# Necrosy Report: Isolation of Klebsiella pneumoniae Among Borneo Orangutan (*Pongo pygmaeus*) at Bukit Merah Orang Utan Island (BMOUI), Perak, Malaysia

Nurul Iman[1], Yasmin Khairani Muhammad Ismadi[3], Nurul Aili Zakaria[1], Muhammad Fahmi Ramli[2], Badrul Munir Md-Zain[2,4], Zakuan Zainy Deris[3], Farida Zuraina Mohd Yusof[1,5*]

[1] School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450, Shah Alam, Selangor, Malaysia
[2] Bukit Merah Orang Utan Island Foundation, Bukit Merah, 34400, Perak, Malaysia
[3] Department of Medical Microbiology and Parasitology, School of Medical Sciences, Universiti Sains Malaysia, 16150, Kelantan, Malaysia
[4] Department of Biological Sciences and Biotechnology, Faculty of Sciences and technology, Universiti Kebangsaan Malaysia, 43600, Bangi, Selangor, Malaysia
[5] Integrative Pharmacogenomics Institute (iPROMISE), 42300 Bandar Puncak Alam, Selangor, Malaysia
fzuraina@uitm.edu.my

**Abstract.** Necropsy findings of three deceased orangutans from BMOUI were assessed to explore *Klebsiella pneumoniae* infections among captivated nonhuman primates (NHP) in Malaysia. The diagnosis was developed based on reported symptoms and necropsy findings. Necropsy examination revealed significant lesions correlated with bronchopneumonia, bronchitis, pericarditis, and myocarditis in all three cases. *K. pneumoniae* was isolated from multiple organs of all orangutans. The emergence of *K. pneumoniae* infection cases among endangered species raised a major concern among veterinarians and zoologists globally. This case study contributes invaluable information on the *K. pneumoniae* infection in NHP species.

**Keywords:** BMOUI, Endangered, *Klebsiella pneumoniae*, Orangutan.

## 1.0 Introduction

Bukit Merah Orang Utan Island (BMOUI) located in Perak State of Peninsular Malaysia has been build up in year 2000 and currently housing 15 of orangutans. The island was geographically surrounded by man-made freshwater lake and it is covered by secondary tropical forest [1]. In BMOUI, the orangutans will have access to outdoor enclosures during the day and will be kept in indoor enclosure during the night time [2]. The visitors will have opportunity to observe the orangutans from a 200 meters-long tunnel where the orangutans are freely roaming in their natural habitats (Fig. 1). The semi-wild environment in BMOUI encourage natural behaviors among orangutans [3].


S. Gandaseca et al. (eds.), *Proceedings of the International Conference on Science, Technology and Social Sciences – Biology Track (ICONSTAS-BIO 2023)*, Advances in Biological Sciences Research 43,
https://doi.org/10.2991/978-94-6463-536-2_13

BMOUI has been set up as one of research and education center to increase awareness among publics on the endangered orangutan species [2, 4].


**Fig. 1.** (a) Bukit Merah Orang Utan Island; (b) View of orangutan from the visitor's tunnel in BMOUI.

## 2.0 Literature Review

The most common infection reported among NHPs is the upper respiratory tract and the bacterial pathogens may include *Streptococcus* spp. *Escherichia coli, Proteus* ssp., *Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus* spp. The captivated animals in zoos and conservation centers are prone to infection by aerosol and exposure to contaminated fomites and equipment [5, 6]. The animals usually showed a wide range of symptoms such as diarrhea, hypothermia and pyrexia [7]. According to the record obtained from Hayashi et al. [4] from BMOUI, five percent of cases were reported regarding respiratory tract infection annually and the etiology of the infection was not clearly mentioned.

*K. pneumoniae* is the causative agent of various infections, including upper tract infection (URTI), liver abscesses, sepsis, meningitis, mastitis, and pneumonia in most of wild and domestic animals. This Gram-negative bacterium is commonly distributed in the environment but is also considered a nosocomial pathogen, part of the oral microbiome and fecal [8, 9]. *K. pneumoniae* infection is common in immunocompromised patients as the nature of the bacterium is opportunistic pathogen [10]. Patients with co-morbid disease such as diabetes, hypertension and chronic kidney failure also are at the risk of infection. Recently, a new hypervirulent *K. pneumoniae* (hvKP) has been discovered in culture and increasingly reported in Southeast Asia in the last two decades [11]. The hvKP organisms have evidence of a more than 5 mm stretching viscous mucous capsule showing a positive string test grown on agar plate. The hvKP infection usually more severe and spread more rapidly throughout the body. Moreover, the hvKP infection attributed to multi-drug resistant strain which has led to high numbers of treatment failure cases [12].

