CASE REPORT

MELIOIDOSIS IN CAPTIVATED ORANGUTAN (*PONGO PYGMAEUS*) AT BUKIT MERAH ORANG UTAN ISLAND (BMOUI), PERAK, MALAYSIA

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ABSTRACT. The post-mortem reports of two deceased captivated Bornean orang utan (*Pongo pygmaeus*) in Bukit Merah Orang Utan Island (BMOUI), Bukit Merah, Perak, Malaysia, were investigated to assess the diagnosis of melioidosis among non-human primates. Before death, the orang utans showed symptoms such as weakness, inactivity, loss of appetite, reduction in water intake, and signs of respiratory distress. The orang utans were treated with ceftazidime and trimethoprim-sulfamethoxazole. However, the symptoms persisted until they succumbed to death. Necropsy was conducted at Veterinary Research Institute (VRI), Perak. The lung was removed and sampled immediately to minimize the possibility of cross-contamination with normal gut flora. The necropsy findings revealed gross changes associated with an on-going bacterial infection. Microbial investigation confirmed the presence of *Burkholderia pseudomallei* in the pus of the spleen and gum tissue. This is an update of cases of fatal animal melioidosis among the orang utan species in Malaysia after decades. This case report highlights the need to reduce the mortality of orang utan and improved conservation management of the critically endangered species in Malaysia.

Keywords: Orang utan, captivated, animal melioidosis, *Burkholderia pseudomallei,* conservation management

INTRODUCTION

Melioidosis represents one of the most common infectious diseases in zoo animals (Kasantikul *et al.,* 2014). Melioidosis contributes to biological diversity loss due to the mortality of zoo animals (Alwis *et al*., 2020). The causative agent of melioidosis is *Burkholderia pseudomallei,* a facultative anaerobic saprophytic bacterium usually found in soil and water (Wiersinga *et al.,* 2018). Most cases of melioidosis are attributed to the exposure of animals to contaminated water and soil (Musa *et al*., 2012).

In Thailand, cases of captive non-human primate melioidosis were estimated to be 28% (Kasantikul *et al.,* 2014), while the frequency of animal melioidosis in Malaysia involving non-human primates is under-reported. To date, there are only a few necropsy reports to investigate the cause of death in zoo animals suspected to have died from the melioidosis (Limmathurotsakul *et al*., 2012). Vellayan (1994) reported that more than 95 % of melioidosis cases among animals were diagnosed based on necropsy findings. It is well-known that non-human primate such as orang utans are biologically nearest relatives to

the human in the taxonomy (Trosvik *et al*., 2018) thus are highly susceptible to the most human diseases such as melioidosis, influenza, measles, tuberculosis, leptospirosis, scabies, *Plasmodium* sp., and *Strongyloides* sp. infections (Sherman *et al*., 2021; Hayashi *et al.,* 2018, Carne *et al.,* 2014).

Bukit Merah Orang Utan Island (BMOUI) is located in Bukit Merah, Perak, Malaysia, is an ex-situ conservation centre built exclusively for orang utan species and currently houses 16 of orang utan species. The main objective for setting up the Orang Utan Island was to create public awareness of orang utan species conservation and to ensure their sustainability in the world (Hayashi *et al*., 2018). Disease control and prevention is part of the animal health program, thus, an investigation regarding melioidosis among orang utans was carried out in Bukit Merah Orang Utan Island (BMOUI) to access this problem.

Case History

The two orang utans were 43-year-old male and 11-year-old female, which were permanent residents of BMOUI. The orang utans were weak and physically inactive for approximately 3 days before death, while other signs were also shown during the time including loss of appetite, reduction in water intake, vomiting, and respiratory distress manifested as difficulty in breathing. The orang utans were immediately administered with ceftazidime (Cefatum Inj.), 100 mg/kg intramuscularly every 8 hours daily, and trimethoprim-sulfamethoxazole (Co-trimoxazole®) 80/400 mg/kg orally every 12 hours daily, with regular monitoring of their conditions. However, the orang utans died 3 days after the onset of clinical signs.

