Jurnal Veteriner
pISSN: 1411-8327; eISSN: 2477-5665
Terakreditasi Nasional, Dirjen Penguatan Riset dan Pengembangan,
Kemenristek Dikti RI S.K. No. 36a/E/KPT/2016

Juni 2022 Vol. 23 No. 2 : 211 - 216
DOI: 10.19087/jveteriner.2022.23.2.211
online pada http://ojs.unud.ac.id/index.php/jvet

Clinical Findings and Bacterial Identification in Eight Pythons with Respiratory Disorders in Bali

(*TEMUAN KLINIS DAN IDENTIFIKASI BAKTERI PADA DELAPAN ULAR SANCA DENGAN GANGGUAN RESPIRASI DI BALI*)

Puveanthan Nagappan Govendan[1], Steven Dwi Purbantoro[1], Erika[1], Yedija Putra Kusuma Wardana Rumbay[1], Aida Louise Tenden Rompis[2]*

[1]Undergraduate students Faculty of Veterinary Medicine,
[2]Laboratorium of Veterinary Microbiology,
Faculty of Veterinary Medicine, Udayana University,
Jl. Sudirman, Sanglah, Denpasar, Bali, Indonesia 80234
*Email: aida.lt.rompis@gmail.com

ABSTRACT

The study was aimed to identify bacteria associated with upper respiratory disorders in pythons. Epiglottis-tracheal swabs were collected aseptically from eight pythons (*Malayopython reticulatus*, *Python bivittatus*, and *Python regius*) with respiratory disorders. Common clinical signs were presented with frothy nasal discharge, nasal discharge, wheezing, mild coughing, crackle sound and star-gazing. Samples were cultured and sub-cultured on Blood Agar (BA) and MacConkey Agar (MCA). Colonies were identified by performing Gram staining, standard biochemical and phenotypic tests procedures. One colony was identified using API 20NE kit. Of all seven different colonies that were previously isolated, *Neisseria sp.*, *Escherichia coli*, *Staphylococcus sp.*, *Klebsiella sp.*, and *Burkholderia cepacia* were identified. We suggest that bacterial infections among captive pythons could be closely related to the immune system because of the commensal flora becoming pathogenic.

Keywords: bacteria; epiglottis-tracheal swab; python; respiratory disorders

ABSTRAK

Penelitian ini bertujuan untuk mengidentifikasi bakteri yang menyebabkan gangguan respirasi atas pada ular sanca. *Epilogittis-tracheal swab* dilakukan secara aseptis terhadap delapan ular sanca (*Malayopython reticulatus*, *Python bivittatus*, dan *Python regius*) dengan gangguan respirasi. Gejala klinis yang ditemukan pada pemeriksaan klinis adalah leleran hidung yang berbusa, leleran nasal, bersin-bersin, batuk ringan, *crackle sound*, dan *star-gazing*. Sampel dikultur dan disubkultur pada *Blood Agar* (BA) dan *MacConkey Agar* (MCA). Koloni diidentifikasi dengan cara pewarnaan Gram, prosedur biokimia standar dan uji fenotipik. Satu koloni diidentifikasi menggunakan API 20NE *kit*. Dari tujuh koloni yang telah diisolasi, *Neisseria sp.*, *Escheria coli*, *Staphylococcus sp.*, *Klebsiella sp.*, dan *Burkholderia cepacia* telah diidentifikasi. Kami menyimpulkan bahwa infeksi bakteri pada ular sanca terjadi karena keadaan *immunocompromised* dan menyebabkan pertumbuhan yang berlebihan pada flora komensal respirasi.

Kata-kata kunci: bakteri; *epiglottis-tracheal swab*; ular sanca; gangguan respirasi

INTRODUCTION

Herpetoculture has been growing rapidly in recent years (Ebani and Fratini, 2005; Bastos, 2012; Putranto *et al*., 2016). Python is a large snakes which are among the reptiles kept as exotic pets among the herpetoculture community. Python is one of the most commonly kept as pets, considered primitive snakes with two lungs. Diseases among captive reptiles are common and closely related to the husbandry.