*K. pneumoniae* has been reported as rare in NHP species [13] but has been associated with significant morbidity and mortality among them. An extensive investigation of rapid diagnosis of *K. pneumoniae* infection in animals is obligated in any conservation centers in order to establish the effective preventive and control measures on the spread of infectious diseases among animals. Furthermore, information related to the infection among NHPs is very limited thus, this case study reports the necropsy findings of the *K. pneumoniae* among orangutans in BMOUI.

## 3.0 Methodology

Preliminary study was performed to investigate the occurrence of *K. pneumoniae* infection in BMOUI. The data was obtained from the medical reports, clinical signs and necropsy reports of previous infection among orangutans. The animal ethics approval for this study has been attained through Committee on Animal Research and Ethics Universiti Teknologi MARA (UiTM CARE:289/2019) and Department of Wildlife and National Parks under research permit (JPHL&TN (IP): 100-34/1.24 Jld 15(36).

## 4.0 Findings

### 4.1 Case history

Three Bornean orangutans who were permanent residents of BMOUI aged from six to 37 years old died over a period of 13 months. Previously, each of the orangutans had been reported to have respiratory distress, including difficulty breathing and coughing, as well as reduced daily activities. The occurrence of mucopurulent nasal discharge was also observed with an increase in body temperature. All of orangutans were observed dehydrated and had a loss of appetite. The orangutans died in BMOUI and the carcasses were immediately sent to VRI, Ipoh, Perak within 24 hours to confirm the cause of death. The necropsy, histopathology and bacteriology findings result presented in this case study were retrieved from the VRI records reported in year 2012 and 2013.

### 4.2 Necropsy findings

Case 1: Based on necropsy findings of the male-9-year-old orangutan (OU1), pus was present at his nose and trachea. The lung was pale in color and there was evidence of generalized hemorrhage at the whole lung. While in the cardiovascular system necropsy, there was evidence of a shrunken heart and jelly-like lesions at the aorta, auricle and ventricles. Necropsy results of the digestive system showed evidence of generalized petechial hemorrhage, rough cut on the surface of the liver and slough off mucous membrane of the stomach. In addition, necrosis of the intestine was seen and there was the

presence of hard feces along the intestine. Shrunken of the spleen and enlargement of the lymph nodes also were observed during the necropsy process. The bone was dry and fragile.

Case 2: The necropsy results of the male-37-year-old orangutan (OU2) revealed the occurrence of congestion and hepatinization of the lobes in the respiratory system. There was evidence of edematous and enlargement of the lung. A layer of pus that covered the entire lung was observed and it was attached to the left ribs. In addition, there was also evidence of congestion and enlargement of the heart and liver of the orangutan. The liver was observed to have very prominent enlargement with rounded border and appeared yellowish at the cut surface. There was evidence of obliteration of the spleen tissue occurred. Other than that, the urinary system examination of the orangutan showed that there was hemorrhage evidence at the cortex region with friable condition.

Case 3: The results of necropsy performed on the female-6-year-old orangutan (OU3) showed the presence of generalized hemorrhagic multifocal whitish nodules throughout the lung surface. Meanwhile, adherence of pericardial sac to the surface of pleura and diaphragm was observed during the necropsy. The thickening of the diaphragm was reported. The heart was in hydro pericardium state where fluid in the pericardial sac was found. There was evidence of endocarditis upon the cut surface of the heart. Otherwise, the liver was slightly pale.

### 4.3 Histopathology findings

Case 1: Histopathological examination on the male-9-year-old orangutan (OU1) revealed the kidney tubules and lung tissue necrosis. The infiltration and inflammatory cells were also found in the kidneys and bronchus area. The presence of fibrin cells was also observed in the lung, indicating chronic bronchitis. While in the liver histopathological examination, there was evidence of accumulation infiltration and inflammatory cells with complete hepatic steatosis. At the same time, the depletion of lymphoid cells and inflammatory cells was observed in the sinuses of the spleen. The findings also showed necrosis of the myocardium occurred.

