish-yellowish nodules were observed in the lungs. Upon dissection of the lower lobe of the lungs, whitish-yellow cream (pus) oozed from the cut surface (Figure 1). Samples of kidney, lungs, and liver were collected, kept in an ice pack, and sent to the bacteriology and histopathology laboratories at the Faculty of Veterinary Medicine, University Putra Malaysia (UPM) for analysis. Sampling was done cautiously to prevent contamination of samples, and instruments used for sample collection were sterilised before contact with organs.

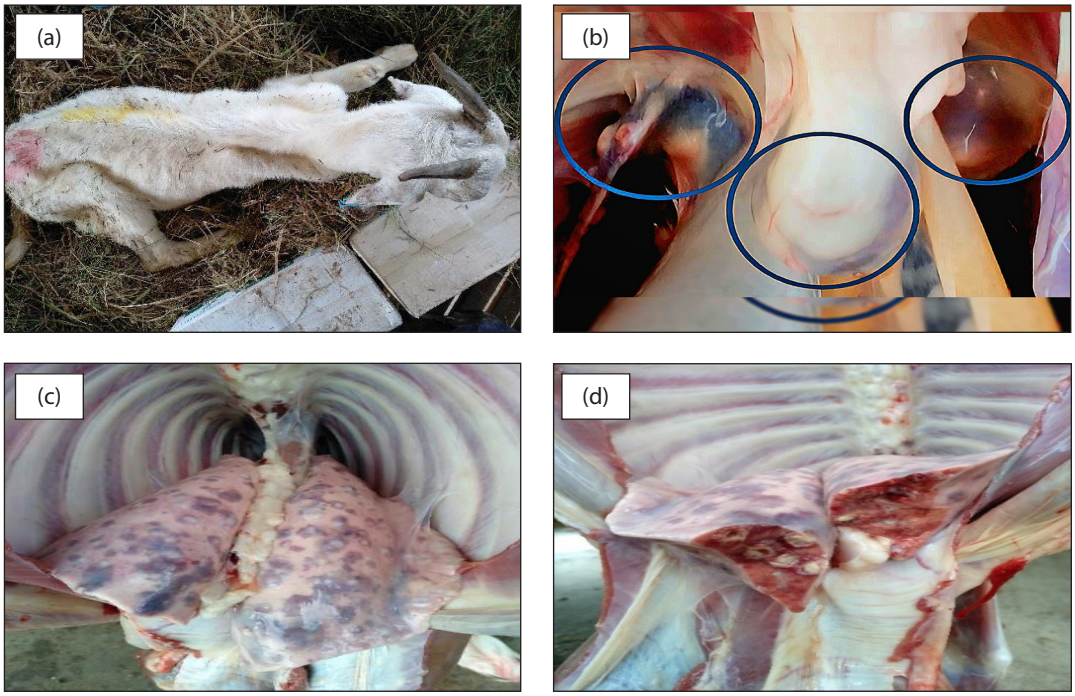


Figure 1. Clinical and post-mortem findings of infected goats on the farm.
(a) BCS of 1.5-2; (b) Generalised whitish-yellowish nodules on the internal organs;
(c) Generalised reddish and whitish-yellow nodules on the lungs;
(d) Whitish-yellow cream (pus) oozing from the cut surface of the lungs.

