CLINICAL REPORT

Veterinary Research Forum. 2025; 16 (6): 361 - 364
doi: 10.30466/vrf.2024.2034234.4345
Journal Homepage: vrf.iranjournals.ir

Veterinary Research Forum

First case of serpentovirus infection in a ball python (*Python regius*) in Thailand: a case report with molecular characterization

Panithi Laoungbua[1]*, Nararat Laopichienpong[2], Apinya Longya[2], Lawan Chanhome[1], Taksa Vasaruchapong[1], Tanapong Tawan[1], Narongsak Chaiyabutr[2]

[1] Snake Farm, Queen Saovabha Memorial Institute, Bangkok, Thailand; [2] Department of Research and Development, Queen Saovabha Memorial Institute, Bangkok, Thailand.

Article Info

Article history:

Received: 02 July 2024
Accepted: 05 October 2024
Available online: 15 June 2025

Keywords:

Nidovirales
Respiratory tract infection
Snake

Abstract

The pet snake industry in Thailand has seen a significant rise in popularity, with the ball python (*Python regius*) becoming a frequently kept species. However, respiratory disease poses a notable health concern, and various viral pathogens, including serpentoviruses (formerly classified as nidoviruses), have been implicated. While serpentovirus infections have been reported globally in diverse snake species, no documented cases had previously been identified in Thailand. This case report describes a 9-month-old ball python presenting to the Reptile Science Clinic at the Queen Saovabha Memorial Institute in Bangkok, Thailand, with respiratory distress and emaciation. Despite veterinary intervention, the snake succumbed to the infection within two weeks. Post-mortem examination revealed marked mucus accumulation within the oral cavity and necrotic oral mucosa. Histopathological analysis demonstrated severe catarrhal pneumonia. Molecular investigations confirmed the presence of serpentovirus in the lung tissue of the affected python, with subsequent sequence analysis revealing close homology to known serpentoviruses in ball pythons. This report documents the first confirmed case of serpentovirus infection in a pet snake in Thailand.

Introduction

The pet snake industry has experienced a surge in popularity within Thailand,[1] with the ball python (*Python regius*) frequently presenting at the Reptile Science Clinic of Queen Saovabha Memorial Institute, Bangkok, Thailand, for veterinary care. Respiratory issues are a common complaint among these patients, with potential etiologies spanning from non-infectious to infectious agents, including bacteria, fungi, parasitic worms, and viruses.[2] Viral infections, in particular, represent a significant health risk to ball pythons. Notably, the taxonomy of nidoviruses has been revised to serpentoviruses in recent years,[3] and several viruses, encompassing serpentoviruses, paramyxoviruses, reoviruses, and adenoviruses, have been implicated in respiratory manifestations in snakes.[4] While reptilian ferlavirus infection has been documented in both wild and captive snakes, with viral genomes detected across various organs,[5] serpentovirus infection had not yet been reported in Thailand. However, recent years have witnessed a growing number of reports detailing serpentovirus infection in diverse snake species globally.[4,6-8]

Case Description

A male ball python (*P. regius*) aged 9 months was brought to the Reptile Science Clinic at the Queen Saovabha Memorial Institute in Bangkok, Thailand. Snake was purchased from private snake breeder. After being purchased, the snake was kept in acrylic 36.00 L plastic enclosure, and the wooden substrate was used as an enclosure ground. The tank is lacked of any sheltered areas for the snake to retreat to. The enclosure was kept in a room with a consistent temperature range of 25.00 to 34.00 ˚C year-round, illuminated by natural light. Five months post-acquisition, the owner noted the onset of respiratory difficulties, characterized by open-mouth breathing and clear nasal discharge. Additionally, wooden substrate was observed adhering to the snake's mouth, likely due to the excessive salivation. The snake also refused the pre-killed mice the owner offered for feeding, and the owner observed that the snake was becoming emaciated. Those signs occurred for 1 month without veterinary treatment and during the period the snake was brought to the