Case 2: Findings of histopathological examination on the male-37-year-old orangutan (OU2) signified that necrosis of the kidney tubules occurred beside the accumulation of infiltration and inflammatory cells found in that area. The obliteration of the kidney tissues was also observed. There was also evidence of emphysema, edema and necrosis of the lung. Meanwhile, the accumulation of infiltration and inflammatory cells in the lung indicated bronchopneumonia. The presence of white spot granulomatous type which is characterized by a chronic proliferative interstitial hepatitis was observed in the liver. The interlobular septa were thickened which is highly suggestive of ascariasis. There was an accumulation of infiltration and inflammatory cells seen at the sinusoids. Besides, small fat droplets were also observed in the liver.

Occurrence of necrosis of hepatocytes reported in the histopathological examination results. The spleen sinuses and myocardium of the orangutan were observed to have massive infiltration of inflammatory cells. The lymphoid cells in the spleen are also depleted.

Case 3: Results of the histopathological examination on the female-6-year-old orangutan (OU3) reported severe infiltration and inflammatory cells, mainly neutrophils and histiocytes in the lung. The presence of a few nodular abscesses was found. Degradation of fatty acid and hepatocytes throughout the liver tissue was observed during the examination. There were also several small foci indicating abscesses at the liver. The heart of the orangutan was congested, hyperemia and myocarditis in condition.

### 4.4 Bacteriology findings

For the bacteriology findings, *K. pneumoniae* was isolated from all three orangutans. *K. pneumoniae* and *E. coli* were isolated from all organs of the male-9-year-old orangutan (OU1). While in the male-37-year-old orangutan (OU2) bacteriology reports, *K. pneumonia* was isolated from the kidney and liquid of the abdomen. Other than that, Haemolytic *Streptococcus aureus* was isolated from nasal swabs while *E. coli* was isolated from all organs, trachea swabs, nasal and abdomen fluid. Otherwise, *K. pneumonia* and *Pseudomonas fluorescent* were isolated from lung and liver of the female-6-year-old orangutan (OU3). The summary of all the necropsy findings were presented in table 1.

**Table 1.** Summary of the pathological and bacteriological findings of necropsy in orangutans from BMOUI.

| ID Number | Sex | Age (years) | Lesions | Microorganism Isolated |
|---|---|---|---|---|
| OU1 | Male | 9 | Bronchitis, renal congestion, hepatic steatosis, fibrosis splenitis and pericarditis. | *K. pneumoniae* and *E. coli* were isolated. |
| OU2 | Male | 37 | Emphysema, oedema, necrosis and bronchopneumonia, nephritis, chronic hepatitis, splenitis and pericarditis. | Hemolytic *S. aureus* was isolated from nasal swab. *K. pneumoniae* was isolated from abdomen liquid and kidney. *E. coli* was isolated from all organs, trachea swab, nasal and abdomen fluid. |

| | | | | |
|---|---|---|---|---|
| OU3 | Female | 6 | Hemorrhagic bronchitis, renal congestion, hepatic steatosis, splenitis, congestion, hyperemia myocarditis and endocarditis. | *K. pneumoniae* and *P. fluorescent* were isolated from lung and liver. |

## 5.0 Discussion

It is all-important to incorporate the aspects of wildlife conservation, livestock infectious diseases and human health protection in one account [14]. Recently, this issue has raised significant concern in the conservation of endangered species, especially orangutan. Factors such as captivity and loss of dietary fiber in non-human primates in the sanctuary are believed to associate with loss of native microbiome and convergence toward the modern human microbiome [15]. In veterinary medicine, infection of *K. pneumoniae* is considered rare however some cases have been reported in several NHP species. This pathogen has been detected in the macaque, African green monkey, marmosets and owl monkeys [13]. The *Klebsiella* infection is usually diagnosed by isolating the organism from clinical samples or during necropsy procedures.

The observation of peritonitis, hepatic and pulmonary abscesses in this case study is consistent with cases of *K. pneumonia* among moustache tamarins; however, these animals did not show any clinical symptoms before death. In some cases, animals severely infected with *K. pneumoniae* may not manifest any sign of clinical symptoms as seen in lemurs from a zoo in Japan. In this study, the organism was isolated from all internal organs of OU1 which is similar to cases of pigeons and snakes observed in Leipzig Zoo Germany in year 2003 and 2004. Furthermore, the necropsy findings including liver, hepatic and pulmonary abscesses of all three orangutans in this study were similar to *K. pneumoniae* infection in macaques (*Macaca mulatta*) as reported by Keesler et al. [13]. The yellowish and thick fluid was observed in the abdomen cavity of the macaques during the necropsy procedure suggesting the suppurative peritonitis. Moreover, the pleural surface of the macaque lung was covered with fibrin and filled with neutrophils indicating inflammation. Gross histopathological results of the orangutans OU2 and OU3 are also comparable to a case report related to a colony of chimpanzees that developed inflated and firm lung lobes with the presence of yellow-white foci throughout the parenchyma cells seen during necropsy.