The carcasses were sent to the Veterinary Research Institute for post-mortem examination immediately within 24 hours of animal death in order to avoid the bodies undergo biochemical and physiological changes. Based on the necropsy findings, the body cavity of both orang utans showed evidence of emaciation and accumulation of a yellowish exudate in the peritoneal and thoracic cavity. Other than that, there was also adhesion of pleura of mesenteric fat at the diaphragm, liver, spleen, and serosae surface of the large intestine with the presence of fibrinous and fibrous tissue. There was evidence of passive congestion of the liver with the presence of approximately 1-2 cm diffused whitish fatty liver and nodular lesions on the liver surface. Upon cut surface of the liver, there were nodular lesions, petechial haemorrhage, creamy yellowish calcareous fluid material, and fibrous tissues. There was also evidence of diffused petechial haemorrhage on the mucosal surface of the duodenum with creamy and watery stomach content.

Necropsy results of the respiratory organs revealed lung oedema and congestion, and embolic pneumonia. Diffused whitish nodular lesions, petechial haemorrhages, and pleuritic condition were also observed. For the cardiovascular system, the heart muscles were pale, while other findings included inflammation of the pericardial sac (pericarditis) and fluid in the pericardium (hydropericardium). There were evidence of dried synovial fluid and pale bone marrow. The male orang utan had petechial haemorrhages in the heart muscles, whereas such haemorrhage was observed in the kidney of the female orang utan.

Histopathological examination of the male orang utan revealed an embolism in the lung tissue which consisted of numerous inflammatory cells such as neutrophils, debris, and fibrins. The accumulation of homogenous oedematous fluids was also found in the alveoli, which was suggestive of severe fibrinous embolic

pneumonia and pulmonary oedema. Besides, necrosis and fatty degradation of hepatocytes also occurred in liver tissues. There was also neutrophilic infiltration of the gum tissue of the deceased orang utan. Dead cells, macrophages, and debris were observed in the spleen tissue, while the presence of pus in the spleen tissue signifies the probability of melioidosis or pyogenic bacterial infections as the differential diagnosis of the death.

Histopathological examination of the female orang utan revealed numerous inflammatory cells comprised of dead neutrophils, macrophages, and debris in the spleen tissue. There was also congestion and oedema of the lung with tiny abscesses in both lungs and liver. The evidence of abscesses and pus signifies bacterial infection probably due to melioidosis. Based on the microbial investigation, *B. pseudomallei* was isolated from spleen swabs and pus of both carcasses and in the gum tissue from the male orang utan.

DISCUSSION

From the record obtained from BMOUI in 2018, 10 % of melioidosis cases were reported yearly (Hayashi *et al*., 2018). BMOUI was established in 2000 and 11 orang utans were transferred from Sarawak Forestry and Malacca Zoo. Since then, the procedure of health monitoring of orang utans, diagnosis and treatment protocols were practised. In BMOUI, the full medical examination of the orang utans will be performed every 6 months to monitor their health condition. The blood samples will be sent to the laboratory for the detail assessment. But if the orang utans show any symptoms of infection, the medical examination will be performed immediately to ensure the orang utans get the appropriate treatment. Melioidosis occurrences among orang utans were recorded properly by the management of BMOUI.

The sources of *B. pseudomallei* infection among orang utans in BMOUI are mainly from the contaminated soil. The incidence of melioidosis is usually high during the monsoon season, as this inhabiting soil organism will rise to the topmost of the soil (Chen *et al.,* 2015). However, both deceased orang utans in BMOUI died in May and June, which is a dry season in Malaysia. The southwest monsoon usually starts in May and ends in September with lesser rainfall than the northeast monsoon that occurs in November and ends in March every year (Musa *et al.,* 2015). Two possibilities can be related to the incidence of melioidosis in captive zoo animals. Before signs of acute sepsis appeared, the infected animals might be exposed to the melioidosis agent for an extended period and remain asymptomatic, or the melioidosis agent survives and persists in a harsh environment for months to years. When the animals get infected, the symptoms will show afterward especially when the host is associated with immunocompromising conditions (Inglis & Sagripanti, 2003).

