A PATHOLOGICAL CASE OF *Klebsiella pneumoniae* INFECTION IN A COLONY OF DUSKY LEAF MONKEY (*Trachypithecus obscurus*)

DOROTHY K.S.[1]*, SHAFARIN M.S.[2], S. GEETHAMALAR[1] AND ROSELIZA R.[1]

1 Veterinary Research Institute, 59 Jalan Sultan Azlan Shah, 31400 Ipoh, Perak
2 Makmal Veterinar Wilayah Tengah Salak Tinggi, Bandar Baru Salak Tinggi, 43900 Sepang, Selangor
* *Corresponding author:* dotty77@yahoo.com

ABSTRACT. Seven baby dusky leaf monkeys from the age of one to six months from a colony of eleven died over a period of two weeks, with the predominant finding of gram-negative bacterial septicaemia despite clinical intervention. These animals had lethargy, pale mucosae, and dehydration before death. Necropsy was conducted to investigate the cause of death at the Pathology Section of Veterinary Research Institute (VRI) Ipoh. In general, the animals were observed to be very drawn and anorexic. Numerous traumatic marks were observed all-round the body. Necropsy examination revealed severe bronchopneumonia and pericarditis. Peritonitis was also observed as well. Organ samples were submitted to laboratories for further diagnosis. Bacteriology examinations revealed positive findings for *Klebsiella pneumoniae* in all seven animals. However, virology and parasitology examinations revealed no other findings. This case of *Klebsiella pneumoniae* infection in dusky leaf monkey is currently unreported and is being documented for the first time in Malaysia. It further shows that this microorganism is becoming increasingly important as a cause of morbidity and mortality in captive monkeys. Veterinarians and animal handlers should be aware of this infection and its zoonotic implications.

Keywords: Klebsiella pneumonia, dusky leaf monkey, zoonotic

INTRODUCTION

Klebsiella pneumoniae is an important emerging pathogen in humans and animals. *Klebsiella pneumoniae* from the family Enterobacteriaceae, is a Gram-negative, aerobic, non-motile, rod-shaped encapsulated bacterium.

Klebsiella is among the five gram-negative pathogens most commonly encountered in hospital-acquired infections (Horan T. and Culver D., 1988), and *Klebsiella pneumoniae* is the most frequently occurring species, accounting for 75% to 86% of *Klebsiella* species reported (De La Torre *et al.,* 1985; Hansen D.S. *et al*., 1998; Watanakunakorn C., 1991).

The organism is an important public health concern because of its nosocomial infection and antimicrobial resistance (Podschun R. *et al.,* 1998; Gozalo A. *et al.,* 1991). The non-pathogenic strains are widely distributed in nature as free-living forms in the soil and water or as commensals in the intestinal tract of humans and animals,

constituting a normal faecal and oral microbiome in many non human primates (Old World and New World) (Pisharath HR *et al.*, 2005).

Non-human primates in captivity are susceptible to *Klebsiella*. Infection with *Klebsiella* spp. has been reported to cause pneumonia, meningitis, peritonitis, cystitis, and septicemia (Gozalo A *et al.*, 1991). The infection likely spreads between animals by aerosol and close contact and between cages on fomites, such as clothing, gloves, and scrapers (Ludlage E. *et al.*, 2003).

Klebsiella spp. is considered an opportunistic pathogens that cause diseases when an animal is stressed or exhibits an altered defense mechanism due to many factors (Gozalo A. *et al.*, 1991). Diarrhoea, hypothermia, pyrexia, and a painful abdomen are a few of the clinical signs seen with this infection in New World primates monkeys (Gozalo A *et al.*, 1991; Snyder, S. B. *et al.*, 1970). The organisms have also been isolated from clinical cases of cervicitis and metritis in mares, mastitis in cows, and wound infections, septicemia, and pneumonia in dogs (Pisharath H.R. *et al.*, 2005).

MATERIALS AND METHODS

Animals and management

Seven baby dusky leaf monkeys, from the ages of one to six months, from a conservation centre of eleven died over a period of two weeks. Clinical signs that were exhibited by all seven animals include difficulty in breathing, inappetence, diarrhoea, lethargy, pale mucous membrane and dehydration. Two out of seven animals were treated with azithromycin and probiotics. The other remaining five animals were not treated.