Sporadic infection of *K. pneumoniae* has been associated with sudden death and various clinical symptoms such as prostration, fever, cough, mucopurulent discharge, pneumonia, peritonitis and sepsis in marmoset's research colonies [16]. The symptoms shown by the orangutans from BMOUI also similar to the symptoms shown by captivated dusky leaf monkeys from a conservation center in Malaysia. The animals showed difficulty in breathing, reduction in food intake and lethargy before death. The pathological findings of the seven animals presented lesions as bronchopneumonia, hemorrhagic hepatitis and splenitis [7]. Similar symptoms also were observed in feline

cases where the animals showed high body temperature, respiratory distress, loss of appetite, frequent sneezing, severe nasal discharge and mucus production [17]. Moreover, Keesler et al. [13] added that the infection is already severe whenever the clinical symptoms appear thus delaying the treatment. *K. pneumoniae* was described to be secondary infection after pneumonia in most bacteremia cases as detected in Hainan black goat in China [12]. The bacteria were retrieved from spleen and lung of the animal. The organs were congested and in edema state which were very similar to condition observed in vital organs of the orangutans from BMOUI.

The treatment of *K. pneumoniae* infection may be challenging since it has been associated with multi-drug resistant pathogens, thus increasing the possibility of treatment failure in humans and animals. Moreover, if the infected animals were asymptomatic thus it would significantly increase the fatal risk [18]. This pathogen also has been described to be unsusceptible to the most widely used antibiotics such as β-lactams and cephalosporin [18]. In 2021, a few of *K. pneumoniae* isolates have reported to be resistant against gentamicin and trimethoprim antibiotics. The current recommendation of antibiotics for *K. pneumoniae* treatment in animals includes azithromycin, co-trimoxazole, penicillin and cephalosporin [7]. Previously, an infant orangutan in BMOUI that was infected with URTI based on the clinical symptoms and vital signs was successfully treated with ceftriaxone. Otherwise, the actual cause of infection was not investigated [19]. Meanwhile recently, hvKP strain has been successfully recovered from the vital organs including in the nerve system of African marmosets in Thailand. The retrieved isolates showed intermediate susceptibility to co-amoxiclav antibiotics otherwise sensitive to amikacin, cephalexin, doxycycline, gentamicin and enrofloxacin. Surprisingly, the deceived marmosets did not show any symptom beforehand and all the four marmosets were found dead in the cages with the evidence of salivary foam in their mouth.

Owing to the evidence that *K. pneumoniae* has been successfully isolated from variety of environmental sources as soil, water and vegetation indicated that contaminated environment sources contributed to the *K. pneumoniae* infection. Thus, hygiene strategy must be implemented that only treated water must be used in feeding and bathing of the animals in order to control the infection. Moreover, this organism also has been isolated from contaminated animal food [20]. There was evidence that the bacteria were retrieved from undercook food and fresh vegetables and fruits. This finding may correlate to the fact that the captive orangutans consumed fruits and vegetables provided by humans in the sanctuary [4]. Other potential source of *K. pneumoniae* infection was identified come from the bedding of the animals such as sand, sawdust and straw and facilitated by aerolization factor hence frequent bedding changing despite of proper management of domestic waste disposal were suggested to maintain the health of the animals. In 2019, Marques *et al*. firstly reported the presence identical colony of *K. pneumoniae* in the fecal of co-inhabiting human and dog indicated the zoonotic transmission occurred within the household. This finding was supported by a study done by researchers as they demonstrated *K. pneumoniae* retrieved from companion animals were observed to resemble traits as in humans.