***Correspondence:**

Panithi Laoungbua. DVM
Snake farm, Queen Saovabha Memorial Institute, Bangkok, Thailand
E-mail: Panithi.l@redcross.or.th

clinic. Upon presentation, the snake weighed 874 g and exhibited copious oronasal mucus production, cyanosis of the oral mucous membranes, audible respiration, stomatitis, and epiglottitis. Outpatient management was initiated, consisting of ceftazidime (20.00 mg kg^{-1}, every 3 days, intra-muscular injection; Siam Bheasach, Bangkok, Thailand) for five times, vitamin C (20.00 mg kg^{-1}, every 3 days; intramuscularly; T.P. Drug Laboratories, Bangkok, Thailand).[9] In addition, the oral cavity and choana were routinely debrided of necrotic tissue and discharged to facilitate respiration. Despite therapeutic intervention, the snake succumbed approximately 2 weeks post-presentation. Following a brief period of freezing, the carcass was submitted to the clinic for post-mortem examination.

Gross examination revealed the accumulation of mucous secretions within the oral mucosa and choana. Additionally, areas of the maxillary mucosa exhibited thickening and necrosis (Fig. 1A). The lungs were notably thickened and edematous, with copious mucoid to caseous exudate filling the pulmonary cavities (Figs. 1B and C). No other significant gross lesions were observed in the remaining organs. Histopathological examination under a light microscope (Olympus CX23, Olympus Corporation, Tokyo, Japan) of formalin-fixed, paraffin-embedded lung tissue sections stained with Hematoxylin and Eosin demonstrated severe, diffuse faveolar congestion and marked catarrhal exudate within the faveolar spaces. Mild necrosis of the faveolar epithelium was also noted (Fig. 2). These findings supported a diagnosis of severe catarrhal pneumonia.

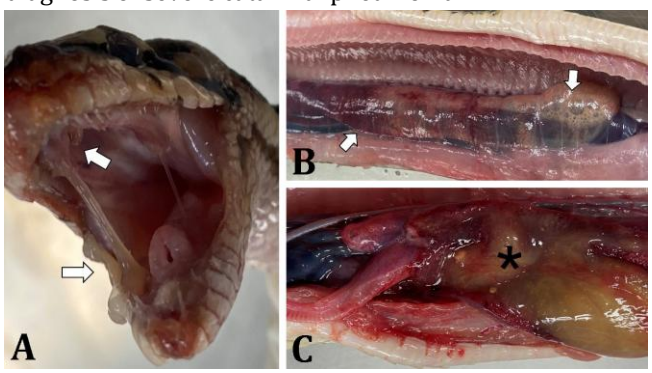

Fig. 1. Macroscopic findings of the deceased ball python (*Python regius*). **A)** Oral mucosa and choana passage were accumulated with mucous secretion (arrows); **B)** The vascular part of lung is thickened and swollen with noted frothy exudate(arrows); **C)** Noticeable abundant mucous to caseous discharge in the lung cavity (asterisk).