Respiratory infection is a common finding in captive snakes and an important cause of morbidity and mortality (Hilf *et al*., 1990;

Uccellini *et al.*, 2014). Etiology of the respiratory disorder could be caused by a bacterial infection. Besides bacteria, parasites, protozoa, pentasomids, and ectoparasites have been known to be zoonotic agents for both pet reptiles and human (Mendoza-Roldan *et al*., 2020). Bacterial infections in the respiratory system in reptiles could be caused by immunocompromised conditions (Knotek and Jekl, 2015). Data on normal flora of snakes in captivity is scarce (Lukac *et al*., 2013). The most common pathogens isolated in respiratory-infected reptiles belong to the genera *Pseudomonas spp*., *Klebsiella spp*., *Proteus spp*., *Aeromonas spp*., *Salmonella spp*., and *Staphylococcus spp*. (Schumacher, 2003).

Bacterial infection are potential to be zoonotic pathogens from snakes (Mitchell, 2011; Romero *et al*., 2015). This bacterial pathogens can infect humans coming in contact with the infected snakes. Since the numbers of snakes are increasing day by day among the herpetoculture, the possible pathogens should be known (Ebani and Fratini, 2005; Mitchell, 2011; Bastos, 2012). *Salmonella* is one of the most well-known Gram-negative bacteria found in reptiles (Rabinowitz *et al*., 2007; Mitchell, 2011; Abba *et al*., 2016). The awareness should be rised to minimize such infections are possible to be transmited to humans.

Definitive diagnosis is important to maximize therapeutic success and microbiology test is one of the diagnostic methods which could be performed for the bacterial identification (Hernandez-Divers, 2008). However, there is no report about bacteriology studies among respiratory infected in captive pythons in Bali. Thus, we investigated the etiology of respiratory infection in pythons through bacterial identification.

RESEARCH METHODS

Eight captive pythons of three species (*Malayopython reticulatus* (n=3), *Python bivittatus* (n=1), and *Python regius* (n=4)) with clinical signs of respiratory disorder were collected from private keepers and samples were collected for in this study. All pythons had primary complaint of respiratory disorders. Both physical and clinical examinations were conducted and recorded. Data of sex, age (for captive-bred animals) and clinical findings are presented in Table 1.

A blunt spatula was used to open the oral cavity. Sample collections were performed from all snakes while being handled by one or two assistants. Samples were collected by performing epiglottis-tracheal swab by placing a sterile cotton swab in the epiglottis and rolled over several times before direct aerobic culturing onto Blood Agar (BA) and MacConkey Agar (MCA) (Purbantoro *et al*., 2018). Samples were incubated at 35-37 ^{o}C and evaluated for its growth in 18-24 hours (Jho *et al*., 2011; Purbantoro *et al*., 2018). After 18-24 hours, the colonies were evaluated and selected to be sub-cultured on the BA and MCA to get the pure colonies.

A negative and/or non-significant growth (NSG) culture result on both two media were exempted and considered negative. Bacterial isolation in some snakes yielded more than one colony. Bacterial identification was done by performing Gram staining, standard conventional biochemical and phenotypic tests procedures. One sample of *M. reticulatus* was identified using API microorganism identification (API 20NE kit, Bio Merieux, Marcy-l'Étoile, France) to identify the non-fermenting Gram-negative bacilli bacteria (Purbantoro *et al*., 2018). Isolates were identified to genera or species when possible.

RESULTS AND DISCUSSION

Results in Table 2 shows morphology and the scoring result of the colony number on each media and identification result. Three samples were considered negative. A total of five bacteria were identified from seven different colonies: *Neisseria sp*., *E. coli*, *Staphylococcus sp*., *Klebsiella sp*., and *Burkholderia cepacia* (Purbantoro *et al*., 2018) were isolated and identified in this study.