As a matter of fact, that animals can be potential reservoirs of this pathogen and there are chances of transmit the infection to humans as well [8, 21]. It is well-known that non-human primates such as orangutan are biologically nearest relatives to humans in the taxonomy therefore increasing the risk of the infection. Further investigation should also be warranted regarding the involvement of human factors such as zookeepers or visitors close-contact which may lead to transmission of the pathogens to the animals in the sanctuary. Nadia et al. [22] investigated the risk of exposure between zookeepers and animals. They demonstrated significantly high value (44.4%) of *Leptospira* seroprevalence among participants in the study and ruled out that high probability of diseases transmission occurred. Thus, they suggested appropriate animal handling must be practiced in the center including the usage of personal protective equipment (PPE).

## 6.0 Conclusion & Recommendations

Since *K. pneumoniae* infection has recently become a burden to public health, it is crucial to better understand the etiology and epidemiology of this infection among animals, especially among endangered species. Even worse, this infection has been associated with the emergence of hypervirulent strains and resistance to most antimicrobial agents. Thus, it is very pertinent to have an insightful understanding of this infection in order to establish appropriate management.

**Acknowledgement**. We would like to offer our gratitude to Almarhum Tan Sri Datuk Mustapha Kamal Abu Bakar and the Board of Trustee of BMOUI Foundation who provided the opportunity to carry out research in BMOUI. We also would like to thank VRI Ipoh Perak for giving permission and sharing the necropsy results with us. The first author received scholarship from BMOUI Foundation and this study was funded by Malaysian Ministry of Education in Fundamental Research Grant Scheme for Research Acculturation of Early Career Researchers: 600-IRMI/FRGS-RACER5/3(101/2109).

## References

1. Mohamad, N. I., Zakaria, N.A., Dharmalingam, S., Deris, Z. Z. & Mohd Yusof, F. Z.: Surveillance of *Burkholderia* spp. in Bukit Merah Orang Utan Island (BMOUI), Perak, Malaysia. Korean Journal of Microbiology **58**(3), 142-149 (2022)
2. Kamaluddin, S. N., Matsuda, I. & Md-Zain, B. M.: Activity budget and postural behaviors in orangutans on Bukit Merah Orang Utan Island for assessing captive great ape welfare. Journal of Applied Welfare Science **25**(3), 244-255 (2022)
3. Abdullah, N. I., Dharmalingam, S. & Md-Zain, B. M.: A behavioural study on the Bornean orangutan (*Pongo pygmaeus*) in a semi-wild environment at Bukit Merah Orang Utan Island, Perak. Journal of Sustainable of Natural Resources **2**(2), 22-30 (2021)
4. Hayashi, M., Kawakami, F., Roslan, R., M. Hapiszudin, N., & Dharmalingam, S.: Behavioral studies and veterinary management of orangutans at Bukit Merah Orang Utan Island, Perak, Malaysia. Primates **59**, 135-144 (2018)