Tissue samples were collected during necropsy (liver, heart, kidney, and testis) and send to the Research and Development Department of Queen Saovabha Memorial Institute, Bangkok, Thailand for RNA extraction. The RNA was extracted from the snake tissue samples using Trizol reagent (Invitrogen, Carlsbad, USA) according to the manufacturer's instructions. Briefly, 100 mg tissue was frozen in liquid nitrogen, ground into a fine powder, and homogenized with 1.00 mL Trizol. The RNA was reverse transcribed into the complementary DNA (cDNA) using the RevertAid First Strand cDNA Synthesis kit (Thermo Scientific, Vilnius, Lithuania) with Oligo (dT)18 primer. A partial DNA fragment of ball python serpentovirus was amplified using specific primer set Ball Python nidovirus (BPNV) 4Fwd: 5'-CCACAACCCGACAGTCAGTA-3' and BPNV 4Rev: 5'-GTACGTAGTCTTGCCAGTTCC-3'[10] with approximate size of 700 bp. The *brain derived neurotrophic factor* (*BDNF*) gene was used as a positive polymerase chain reaction (PCR) control marker with primers BDNF_KU_F: (5'-CAGCTTGGCTTATCCTGGTC-3') and BDNF_KU_R: (5'-CTTTGTGCTGCACTTGGTCTC-3')[11] with approximate size of 500 bp. The PCR amplification was performed using 15.00 µL of 2.00 X Taq Master Mix, containing 2.00 X ViBuffer A (100 mM KCl, 20.00 mM TrisHCl (pH: 9.10 at 20.00 ˚C), and 0.02% Triton™ X-100), 0.40 mM dNTPs, 3.00 mM $MgCl_2$, and 5.00 µm of the primers (Vivantis Technologies Sdn Bhd, Subang Jaya, Malaysia). The PCR conditions were as follows: An initial denaturation at 94.00 ˚C for 3 min, followed by 35 cycles of 94.00 ˚C for 30 sec, 55.00 ˚C for 30 sec, and 72.00 ˚C for 1 min, and a final extension at 72.00 ˚C for 7 min. The nucleotide sequence of DNA fragment was determined using DNA sequencing services (Macrogen, Seoul, South Korea), and nucleotide sequence was searched for homologies with the nucleotide sequence of serpentovirus in the National Center for Biotechnology Information database. The level of sequence divergence was estimated using uncorrected pairwise distances (*p*-distances) with MEGA Software (version 10.2.2; Biodesign Institute, Tempe, USA).

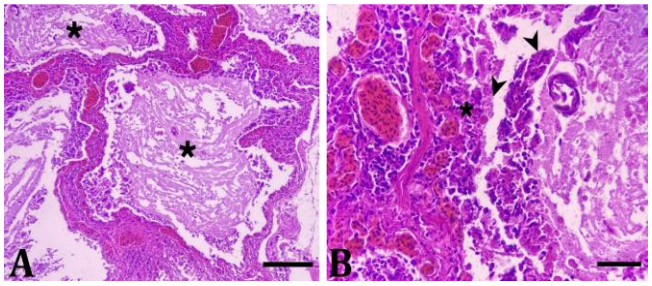

Fig. 2. Longitudinal section of ball python (*Python regius*) lung using Hematoxylin and Eosin staining, **A)** Accumulation of catarrhal exudate and cell debris in faveoli lumen (asterisks);(Bar = 200 µm). **B)** A necrotic of faveolar epithelium (arrowheads) and septal wall epithelial hyperplasia with mixed inflammatory infiltrates (asterisk). (Bar = 50.00 µm).

Agarose gel electrophoresis using the BPNV 4Fwd and BPNV 4Rev primers revealed a DNA fragment of approximately 700 bp in the lung tissue matching the positive control serpentovirus. No specific DNA fragment was observed in the other tissue samples (liver, heart, kidney, and testis; Fig. 3). To investigate the failure of

PCR amplification in the other tissues, the BDNF primers were used separately under the same PCR conditions. The expected DNA fragment of approximately 500 bp was amplified, indicating that the PCR process was functioning correctly.

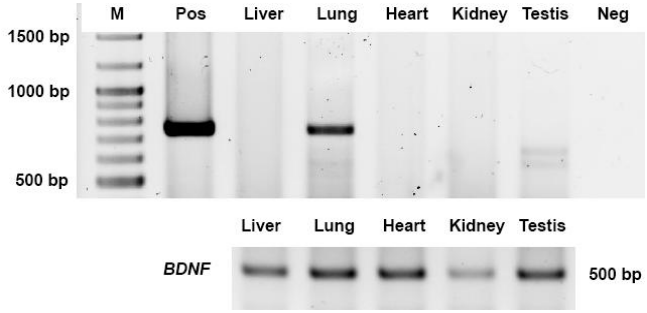

Fig. 3. Agarose gel electrophoresis of polymerase chain reaction products in tissue samples of ball python (*Python regius*) using BPNV 4Fwd - BPNV 4Rev and BDNF_KU_F - BDNF_KU_R primers. Molecular size of DNA is indicated in the left lane. M: VC 100-bp Plus DNA ladder; Pos: Positive control; Neg: Negative control; BDNF: Brain derived neurotrophic factor