These monkeys are primarily folivorous, that is, a herbivore that specialises in eating leaves and thrives on consumption of flowers, shoots, seedlings, leaves and fruits. They also consume unripe fruits and plant leaves. The monkeys were maintained on vegetables like sweet potato, cabbage, lettuce, carrots, green beans, along with various soft fruits.

The monkeys are kept and taken care of in captivity along with their mothers. Upon death, the carcasses of these seven baby dusky leaf monkeys were sent to VRI for post-mortem to confirm the cause of death.

Post-mortem and laboratory findings

Necropsy was carried out on all 7 carcasses about 24 hours after death. Sample organs were submitted to virology, bacterialogy and parasitology laboratories of VRI for examination and diagnosis.

Samples of gross lesions were sent to the virology laboratory for the isolation and identification of viral infections. Organ samples were submitted to the parasitology laboratory for isolation and identification of internal parasites. Histopathology examination was also carried out. Culture of gross lesions for aerobic bacteria employing blood agar and MacConkey media were carried out at the bacteriology laboratory. Preliminary observations on bacteria isolates were colony morphology and appearance on Gram stain. Biochemical tests including motility, indole, and oxidase tests were used

to identify the bacteria isolates at the species level.

RESULTS

All seven baby dusky leaf carcasses were examined carefully during necropsy for both ante-mortem and post-mortem lesions. The lesions observed were presented in Table 1 and Table 2.

At the ulna-radial bone, the socket joint was chipped off. Traumatic wounds were also seen at the skull to the cervical vertebrae (from C1 – T2 bone).

Two monkeys had diffuse fibrinopurulent peritonitis with multifocal areas of serosal adhesions involving the lungs, liver, kidney, heart and spleen. One of these two monkeys were also observed to have a very obvious post-surgery suture

Table 1. Summary of the general examination findings

Identification number	Sex	Age (months)	Lesions
4158	2 males 1 female	1 month	Pale, gaunt, rashes all over body, pustules of skin at forehead, around mouth eyes. Swelling at the mouth. Bruises at all extremities. Dehydrated and swelling at anus region with yellowish bloody diarrhoeal stains.
4209	Male	6 months	Drawn and pale, anorexic, dehydrated. Post-surgery sutured wound site at abdominal cavity with five visible sutures.
4210	Male	1 month	Bruises observed from all extremities of head to toe. Visible blue black bruising marks on both hands and legs. Dehydrated and swelling at anus region with brownish diarrhoeal stains.
4412	Female	1 month	Dehydrated and swelling at anus region with yellowish bloody diarrhoeal stains. Visible blue black bruising marks. Haggard with ruffled fur. Alopecic at chest and abdominal cavity, reddening and pustules at abdomen, forehead, around mouth and eyes.
4413	Female	1 month	Visible blue black bruising marks. Haggard with ruffled fur. Reddening and pustules at abdomen, forehead, around mouth and eyes. Fracture and wounds at foreleg.

Table 2. Summary of the pathological findings and bacterial culture results

Identification number	Lesions	Microorganism Isolated
4158	Pericarditis, bronchopneumonia, multiple organ bacteraemia	*Klebsiella pneumoniae*
4209	Pericarditis, bronchopneumonia, multiple organ bacteraemia, enteritis, traumatic injuries marks, periitonitis, splenitis, nephritis	*Klebsiella pneumoniae*
4210	Pericarditis, bronchopneumonia, multiple organ bacteraemia, enteritis, traumatic injuries marks, hepatitis, cystitis, splenitis	*Klebsiella pneumoniae*
4412	Pericarditis, bronchopneumonia, multiple organ bacteraemia, enteritis, severe multiple traumatic injuries marks, hepatitis, cystitis, meningitis, splenitis, gastritis	*Klebsiella pneumoniae*
4413	Pericarditis, bronchopneumonia, multiple organ bacteraemia, enteritis, severe multiple traumatic, injuries marks, hepatitis, cystitis, meningitis, peritonitis, gastritis, splenitis, nephritis	*Klebsiella pneumoniae*

site at its stomach. In addition, these seven animals had moderate to severe splenitis, pericarditis, nephritis, and enteritis.

Cystitis was also noted in one of the monkeys also. When the bladder was emptied, bloody urine was collected. Urinalysis results revealed RBC sediments and leukocytes in the sample indicating infection.