Respiratory infection could be diagnosed with anamnesis, inspection, auscultations, clinical signs, nasal or tracheal sample culture and radiology. Common clinical signs of respiratory distress are nasal discharge, wheezing, increased respiratory efforts, dyspnea, open-mouth breathing and stargazing (Schumacher, 2003; Purbantoro *et al*., 2018). All pythons in this study were presented with wheezing and bubbly nostrils. Other common signs were also sneezing and crackles sound during inhaling and exhaling. Less common signs were presented with mild coughing and star gazing. Infection in the respiratory system

Table 1. The signalement and clinical signs of eight (8) captive pythons in Bali.

Species	Age (years)	Sex	Clinical signs
Malayopython reticulates	U	F	wheezing, frothy nasal discharge
Phyton bivittatus (a)	4	F	Mild coughing, wheezing, sneezing, frothy nasal discharge
Phyton regius	5	F	Wheezing, sneezing, frothy nasal discharge, crackle sound
Phyton regius	4	F	Wheezing, sneezing, frothy nasal discharge, crackle sound
Phyton regius	5	F	Wheezing, sneezing, frothy nasal discharge, crackle sound
Phyton regius	3	F	Wheezing, sneezing, frothy nasal discharge, crackle sound, nasal discharge
*Malayopython reticulatus**	U	F	Wheezing, sneezing, frothy nasal discharge, crackle sound
Malayopython reticulatus	U	F	Wheezing, sneezing, frothy nasal discharge, crackle sound, star-gazing

Note: F: Female; M: Male; U: Unknown; a: albino; *data published in Purbantoro *et al.* (2018)

Table 2. The bacterial characteristics from eight (8) captive pythons in Bali.

BA	MCA	Identification result
Yellow (+)	Cream NLF (+++)	*Neisseria sp.*
Pinpoint (+++)	Negative	*Escherichia coli*
Negative	Negative	Negative
White (small) (+)	Greenish Mucoid (+++)	*Staphylococcus sp.*
Grey Mucoid (+++)	Negative	*Klebsiella sp.*
Pinpoint (++)	Negative	*Klebsiella sp.*
White (big) (++)	Pinpoint NLF	Unidentified
Pinpoint (++)	Negative	Unidentified
NSG	Negative	Negative
NSG	Negative	Negative
Pinpoint (+++)	Cream dry NLF	*Burkholderia cepacia**
Cream haemolytic (+)	NSG	*Escherichia coli*

Note: + shows the quantity of the colony; BA: Blood Agar; MCA: McConkey Agar; NSG: Non-significant Growth; NLF: non-lactose fermented. *Purbantoro *et al.* (2018)

among captive reptiles could cause high morbidity and mortality (Uccellini *et* al., 2014; Iannaccone *et al.*, 2017). Tracheal wash is a method that could be performed for the bacteriology sample cultures (Abbas, 2013; Schmidt *et al*., 2013). Studies from Schmidt *et al*. (2013) showed a high number of snakes with respiratory infection showed positive growth of bacterial from tracheal wash during bacteriology culture. Tracheoscopy could also be performed on snakes for microbiology sample collections (Knotek and Jekl, 2015).

Gram-negative bacterial pathogens are more commonly found in snakes compared to Gram-positive bacterial pathogens (Hanks *et al*., 2017; Panda *et al*., 2018). Bacterial pathogens are more common during colder seasons compared to hotter seasons (Blaylock, 2001). *Neisseria sp.* found in this study is known as a non-pathogenic oral flora in an Indian cobra (*Naja naja*) (Panda *et al.*, 2018). The study reported that the species of the genus *Neisseria* includes *N. sicca* and *N. flavescens*, which are classified as non-pathogenic *Neisseria* species. A study of

Plowman *et al.* (1987) assumed that *Neisseria sp.* could be transmitted through a bite puncture. *Neisseria sp.* was cultivated from the oral cavity (Panda *et al.*, 2018) from an Indian cobra (*N. naja*), bite wounds transmission from an oral cavity which was infected in a rhinoceros iguana (*Cyclura cornuta*) (Plowman *et al.*, 1987) and an Uromastyx lizard (*Uromastyx acanthinura nigriventris*) with an acute respiratory distress syndrome (Iannaccone *et al.*, 2017). Predisposition factor with the presence of *Neisseria sp.* could be related to the poor nutritional and metabolic status (Plowman *et al* ., 1987). This could have led the individual becomes immunocompromised and prone to be infected by these pathogens.