Melioidosis infection has an extensive range of symptoms similar to septic shock, community-acquired pnemoniae, and tuberculosis, often leading to misdiagnosis with other infections (Currie *et al.,* 2010; Inglis *et al*., 2006). The symptoms often start as disturbances from the chest such as difficulty in breathing, productive cough, and fever (Elschner *et al.,* 2014; Nathan *et al*., 2018). Some of the animals show symptoms of anorexia, diarrhoea, vomiting, and paralysis (Oudah *et al*., 2006; Elschner *et al.,* 2014) which is similar to the symptoms showed by the deceased orang utan in BMOUI. Findings from this case show that there is no evidence of tuberculosis based on acid fast staining and quick dip Giemsa staining performed to the male

orang utan. Mycobacterium tuberculosis also was not isolated after two weeks of incubation.

The incubation period of melioidosis infection is usually between 1-21 days (Puthucheary, 2009; CDC, 2012). During the incubation period, the infected individual may either show some symptoms or remain asymptomatic (Puthucheary, 2009). Previously at BMOUI, a few other unhealthy orang utans were suspected of having melioidosis infection based on respiratory signs typical of the disease; however, the bacteria were not isolated at necropsy. In the early stage of illness, they had fever for about 1 to 2 days up to a week and were treated accordingly. The symptoms disappeared for a while and later recurred with more severe illness. At this stage, there was less likelihood for the ill orang utan to be cured. Kasantikul and colleagues (2014) reported that orang utan might be dead after 1 to 7 days after the onset of infection due to acute septicaemia. Septicaemia with high fever is an indicator of acute melioidosis (Waiwarawooth *et al.,* 2008).

The preferable treatment for melioidosis septicaemia is intravenous administration of antibiotics (Inglis *et al*., 2006). The treatment of animal and human melioidosis is similar. Based on empirical evidence, the antibiotic suggested for the treatment of melioidosis is ceftazidime or carbapenem. In the intensive phase of treatment, ceftazidime and carbapenem are the first line of antibiotics that will be prescribed to the patients for approximately 10 to 14 days. The treatment might be longer depending on the current condition of the patients (Chaowagul *et al*., 2005; Pitman *et al.,* 2015). During eradication phase treatment, patients will be treated with oral trimethoprim-sulfamethoxazole, doxycycline or amoxicillin-clavulanate for at least 2 to 20 weeks to be completed (Chierakul *et al.,* 2006). The relapse occurrence among patients is usually associated with the failure of patients to complete the course. Chaowagul *et al.* (2005) claimed that if the oral treatment is taken for less than 8 weeks, the relapse rate arises from approximately 10 % to 30 %. Again, the choices of antibiotics vary between countries. In Australia, monotherapy of trimethoprim-sulfamethoxazole is prescribed to the patients, while in Thailand, trimethoprim-sulfamethoxazole is given in combination with doxycycline (Chaowagul *et al.,* 2005). However, due to the high cost and difficult access, the veterinarians prefer conventional regimens such as trimethoprim-sulfamethoxazole and doxycycline (Choy *et al*., 2000).

Meanwhile, *B.pseudomallei* is known to be resistant to vast number of antibiotics for example aminoglycosides, polymyxins, and many beta-lactams antibiotics (Piliouras *et al*., 2002). Thus, the tendency of relapse occurrence while in the treatment is very high. This is because the infected person is unable to fully eliminate the bacteria at the first infection due to the acquired resistance or the person becomes re-infected with the same strain or different strain (Sam *et al.,* 2009). Relapse once reported in melioidosis case involving pig-tailed macaque in Thailand where the same animal was diagnosed with melioidosis again after 6 months due to insufficient duration and dose of treatment. The pig-tailed macaque was administered with ceftriaxone added with ceftazidime for less than two weeks (Kasantikul *et al.,* 2014). Furthermore, Estes *et al.* (2010) reported approximately 13 % to 16 % of clinical isolates from Thailand resistance to trimethoprim-sulfamethoxazole, chloramphenicol (7 %) followed by doxycycline (2 %). Resistance to ceftazidime and amoxicillin-clavulanate has also been recorded recently (Estes *at al.,* 2010). Thus, this situation leads to limitation of therapeutic options for melioidosis treatment. In this case, both orang utans were treated with