Bacteria cultures from the peritoneal exudates and cut sections of organs such as lungs, liver, kidney, heart and spleen from all seven dusky leaf monkeys revealed growths of *Klebsiella pneumoniae*.

Microscopic examination of samples from all the seven monkeys found large numbers of rod shaped bacteria in the lungs, liver, spleen, heart, kidneys with mild to moderate hypertrophy of endothelial

Table 3. Summary of the Biochemical Test and Identification of *Klebsiella pneumoniae* on bacterial culture.

Samples Submitted	Biochemical Test	Klebsiella pneumoniae
Lungs, Liver, Kidney, Heart, Spleen, Peritoneal exudate	**Characteristics**	
	Capsule	positive
	Catalase	positive
	Simmons Citrate	positive
	Gas	positive
	Gram Staining	negative
	Indole Production	negative
	Triple Sugar Iron (TSI) gas	O/A
	Hydrogen Sulfide	negative
	Methyl Red (MR)	negative
	Voges Proskauer (VP)	positive
	Urease Hydrolysis	positive
	Fermentation of	
	Dulcitol	positive
	Xylose	positive
	Sucrose	positive
	Malonate Utilization	positive
	Enzymatic Production	
	Arginine Dihydrolase	negative
	Lysin Decarboxylase	negative
	Ornithine Decarboxylase	negative
	Esculin Hydrolysis	positive

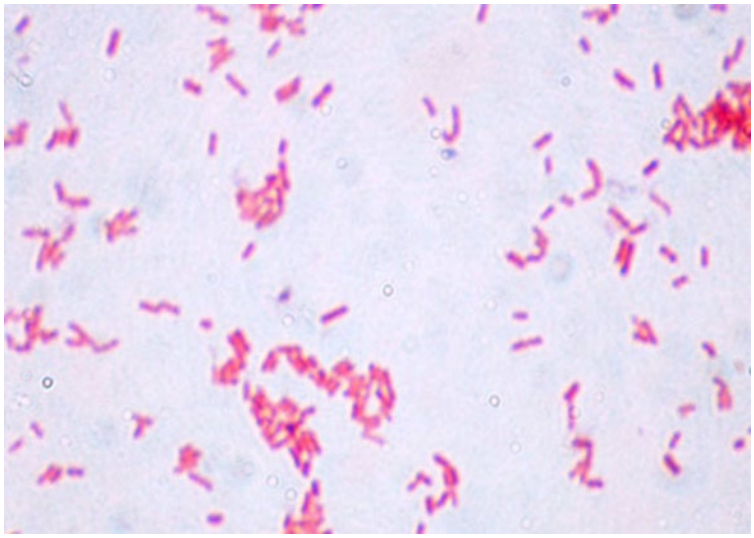

Figure 1. Bacterial rods were present within numerous cut sections of organs such as lungs, liver, kidney, heart and spleen. Giemsa stain imparts a purple color to the polysaccharide capsule of *Klebsiella* sp. (Giemsa; original magnification ×400).

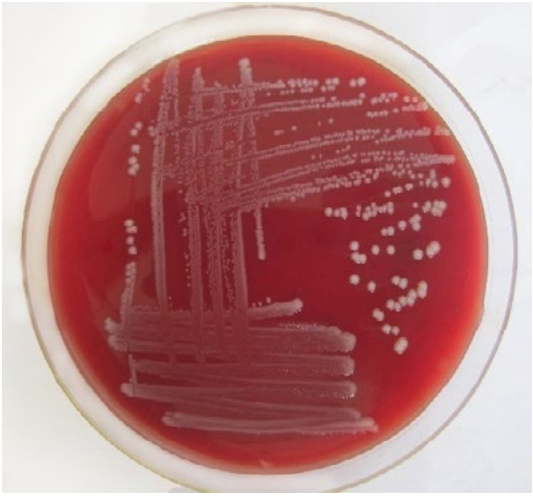

Figure 2. *Klebsiella pneumoniae* showing non haemolytic grey-white, mucoid colonies on Blood Agar.

Figure 3. *Klebsiella Pneumoniae* showing non haemolytic grey-white, mucoid colonies on lactose pink colonies on Mc Conkey Plate.

Figure 4. A Dusky Leaf Monkey before performing necropsy. Traumatic wound marks all over its body.