To identify the serpentovirus in the ball python lung sample, nucleotide sequence comparisons were conducted using the BLASTn (Basic Local Alignment Search Tool for nucleotides) against the National Center for Biotechnology Information database (https://www.ncbi.nlm.nih.gov/). The obtained sequence exhibited > 95.00% identity ($p < 0.001$) with the United States ball python serpentovirus.

The sequences of the identified serpentovirus were aligned, and a phylogenetic analysis was constructed based on the partial nucleotide sequences of open reading frames 1a and 1b. This analysis included serpentoviruses from ball pythons in Thailand and other serpentoviruses from different geographical locations (Fig. 4). The serpentovirus in this study was grouped within the same clade as the US ball python serpentovirus and it was distinct from other snake serpentoviruses. Comparative analysis of sequence divergence revealed a minimum interspecific divergence of 0.032 ± 0.007 between the ball python serpentovirus in Thailand and the US ball python serpentovirus. In contrast, the maximum interspecific sequence divergence was observed with the reticulated python (*Malayopython reticulatus*) at 0.476 ± 0.020.

Discussion

In our case, gross examination and histopathological results align with the clinical presentation of snakes infected with various serpentovirus strains documented range of respiratory signs, including catarrhal inflammation, pneumonia, and respiratory distress.[8] However, differential diagnoses for this case could include other respiratory pathogens that could not be ruled out, such as bacteria or fungi. Further diagnostic tests, such as imaging study, viral isolation, and immunohistochemistry, would also be necessary.[2,6]

The serpentovirus identified in this case exhibited high sequence identity with a previously identified virus in the US. Given that the ball python (*P. regius*) is not indigenous to Thailand, it was hypothesized that the virus's introduction likely occurred through the illegal importation of exotic snakes, despite the absence of official ball python importation records in Thailand. In our report, the snake was bought from the private snake breeding facility without any information about the snake husbandry that could contribute to disease transmission.[1] Serpentoviruses are primarily transmitted through respiratory secretions and oronasal discharge, facilitating spread through close contact and potentially *via* aerosols.[3,10] The presence of serpentovirus RNA in oral swabs and tracheal washes further supports this route.[4]

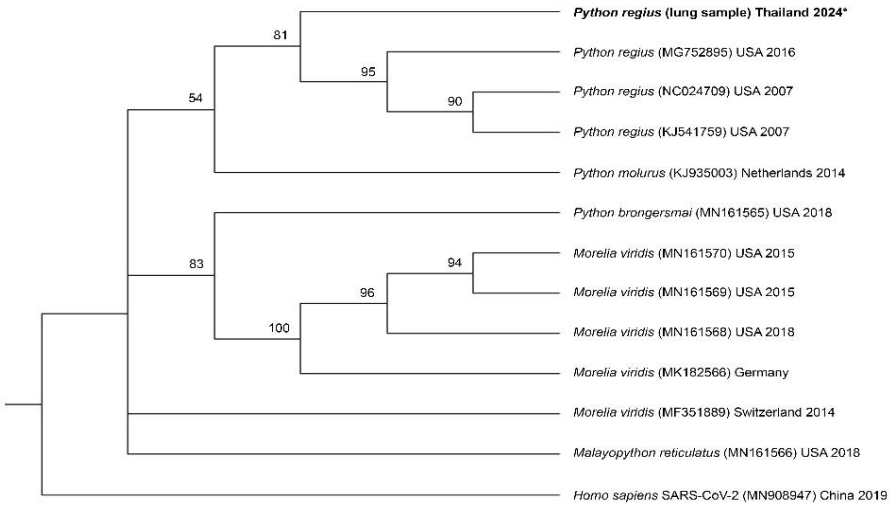

Fig. 4. Phylogenetic tree demonstrating the relationship between the serpentovirus identified in a ball python (*Python regius*) in Thailand and other known serpentoviruses, constructed using the Maximum Likelihood method (The Hasegawa-Kishino-Yano + Gammar model) with 1,000 bootstrap replicates.