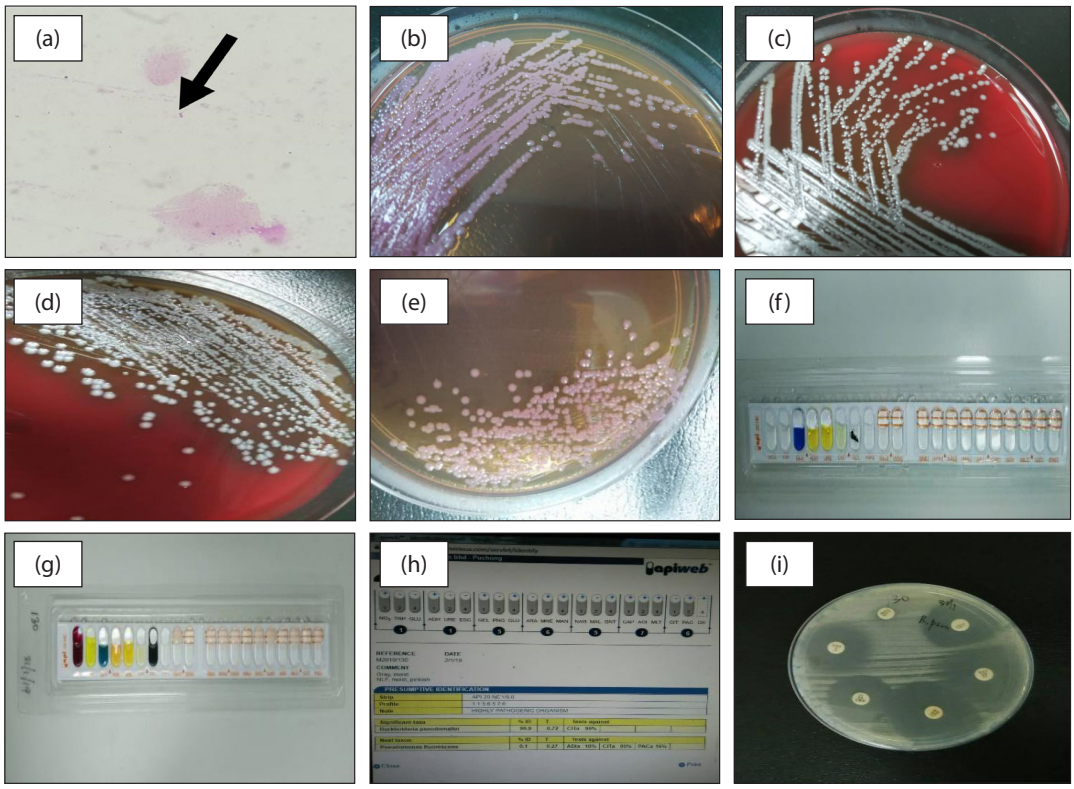
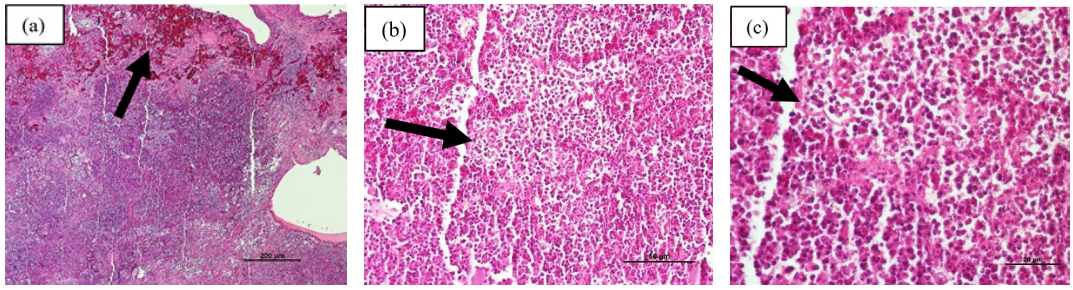


Figure 2. Bacteriology results (a) Gram staining revealed a gram negative intracellular rods is indicative of B. *pseudomallei* (b) MacConkey bacteriology culture of lung sample (c) Blood agar bacteriology culture of lung sample (d) MacConkey bacteriology culture of kidney sample (e) Blood agar bacteriology culture of kidney sample (f) API kit pre-screening (g) API kit post-screening (h) API kit results (i) Antibiotic sensitivity test



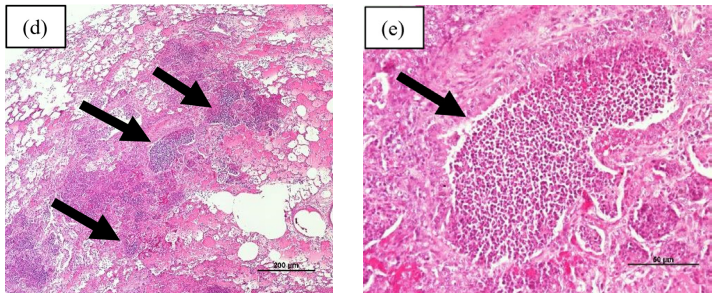


Figure 3. Hematoxylin and eosin staining of histopathology result (a) Perivascular neutrophilic infiltrates (200 µm; (b) Aggregation of neutrophils, necrosis of the lining epithelium (60 µm; (c) Close-up of the aggregation of neutrophils, and necrosis of the lining epithelium (20 µm; (d) Formation of micro abscesses (200 µm; (e) Close-up of the formation of a micro abscess (60 µm)