Figure 5. At ulna-radial bone, showing joint was chipped and surrounded with traumatic wound at the muscles surrounding.

Figure 9. Puncture wounds were also observed at both hands of these baby dusky leaf monkeys.

Figure 6. Fractures at its entire left foreleg with its posterior metacarpus totally crushed.

Figure 7. Several puncture wounds were observed at the feet and toes of a baby dusky leaf monkey.

Figure 8. Puncture wounds were also observed at both hands of these baby dusky leaf monkeys along the vein.

Figure 10. One baby monkey was also observed with a post-surgery suture site at its stomach which had undergone suture breakdown in the interior muscle layer.

Figure 11. Pustules of skin at forehead, chest, around mouth and eyes. Swelling at the mouth. Bruises at all extremities.

Figure 12. Bruising and traumatic wounds were also observed at the skull to the cervical vertebrae (from C1 – T2 spinal bone).

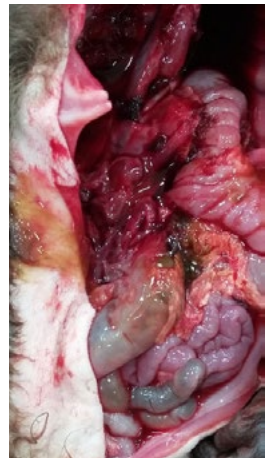

Figure 13. Serosal adhesions were observed surrounding the lungs, liver, kidney, heart and spleen.

Figure 14. Diffuse fibrinopurulent peritonitis with multifocal areas of serosal adhesions involving the lungs, liver, kidney, heart and spleen.

Figure 15. Peritoneal exudate that was collected from the peritoneal region.

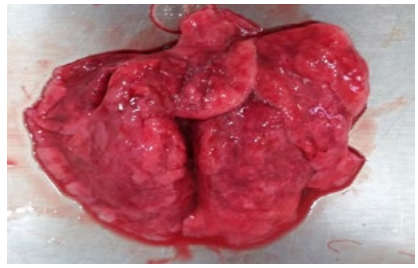

Figure 16. Bronchopneumonia were observed in all seven monkeys.

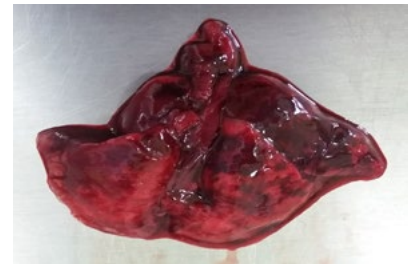

Figure 17. Haemorrhagic Hepatitis were also observed in all seven monkeys.

Figure 18. Haemorrhagic nephritis were observed in all seven monkeys.

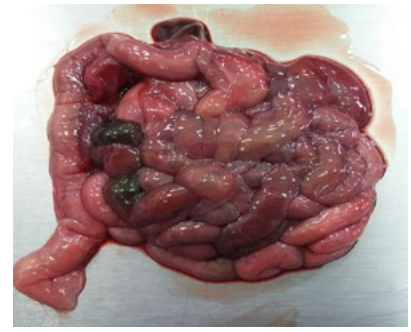

Figure 19. Severe Haemorrhagic Enteritis were observed in all seven monkeys.

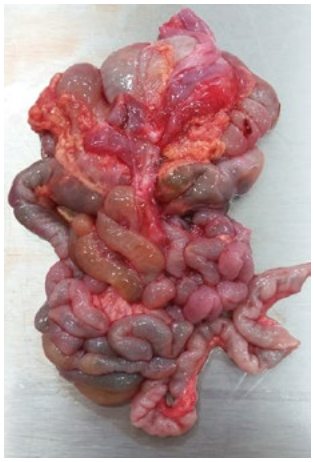

Figure 20. Severe haemorrhagic enteritis in the entire tract including the small and large intestines in all animals.

Figure 21. Internal intestinal walls showed severe haemorrhage with evidence of soft brownish stools and slight pettechial haemorrhage. This indicates that the monkeys were suffering from diarrhea.

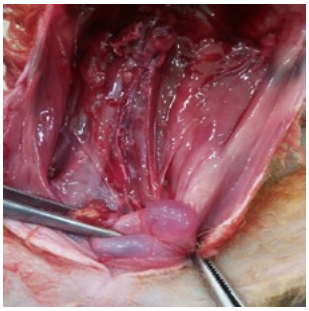

Figure 22. Cystitis was noted in this monkey also.