Staphylococcus sp., *Klebsiella sp.*, and *Pseudomonas sp.* (now known as *Burkholderia sp.*) are organisms commonly isolated from reptiles with respiratory infection (Hilf *et al.*, 1900; Schumacher, 2003; Benetka *et al.*, 2007; Plenz *et al.*, 2014; Hanks *et al.*, 2017). Those are known as commensal micro-floras from the upper airway in snakes (Hilf *et al.*, 1990; Schumacher, 2003). *Pseudomonas sp.* and *Staphylococcus sp.* was also cultured found from oral cavity among African snakes (Blaylock, 2001). Bacteria *E. coli* could be isolated from cases with signs of upper respiratory infection and pneumonia (Hilf *et al.*, 1990; Ebani and Fratini, 2005).

Commensal bacteria were assumed infecting the snakes by overgrowing and became pathogens under adverse conditions (Schumacher, 2003). Overgrowth of the bacteria perhaps occurred because snakes could be in compromised immune system condition (Plenz *et al.*, 2014). Compromised immune system occurs with chronic illness, poor nutrition, or other factors (improper husbandry) (Hilf *et al.*, 1990; Schumacher, 2003). The pathogens mainly occur in individuals with compromised immune state (Ebani and Fratini, 2005; Vanin *et al.*, 2013).

Snakes with respiratory distress had a significant greater number of Gram-negative bacilli (Santos *et al.*, 2008). As our previous study found, *B. cepacia* has an involvement to the respiratory tract in reptiles (Purbantoro *et al.*, 2018). Similar result of *B. cepacia* finding and similar clinical signs were also reported by the studies of, Santos *et al.* (2008) and Vanin *et al.* (2013). API system 20 NE was performed in one more case with positive results of *Burkholderia cepacia* (Ebani *et al.*, 2008).

Escherichia coli, a digestive normal flora, was assumed as a contaminant in this study. Swabs could be contaminated by oral commensals while submitting swab into trachea (Hernandez-Divers, 2008). However, there are possibilities of *E. coli* infecting snakes systemically. *Escherichia. coli* growing on cultures have been recorded in several cases when sample swabs were cultured from oral and nasal (Blaylock, 2001; Jho *et al.*, 2011; Benetka *et al.*, 2007). Contamination even with sterile cotton swabs is possible during sample collections.

All the findings in this study were also collected from oral swabs of snakes. These bacterias could be microflora of the snakes (Panda *et al.*, 2018). Stress, improper husbandry and cleanliness of the enclose all plays a huge role on the bacterial infections. During inhalation these bacterias could be inhaled causing the pathogens to multiply in the respiratory tract. Infected snakes are best to be isolated so spreading of the pathogens are minimized among the reptile collections.

Reptile owners, veterinarians, zoologist and zoo keepers should be informed about the risk of zoonotic possibilities of reptile-human transmission (Rabinowitz *et al.*, 2007; Ullmann *et al.*, 2016; Ebani, 2017). All pathogens identified in this study could be a potential pathogen to human health (Mitchell, 2007). Personal hygiene and cleanliness would reduce the risk of this infections (Abba *et al.*, 2017; Rabinowitz *et al.*, 2007; Mitchell, 2011).

CONCLUSION

Captive snakes among the herpetoculture are prone to bacterial infections. Respiration infection caused by bacterial pathogens are one of the most common problems in captive reptiles. *Neisseria sp.*, *E. coli*, *Staphylococcus sp.*, *Klebsiella sp.* and *Burkholderia cepacia* were identified by bacteriology isolation from the respiratory tract of the captive pythons among the herpetoculture in Bali.

SUGGESTION

For further efficiency, snakes with respiratory infection should be treated with the suitable antibiotic and needed further antibiotic sensitivity tests. Personal hygine should be at core to reduce the risk of zoonotic transmission of the pathogens from captive reptiles to humans.