RESULTS AND DISCUSSION

Gram-staining revealed that the organism is an intracellular, bipolar-staining, Gram-negative rod with a characteristic "safety pin" appearance, which is a distinct characteristic of *B. pseudomallei* (Figure 2a). Samples of lung and kidney were inoculated on blood and MacConkey agar. Partial hemolysis was observed on the blood agar, indicating an Alpha-haemolysin reaction, more distinctively on the kidney sample compared to the lung sample (Figure 2c & 2e). The bacterial colony on blood agar appeared white, circular, smooth, and glistening. Colony on MacConkey, however, appeared light pinkish to purplish, circular to mildly elongated, smooth, and glistening. The absence of colour changes in MacConkey agar suggests that it is a non-lactose fermentative bacterium. MacConkey agar can selectively isolate gram-negative bacteria, whereby the crystal violet and bile salts inhibit the growth of gram-positive bacteria (Figure 2b & 2d). Other bacterial isolation and identification tests reported to be effective are those using Ashdown's media, whereby a consistently distinctive colony morphology and odour can be observed (Prakash *et al*., 2014; Sprague & Neubauer, 2004; Sprague, 2022). The Biomerieux API Kit is an automated method using software for bacterial identification through the detection of enzymatic activity by observing the colour changes of the kit. This method has been reported to have a high level of accuracy in identifying *B. pseudomallei* in API 20NE (99%) and API 20E (98%) kits (Lowe *et al.*, 2002). In this case, however, higher accuracy was obtained for API 20 NE of 99.9% and 0.01% possibility of *Pseudomonas fluorescens* (Figure 2h). This concludes a confirmatory diagnosis of the causative agent of *B. pseudomallei,* which led to chronic melioidosis due to the presence of pus exudates in the lungs.

Micro-abscesses and neutrophil infiltration were observed in the lung cells (Figure 3). The formation of a micro-abscess could indicate that the case has progressed to the later stages. Post-mortem findings of the lung earlier described (Figure 1d) and histopathological findings (Figure 3b & 3c) indicated that the goat may have had reduced lung function. A histopathological study of lung cells' response to *B. pseudomallei* revealed that the infection starts with oedema, perivascular neutrophilic infiltrates, congestion of interlobular and small clusters of macrophage vessels (day 1), followed by luminal neutrophil aggregation, necrosis of the lining epithelium (day 2), fibrinous exudate in the alveolar spaces (day 7), and fibrous connective and

granulation tissue (day 21) (Soffler *et al.* 2014). This leads to an increased pulmonary circulation system, which is necessary to provide sufficient oxygen to the animal. According to clinical and histopathological findings, the cause of death in this case was highly likely due to lung failure. The post-mortem findings of the current case study were concurrent with the reported findings, which aided in the diagnosis of the case. However, in a herd of many animals, some clinical signs for early detection may be overlooked. Post-mortem findings should be alarming enough for herd serology testing and further diagnosis to be done for the prevention and eradication of the disease.

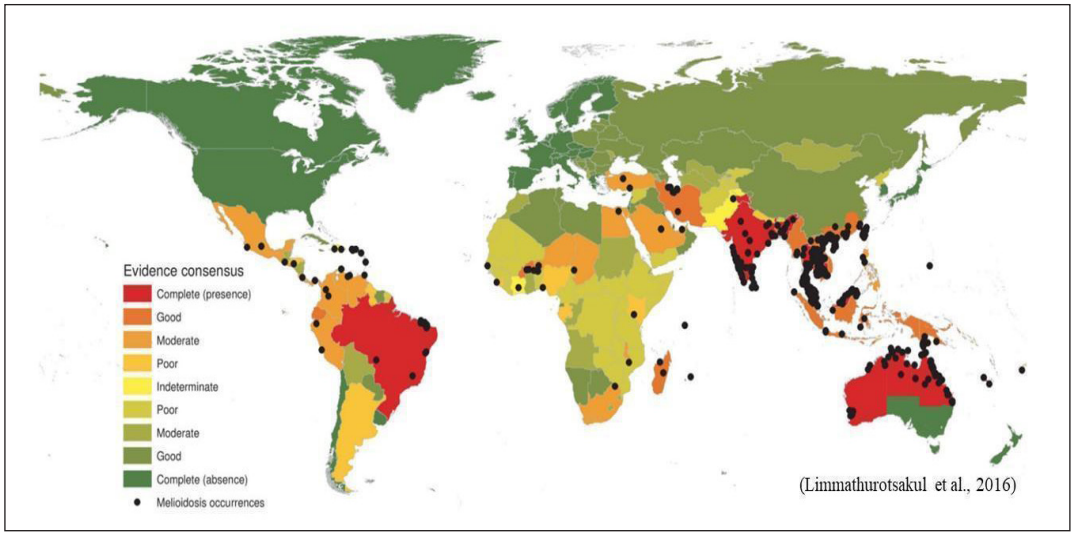


Figure 4. Global evidence consensus and geographic locations of occurrence data of Melioidosis from 1910 to 2014