Figure 23. Urinalysis showed RBC sediments and leukocytes.

Figure 24. Severe Haemorrhagic Gastritis were also generally observed in these monkeys too.

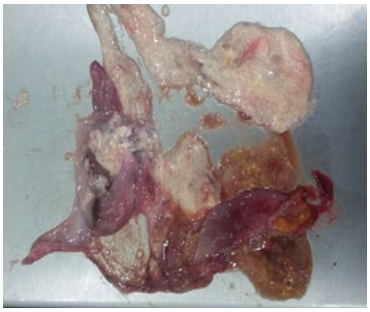

Figure 25. Stomach walls were very thin. The mucosal layers were haemorrhagic with traces of bloody milk curd.

Figure 26. Meningitis with severe haemorrhages at the cerebellum region were noted indicating signs of trauma.

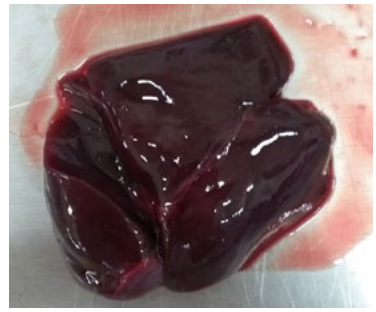

Figure 27. Severe Haemorrhagic Splenitis in all monkeys.

cells and minimal inflammation around the vessels. The bacilli were gram negative and 3 µm to 5 µm in length.

Biochemical tests following preliminary tests on bacteria isolates for colony morphology and appearance on Gram staining confirmed that the bacteria isolates showed growths of *Klebsiella pneumoniae*. The identification of the bacteria isolates at species level is presented in Table 3.

DISCUSSION

Non-human primates in captivity, including the Dusky Leaf are very susceptible to several zoonotic bacterial pathogens causing diseases in both humans and animals. These pathogens include the Gram-positive organisms, particularly the *Mycobacterium* spp. (Hariharan H., 1988; Henrich M. *et al.*, 2007; Brammer D.W. et.al., 1995), and Gram-negative organisms of the family *Enterobacteriaceae*. Infections due to enteric organisms include those caused by *Shigella* (Juan-Sallés C. *et al.*, 1999), *Yersinia pseudotuberculosis* (Buhles W.C. Jr *et al.*, 1981; Plesker R. *et al.*, 1992; Iwata T. *et al.*, 2010) and *Klebsiella pneumoniae* (Richard C., 1989).

The genus *Klebsiella*, are Gram-negative, rod-like shaped bacteria, non-motile and usually encapsulated, belonging to the family *Enterobacteriaceae* (Podschun R. *et al.,* 1998; Simmons J *et al.*, 2012). *Klebsiella pneumoniae* is associated with significant morbidity and mortality in NHP housed in captivity.

Infections with *Klebsiella* spp. have been reported to occur in Old and New World monkeys causing pneumonia, meningitis, peritonitis, cystitis, and septicemia (Gozalo A. *et al.*, 1991; Marina G. Bueno *et al.*, 2015). *Klebsiella pneumoniae* has also been reported to usually exhibit diffuse fibrinopurulent bronchopneumonia, suppurative bronchitis, and pleuritis, with the lung presenting multifocal necrosis and exudation with alveolar congestion, hemorrhage, and oedema (Simmons J. *et al.*, 2012). In this study, the pathological findings are consistent with these references.

Klebsiella spp. have two common habitats: the mucosal surfaces of mammals and the environment. In the environment, it can be found at the water surface, in sewage, soil and on plants (Simmons J. *et al.*, 2012; Hartman L.J. *et al.*, 2009). The natural nature of this organism being widely distributed as free-living forms in both soil and water or as commensals in the intestinal tract of humans and animals makes it an easy infection to spread if not managed properly. However situations that trigger stress, such as transportation, quarantine, malnutrition, and overcrowding, seem to predispose animals to the disease (Simmons J. *et al.*, 2012).