REFERENCES

Abba Y, Ilyasu Y, Yusoff, Noordin MM. 2016. Bacterial co-infections in a captive *Python bivittatus* with septicemia. *Sokoto Journal of Veterinary Sciences* 14(2): 67-71. http://dx.doi.org/10.4314/sokjvs.v14j2.10

Abba Y, Ilyasu Y, Nordin MM. 2017. Isolation and identification of bacterial populations of zoonotic importance from captive non-venomous snakes in Malaysia. *Microbial Pathogenesis* 108: 49-54. http://dx.doi.org/10.1016/j.micpath.2017.04.038

Abbas MD. 2013. Diagnosis of ferlaviruses in snakes and characterization of isolates based on gene sequences. (*PhD Dissertation)* Stuttgart. University of Hohenheim Institute of Enviromental and Animal Hygine and Veterinary Medicine.

Bastos HM. 2012. Salmonella associated with snakes (suborder serpents). *Clinica Veterinaria Vila Sabrina*, Sao Paulo Pp. 81-98. http://doi.10.5772/30639

Baylock RSM. 2001. Normal oral bacterial flora from some southern African snakes. *Journal of Veterinary Research* 68: 175-182.

Benetka V, Grabensteiner E, Gumpenberger M, Neubauer C, Hirschmuller B, Mostl K. 2007. First report of an iridovirus (Genus *Ranavirus*) infection in a leopard tortoise (*Geochelone pardalis pardalis*). *Veterinary Medicine Austria* 95: 243-248.

Hernandez-Divers SJ. 2008. Snake Clinical Procedures and Diagnostics. *North American Veterinary Conference (NAVC) Conference Exotic-Reptiles* 1752-1756.5.

Ebani VV. 2017. Domestic reptiles as source of zoonotic bacteria: a mini review. *Asian Pacific Journal of Tropical Medicine*. http://doi.org/10.1016/j.apjtm.2017.07.020

Ebani VV, Fratini F. 2005. Bacterial zoonoses among domestic reptiles. *Annali della Facolta di Medicina Veterinaria* 58: 85-91.

Ebani VV, Fratini F, Ampola M, Rizzo E, Cerri D, Andreani E. 2008. *Pseudomonas* and *Aeromonas* isolates from domestic reptiles and study of their antimicrobial in vitro sensitivity. *Veterinary Research Communications* 32(Suppl 1): S195-S198. http://doi.101007/s11259-008-9160-9

Hanks LLH, Layton ML, Ossiboff RJ, Parker JSL, Dubovi EJ, Stenglein MD. 2017. Respiratory disease in ball python (*Python regius*) experimentally infected with ball python nidovirus. http://doi.org/10.1016/j.virol.2017.12.008

Hilf M, Wagner RA, Yu VL. 1990. A prospective study of upper airway flora in healthy boid snakes and snakes with pneumonia. *Journal of Zoo and Wildlife Medicine* 21(3): 318-325.

Iannaccone M, Rossi G, Magi GE, Campolo M. 2017. Acute respiratory distress syndrome in a uromastix (*Uromastyx accanthinura nigriventris*, 1820). *Journal of Exotic Pet Medicine*, http://dx.doi.org/10.1053/j.jepm.2017.05.007.

Jho YS, Park DH, Lee JH, Cha SY, Han JS. 2011. Identification of bacteria from the oral cavity and cloaca of snakes imported from Vietnam. *Laboratory Animal Research* 27(3): 213-217.

Knotek Z, Jekl V. 2015. Pulmonoscopy of snakes. *Veterinary Clinics: Exotic Animal Practice* http://dx.doi.org/10.1016/j.cvex.2015.04.007

Lukac M, Matanovic K, Barbic L, Seol B. 2013. Combination treatment of a pseudomonad abscess in a western black-tailed rattlesnake *Crotalus molossus molossus*: a case report. *Vet Med* 58(12): 637-640.