ceftazidime at 100 mg/kg given daily every 8 hours where maximum dose is 6 g daily and trimethoprim-sulfamethoxazole at 80/400 mg/kg given every 12 hours daily for 3 days. The dose of trimethoprim-sulfamethoxazole administered to the animal is lower compared to that of the human dose for melioidosis therapy. During the intensive phase therapy of human melioidosis, the recommended dose of ceftazidime is 100 to 120 mg/kg/day every 6 to 8 hours. The additional trimethoprim-sulfamethoxazole at 320/1600 mg/day for < 40 kg body weight in two divided doses is indicated in the presence of abscesses. Nevertheless, the attempt to drain the abscesses should be part of the management whenever appropriate (Dance, 2014). The dosage of antibiotics in orang utan is calculated based on the body weight and size of the animals with the consideration of their metabolic rates and physiological process. In the cases of both orang utans, the dosages of antibiotics were given as in the prophylaxis treatment so that the risk of toxicity in the animals could be minimized.

The current antibiotics dosing practices by BMOUI in the post-exposure prophylaxis treatment of melioidosis includes trimethoprim-sulfamethoxazole 160/800 mg for body weight less than 40 kg, 240/1200 mg for body weight between 40 kg to 60 kg and 320/1600 mg for body weight more than 60 kg. The antibiotic is prescribed within 24 hours of high probability exposure to *B. pseudomallei* especially during every rainy season. While in the intensive phase treatment, the dosage of trimethoprim-sulfamethoxazole administered is 80/400 mg/kg/day in twice-daily doses for 2 months with the addition of ceftazidime dose administered to infant and adult orang utans is 25 to 100 mg/kg/day and 1 to 6 g/kg/day respectively in thrice daily doses for 3 weeks.

Besides the lungs, visceral abscesses in the liver, spleen, adrenals, kidney, and soft tissues are the other most common melioidosis infection sites in animals (Nathan *et al*., 2018). In the present case report, the gross findings and pathological changes in the visceral organs were suggestive of the disease. This was followed by the isolation of the causative agent for melioidosis from the spleen and liver. The appearance of "Swiss cheese" abscesses in the liver and spleen is typical of bacteraemia melioidosis (Nathan *et al*., 2018). One of the earliest reports of fatal melioidosis involving orang utan was recorded in Zoo Melaka in 1991 (Zainal *et al*., 1991), where the *B. pseudomallei* was detected in the lungs, liver, and spleen of a female orang utan of 11 months old. The lesions in the digestive tissues of the deceased orang utans in BMOUI were similar to the previously reported cases in Zoo Melaka (Zainal *et al*., 1991). Besides, our findings involving the adhesion of internal organs to the diaphragm is consistent with that of Kasantikul *et al.* (2014), where such pathological changes were associated with melioidosis. Other similar necropsy results included the presence of fibrin purulent pleuropneumonia with multifocal abscesses in the spleen and liver of deceased orang utan.

CONCLUSION

In the conservation of critically endangered species as the orang utan, it is pertinent to report cases of fatal melioidosis as soon as possible to the relevant bodies. In Malaysia, the population of this species is decreasing and at the risk of extinction. Therefore, the infection needs to be detected at an early stage, followed by the administration of appropriate treatment to reduce the mortality rate. The standard protocol for melioidosis management among captivated orang utans also needs to be refined and established. In this case, the cause of death

of the orang utans were acute septicaemia due to *B. pseudomallei* infection.

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