The diseases caused by *Klebsiella pneumoniae* infection vary depending on the host specie. Pneumonia and septicemia have been associated with outbreaks in humans and NHP (Richard C., 1989). *Klebsiella pneumoniae* is also known to be an important emerging nosocomial human pathogen (Gozalo A. *et al.*, 1991). Consequently making it a relevant and important public health issue particularly because of its antimicrobial resistance (Podschun R. *et al.* 1998).

Treatment, tender loving care and close monitoring by veterinary personnel is critical

and crucial for the survival of young primates that have been subjected to any major stress factor such as transport and surgery. Empiric antibiotic therapy with azithromycin, trimethoprim/sulfamethoxazole, penicillin, or cephalosporin (either of the latter two in combination with an aminoglycoside) generally, is indicated. Intensive nursing and other supportive therapy, such as fluid and oxygen administration, may also aid recovery in select cases. As for this case, all the treatment and care was also given as described above. Azithromycin, fluids and probiotics were administered to these seven baby dusky leaf monkeys within the two-week period but the treatment was unsuccessful.

Generally, Klebsiella infection caused by *Klebsiella pneumonia in* wild life have been widely reported worldwide not only in the Old World Non Human Primates and Non Human Primates (NHP) but in many other species as well. This is because the organism itself is widely distributed in nature as free-living forms in the soil and water or as commensals in the intestinal tract of humans and animals making it an opportunistic pathogen that cause diseases when there is a stress factor involved (Pisharath H.R. *et al.*, 2005; Simmons J. *et al.*, 2012; Gozalo A. *et al.*, 1991)

The case in this study demonstrates the importance of monitoring the health condition of animals undergoing any form of stress such as transportation, quarantine, malnutrition and overcrowding. Particular attention should also be focused on wildlife habitats that are known to be at the same area as humans (Richard C., 1989). *Klebsiella* infection tends to occur in these situations, causing changes in the bacteria host balance and favoring the emergence of disease (CPSG, 2003).

This fatal case of *Klebsiella pneumoniae* infection in dusky leaf monkey is documented for the first time in Malaysia. It further shows that this organism is becoming increasingly important as a cause of morbidity and mortality in captive monkeys. Veterinarians and animal handlers should be aware of this infection and its zoonotic implications. Caretakers have had a history of an upper respiratory infection. Infants that are hand reared frequently have also been observed to have been presented with aspiration pneumonia from bottle feeding. Therefore, adequate history on presentation of case is very important.

REFERENCES

1. Brammer D.W., O'Rourke C.M., Heath L.A., Chrisp C.E., Peter G.K. and Hofing G.L. (1995). *Mycobacterium kansasii* Infection in squirrel monkeys (*Saimiri sciureus sciureus*). *Journal of Medical Primatology,* **24:** 231-235.
2. Buhles W.C. Jr, Vanderlip J.E., Russell S.W. and Alexander N.L. (1981). *Yersinia Pseudotuberculosis* infection: Study of an epizootic in squirrel monkeys. *Journal of Clinical Microbiology,* **13:** 519-525.
3. Conservation Planning Specialist Group (CPSG). (2003). *Animal movements and disease risk: A Workbook,* 5th ed. Armstrong D., Jakob-Hoff R. and Seal U.S. (eds). CPSG, MN USA.
4. de la Torre M.G., Romero-Vivas J., Martínez-Beltrán J., Guerrero A., Meseguer M. and Bouza E. (1985). Klebsiella bacteremia: an analysis of 100 episodes, *Reviews of Infectious Diseases,* **7(2):** 143-150. doi: 10.1093/clinids/7.2.143
5. Du Y., Luo J., Wang C., Wen Q., Duan M., Zhang H. and He H. (2014). Detection of drug-resistant *Klebsiella pneumonia* in Chinese hares (*Lepus sinensis*). *J Wildl Dis* **50(1):** 109-112. doi: 10.7589/2013-03-059
6. Gozalo A. and Montoya E. (1991). *Klebsiella pneumoniae* infection in a New World Non Human primate. *Laboratory Primate Newsletter* **30(2):** 13-19.