The cause of infection may have been due to the endemicity of the region, where positive cases have been found before (Figure 4). Some of the reported factors leading to the emergence of the pathogen include physical factors such as heavy rainfall, humidity, and temperature, as well as chemical factors such as soil composition, the use of fertilisers, and recent soil disturbances such as excavation and ploughing (Cheng & Currie, 2005; Sprague & Neubauer, 2004). The existing herd may have had some kind of immunity against the disease, though it was not sero-prevalent. However, the goats imported from South Africa may not have had exposure to the disease, as the disease was not prevalent in the region, being endemic only in tropical regions (latitudes of 20 °N and 20 °S) (Cheng & Currie, 2005). Therefore, the importation of naïve goats may increase the risk of infection in endemic regions. Although infected goats had been culled prior to the introduction of the new imported herd, the primary presumptive cause of the exposure could have been transmitted due to the excavation work done, which started during the dry season when the soil is dry and dusty. This led to the re-emergence of the pathogen from the soil, leading to the direct inhalation of naïve goats or via secondary

causes. Heavy rainfall may also be another factor leading to the re-emergence of the pathogen in deep soil. The North East Monsoon is generally from December to February, and heavy rainfall due to this monsoon has been reported in Kluang, Johor (Ahmad *et al.*, 2017). The goats were housed intensively and have not been led out to graze from the point of importation; however, the movement of workers in and out of the region could also lead to the transmission of the pathogen to the herd. Although goats were segregated according to breed and not mixed with the existing herd, there may also be a possibility of exposure to the pathogen through the water source, which was pumped from the nearby lake.

Treatment for melioidosis is relatively expensive due to the long antibiotic treatment, as immunosuppressive conditions are common. However, studies on choice of drugs to be used in a tropical setting is lacking and therefore the current case study looked further to test the antibiotic sensitive to *B. pseudomallei*, particularly due to the zoonosis nature of the disease. *B. pseudomallei* has been reported to be resistant to penicillin, ampicillin, first- and second-generation cephalosporins, gentamicin, tobramycin, streptomycin, macrolides, and polymyxins (Sprague, 2022). Antibiotic sensitivity in this study revealed doxycycline, 30 µg (effective), trimethoprim-sulfamethoxazole, 1.25/23.75 µg (effective), amoxicillin-clavulanic acid, 20/10 µg (moderately effective), enrofloxacin, 5 µg (moderately effective), gentamicin, 10 µg (not effective), and chloramphenicol, 30 µg (moderately effective). Thus, based on this case study, it is recommended that doxycycline and trimethoprim-sulfamethoxazole would be a good choice for treatment; however, for most cases, culling would be recommended to prevent the spread of infection.

Previous studies have suggested several mitigation methods to reduce the risk of infection. Movement restrictions need to be adhered to on farms during the rainy season, especially when construction is being carried out in the farm area, the re-emergence of the bacteria may contaminate water sources. (Sprague & Neubauer, 2004). Thus, in this case, adding chlorine to the water system may act as a prevention method. Though chlorine has a bacterial-static effect on *B. pseudomallei*, it can effectively reduce the number of viable bacteria (Sprague & Neubauer, 2004). Besides that, another method of prevention is by elevating the pH level of the soil through treatment with limestone to render an unfavourable environment for the survival of the bacteria (Musa *et al.*, 2015).

CONCLUSION

In a nutshell, the current study observed pathological findings of yellow nodules in several organs including liver, spleen and kidney as well as pus exudate in the lungs leading to the tentative diagnosis of Melioidosis. The confirmatory diagnosis was obtained via bacterial isolation and safety pin like cellular structure as well as the (API) diagnostic kit used with 99% accuracy. The study stresses on the vulnerability of imported goats from Melioidosis naïve regions being exposed to the disease in Malaysia. Therefor the study warrants extra precautions to be taken particularly where construction is done close to the pen to prevent risk of re-emergence of the pathogen.

Conflicts of Interest

None of the authors of this paper has any financial or personal relationship with other personnel or organizations that could inappropriately influence or bias the content of the paper.

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