Mendoza-Roldan, JA, David M, Domenico O. 2020. Opinion Zoonotic Parasites of Reptiles: A Crawling Threat. *Trends in Parasitology* 36(8): 684

Mitchell MA. 2011. Zoonotic diseases associated with reptiles and amphibians: An update. *Vet Clin Exot Anim* 14: 439-456. http://doi.org/10.1016/j.cvex.2011.05.005

Panda SK, Padhi L, Sahoo G. 2018. Oral bacterial flora of Indian cobra (*Naja naja*) and their antibiotic susceptibilities. *Heliyon* 4: e01008. http://doi.org/10.1016/j.heliyon.2018.e01008.

Plenz B, Schmidt V, Grosse-Herrenthey A, Krüger M, Pees M. 2014. Characterisation of the aerobic bacterial flora of boid snakes: applicatin of MALDI-TOF mass spectrometry. *Veterinary Record* 10.1136/vr.102580: 1-7.

Plowman CA, Montali RJ, Phillips Jr LG, Schlater LK, Lowenstine LJ. 1987. Septicemia and chronic abscesses in iguanas (*Cyclura conuta* and *Iguana iguana*) associated with a *Neisseria* species. *Journal of Zoo Animal Medicine* 18(2-3): 86-93.

Purbantoro SD, Erika, Govendan PN, Rumbay YPKW, Rompis ALT. 2018. *Burkholderia cepacia* infection in *Python reticulatus*. *Proceeding of the 20th FAVA Congress & the 15th Kivnas PDHI, Bali*. November 1-3, 2018. Nusa Dua Badung, ***Bali*** – Indonesia

Putranto DI, Yuda P, Zahida F. 2016. Diversity of Imported Reptiles in Yogyakarta. *Biota* 1(3): 117-125.

Rabinowitz PM, Gordon Z, Odofin L. 2007. Pet-related infections. *American Family Physician* 76(9): 1314-1322.

Romero SB, Cizek A, Masarikova M, Knotek Z. 2015. Choanal and cloacal aerobic bacterial flora in captive green iguanas: a comparative analysis. *Acta Veterinaria Brno* 84: 19-24. http://doi.10.2754/avb201584010019

Santos KR, Takahira RK, Rall VLM, Calderon C, Sequerira JL, Silva RJ. 2008. Pulmonary, microbiological and hematological in *Crotalus durissus terrificus* (Serpentes, Viperidae) parasitized by nematodes of the genus *Rhabdias* (Nematoda, Rhabdiasidae). *Arquivo Brasileiro de Medicina Veterinaria e Zootecnia* 60(3): 667-674.

Schmidt V, Marschang RE, Abbas MD, Ball I, Szabo I, Helmuth R, Plenz B, Spergser J, Pees M. 2013. Detection of pathogens in Boidae and Pythonidae with and without respiratory disease. *Veterinary Record* 172:236 http://doi:10.1136/vr.100972

Schumacher J. 2003. Reptile respiratory medicine. *Veterinary Clinics: Exotic Animal Practice* 6: 213-231.

Uccellini L, Ossiboff RJ, de Matos REC, Morrisey JK, Petrosov A, Navarrete-Macias I, Jain K, Hicks AL, Buckles EL, Tokarz R, MacAloose D, Lipkin WI. 2014. Identification of a novel nidovirus in an outbreak of fatal respiratory disease in ball pythons (*Python regius*). *Virology Journal* 11(144): 1-6.

Ullmann LS, Dias-Neto RDN, Cagnini DQ, Yamatogi RS, Oliveira-Filho JP, Nemer V, Teixeira RHF, Biondo AW, Jr JPA. 2016. *Mycobacterium genavense* infection in two species of captive snakes. *Journal of Venomous Animals and Toxins including Tropical Diseases* 22:27 http://doi.10.1186/s40409-016-0082-7

Vanin S, Mazzariol S, Menandro ML, Lafisca, Turchetto M. 2013. Myiasis by *Megaselia scalaris* (Diptera: Phoridae) in a Python Affected by Pulmonitis. *Journal of Medical Entomology* 50(1): 209-211.