7. Hansen D.S., Gottschau A., and Kolmos H.J. **(**1998). Epidemiology of *Klebsiella* bacteraemia: a case control study using *Escherichia coli* bacteraemia as control. *J. Hosp. Infect.* **38:** 119-132.
8. Hariharan H. (1988). Isolation of *Mycobacterium bovis* from monkeys. *Irish Veterinary Journal,* **42(1):** 5.
9. Hartman L.J., Selby E.B., Whitehouse C.A., Coyne S.R., Jaisle J.G., Twenhafel N.A., Burke R.L. and Kulesh D.A. (2009). Rapid real-time PCR assays for detection of *Klebsiella pneumoniae* with the rmpA or magA genes associated with the hypermucoviscosity phenotype: screening of nonhuman primates. *J Mol Diagn* **11(5):** 464-471.
10. Henrich M., Moser I., Weiss A. and Reinacher M. (2007). Multiple granulomas in three squirrel monkeys (*Saimiri sciureus*) caused by *Mycobacterium microti. Journal of Comparative Pathology,* **137:** 245-248.
11. Hong C.W. and Chan N.W. (2010). The potentials, threats and challenges in sustainable development of Penang National Park. *Malaysian Journal Of Environmental Management.* **11(2):** 95-109.
12. Horan T., Culver D., Jarvis W., Emori G., Banerjee S., Martone W. and Thornsberry C. (1988). Pathogens causing nosocomial infections. *Antimicrob. Newslett.* **5:**65-67.
13. Iwata T., Une Y., Lee K., Nakamura S., Taniguchi T. and Hayashidani H. (2010). Seroepidemiological survey of pathogenic Yersinia in breeding squirrel monkeys in Japan. *Journal of Veterinary Medical Science,* **72(8):** 981-984.
14. Juan-Sallés C., Vergés J. and Valls X. (1999). Shigellosis in a squirrel monkey: a clinical history. *Veterinary Record,* **145:**528-529.
15. Ludlage E. and Mansfield K. (2003). Clinical care and diseases of the common marmoset (*Callithrix jacchus*). *Comp. Med.* **53:** 369-382.
16. Bueno M.G., Iovine R.O., Torres L.N., Catão-Dias J.L., Pissinatti A., Kierulff M.C., Carvalho V.M. (2015). Pneumonia and bacteremia in a golden-headed lion tamarin (*Leontopithecus chrysomelas*) caused by *Klebsiella pneumonia* subsp. pneumoniae during a translocation program of free-ranging animals in Brazil. *Journal of Veterinary Diagnostic Investigation* **27(3):** 387-391.
17. Pisharath H.R., Cooper T.K., Brice A.K., Cianciolo R.E., Pistorio A.L., Wachtman L.M., Mankowski J.L. and Newcomer C.E. (2005). Septicemia and peritonitis in a colony of common marmosets (*Callithrix jacchus*) secondary to *Klebsiella pneumoniae* infection. *Contemp Top Lab Anim Sci* **44(1):** 35-37.
18. Podschun R. and Ullmann U. (1998). *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. *Clin Microbiol Rev* **11:** 589-603.
19. Plesker R. and Claros M. (1992). Spontaneous *Yersinia pseudotuberculosis* infection in a monkey colony. *Zentralblblatt Furveterinarmedizin, Reihe B. Journal of Veterinary Medicine. Series B*, **39(3):** 201-208.
20. Richard C. (1989). Epidemiology of *Klebsiella pneumoniae* infections in 2 Colonies of squirrel monkeys and lemurs. *Bulletin de la Societe de Patholgie Exotiqueet de ses Filiales (Paris).* **82:** 458-464.
21. Good R.C. and May B.D. (1973)." Respiratory pathogens in monkeys," Infect Immunity, **3(1):** 87-93.
22. Scott G.B.D. (1992). *Comparative primate pathology.* Oxford, UK: Oxford University Press, 304 pp
23. Simmons J. and Gibson S.V. (2012). Bacterial and mycotic diseases on nonhuman primates. In: *Nonhuman Primates in Biomedical Research. Vol. 2, Diseases*. 2nd ed. Abee C.R. *et al.* (eds.). London, UK: Academic Press, pp 105-172.
24. Snyder S.B., Lund J.E., Bone J., Soave O.A., and Hirsch D.C.. (1970). A study of *Klebsiella* infections in owl monkeys (*Aotus trivirgatus*). *J. Am. Vet. Med. Assoc.* **157(7):** 1935-1939.
25. Watanakunakorn C. (1991). *Klebsiella* bacteremia: a review of 196 episodes during a decade (1980–1989). *Scand. J. Infect. Dis.* **23:** 399-405.

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