5. Dayaram, A., Seeber, P. A. & Greenwood, A. D.: Environmental Detection and Potential Transmission of Equine Herpesviruses. Pathogens **10**(423), 1-10 (2021)
6. Mir, A. Q., Dua, K., Singla, L. D., Sharma, S. & Singh, P. M.: Prevalence of parasitic infection in captive wild animals in Bir Moti Bagh Mini Zoo (Deer Park), Patiala, Punjab. Veterinary World **9**(6), 540-543 (2016)
7. Dorothy, K. S., Shafarin, M.S., Geethamalar, S. & Roseliza, R.: A pathological case of *Klebsiella pneumoniae* infection in a colony of dusky leaf monkey (*Trachypithecus obscurus*). Malaysian Journal of Veterinary Research **10**(2), 68-78 (2019)
8. Effendi, M. H., Bintari, I. G., Aksono, E. B. & Hermawan, I. P.: Detection of blaTEM gene of *Klebsiella pneumoniae* isolated from swab of food-producing animals in East Java. Tropical Animal Science Journal **41**(3), 174-178 (2018)
9. Lee, D., Oh, J. Y., Sum, S. & Park, H. M.: Prevalence of antimicrobial resistance in *Klebsiella* species isolated from clinically ill companion animals. Journal of Veterinary Science **22**(2), e17 (2021)
10. Hwang, J. H., Handigund, M., Hwang, J. H., Cho, Y. G., Kim, D. S. & Lee, J.: Clinical features and risk factors associated with 30-day mortality in patients with pneumonia caused by Hypervirulent *Klebsiella pneumoniae* (hvKP). Annals of Laboratory Medicine **40**(6), 481-487 (2020)
11. Hwang, J. H., Handigund, M., Hwang, J. H., Cho, Y. G., Kim, D. S. & Lee, J.: Clinical features and risk factors associated with 30-day mortality in patients with pneumonia caused by Hypervirulent *Klebsiella pneumoniae* (hvKP). Annals of Laboratory Medicine **40**(6), 481-487 (2020)
12. He, M., Li, H., Zhang, Z., Jiang, J., Li, H., Yang, W., Cheng, Y., Gao, H., Chen, Q., Du, Li., Chen, S., Man, C. & Wang, F.: Microbiological characteristics and pathogenesis of *Klebsiella pneumoniae* isolated from Hainan black goat. Advances in Veterinary Clinical Microbiology **9**(9), 471 (2022)
13. Keesler, R. I., Colagross-Schouten, A., & Reader, J. R.: Clinical and pathologic features of spontaneous *Klebsiella pneumoniae* infection in 9 rhesus macaques (*Macaca mulatta*). Comparative Medicine **70**(2), 183-189 (2020)
14. Michel, A. L., Heerden, H. V., Crossley, B. M., Dahouk, S. A., Prasse, D. & Rutten, V.: Pathogen detection and disease diagnosis in wildlife: Challenges and opportunities. Revue Scientifique et Technique **40**(1), 105-118 (2021)
15. Clayton, J. B., Vangay, P., Huang, H., Ward, T., Hillmann, B. M., Al-Ghalith, G. A., Travis, D. A., Ha, T. L., Bui, V. T., Vo., V. M., Cabana, F., Nadler, T., Toddes, B., Murphy, T., Glander, K. E., Johnson, T. J. & Knights, D.: Captivity humanizes the primate microbiomes. The Proceedings of the National Academy of Sciences (PNAS) **113**(37), 10376-10381 (2016)
16. Guerra, J. M., de A. Fernandes, N. C. C., dos Santos, A. L. M., de Souza Pereira Barrel, J., Petri, B. S. S., Milanelo, L., Tiba-Casas, M. R., Liserre, A. M., Gonçalves, C. R., Sacchi, C. T., Catão-Dias, J. L., & Camargo, C. H.: Hypervirulent *Klebsiella pneumoniae* as unexpected cause of fatal outbreak in captive marmosets, Brazil. Emerging Infectious Diseases **26**(12), 3039-3043 (2020)
17. Aslam, M. W., Lau, S. F., Radzi, R., Omar, S., Kaka, U. & Ahmed, I.: Clinicopathological and radiological features of cats presented with infectious respiratory disease signs: A focus on *Rhodococcus equi* and *Klebsiella pneumoniae*. *Microorganisms* **11**(3), 737.
18. Malachowa, N., Kobayashi, S. D., Porter, A. R., Freedman, B., Hanley, P. W., Lovaglio, J., Saturday, G. A., Gardner, D. J., Scott, D. P., Griffin, A., Cordova, K., Long, D., Rosonke, R., Sturdevant, D. E., Bruno, D., Martens, C., Kreiswirth, B. N. & DeLeo, F.

R.: Vaccine protection against multidrug-resistant *Klebsiella pneumoniae* in a nonhuman primate model of severe lower respiratory tract infection. mBio **10**(6), e02994-19 (2019)
19. Dharmalingam, S.: Respiratory tract infection in infant orangutan (*Pongo pygmaeus*) at Orang Utan Island Bukit Merah Perak Malaysia. Pyrex Journal of Medicine and Medical Science **3**(2), 5-9 (2016)
20. Koster, S. D., Ruiz, J. P. R., Rajakani, S. G., Lammens, C., Glupczynski, Y., Goossens, H. & Xavier, B. B.: Diversity in the characteristics of *Klebsiella pneumoniae* ST101 of human, environmental and animal origins. Frontiers in Microbiology **13**, 838207 (2022)
21. Marques, C., Belas, A., Aboim, C., Cavaco-Silva, P., Trigueiro, G., Gama, L.T. & Pomba, C.: Evidence of sharing of *Klebsiella pneumoniae* strains between healthy companion animals and cohabiting humans. Journal of Clinical Microbiology **57**(6), e01537-18 (2019)
22. Nadia, A. S., Zain, B. M., Dharmalingam, S., Fairuz, A., & Hani-Kartini, A.: Serological survey of Leptospirosis in high-risk rangers and wild animals from ex-situ captive centers. Tropical Biomedicine **36**(2), 443-452 (2019)