Additionally, fecal-oral transmission is also considered possible, as serpentovirus RNA has been detected in the gastrointestinal tract and feces of infected snakes.[12] With multiple potential routes of infection, including respiratory and fecal-oral transmissions, the spread of the virus can be exacerbated by sub-optimal husbandry practices. High animal density, small cages, and housing of multiple species together can increase contact rates and environmental contamination, facilitating disease transmission. In this case, the lack of information about the breeding facility where it was purchased makes it difficult to pinpoint specific factors that may have contributed to the infection. However, it is worth noting that sub-optimal husbandry practices can create the outbreaks, as evidenced by the high mortality rate (85.71%) observed in another ball python breeding facility due to a reptilian ferlavirus infection.[5] This suggests that the potential for viral outbreaks in captive snake populations in Thailand exists. However, the exact source and transmission route of the serpentovirus in this study cannot be confirmed.

While ball pythons are not native to Thailand, three species within the Pythonidae family are endemic to the region,[13] all of which are potentially susceptible to serpentoviruses.[14] Although there is no evidence to suggest that ball pythons have established invasive populations in Thailand, further research should be undertaken to determine the susceptibility of native snake species to serpentoviruses.

In this study, the presence of a serpentovirus in a ball python highlights the potential dangers associated with the exotic pet trade, such as the introduction of novel pathogens, like serpentovirus, into Thailand. Further investigations in exotic snakes and wild snake populations in Thailand are warranted to characterize the epidemiology of serpentovirus infection. The development of effective diagnostics and therapeutics for serpentovirus infection in snakes is also needed to mitigate the impact of this emerging pathogen. These efforts are crucial to safeguard both animal and human health in the face of emerging zoonotic threats.

Acknowledgments

We wish to thank the anonymous snake owner for providing the snake sample to us.

Conflict of interest

The authors declare no conflict of interest.

References

1. Yimming B, Pattanatanang K, Sanyathitiseree P, et al. Molecular identification of cryptosporidium species from pet snakes in Thailand. Korean J Parasitol 2016; 54(4): 423-429.
2. Comolli JR, Divers SJ. Respiratory diseases of snakes. Vet Clin North Am Exot Anim Pract 2021; 24(2): 321-340.
3. Hoon-Hanks LL, Ossiboff RJ, Bartolini P, et al. Longitudinal and cross-sectional sampling of serpentovirus (nidovirus) infection in captive snakes reveals high prevalence, persistent infection, and increased mortality in pythons and divergent serpentovirus infection in boas and colubrids. Front Vet Sci 2019; 6: 338. doi: 10.3389/fvets.2019.00338.
4. Blahak S, Jenckel M, Höper D, et al. Investigations into the presence of nidoviruses in pythons. Virol J 2020; 17(1): 6. doi: 10.1186/s12985-020-1279-5.
5. Piewbang C, Wardhani SW, Poonsin P, et al. Epizootic reptilian ferlavirus infection in individual and multiple snake colonies with additional evidence of the virus in the male genital tract. Sci Rep 2021; 11: 12731. doi: 10.1038/s41598-021-92156-5.
6. Dervas E, Hepojoki J, Laimbacher A, et al. Nidovirus-associated proliferative pneumonia in the green tree python (*Morelia viridis*). J Virol 2017; 91(21). e00718-17. doi: 10.1128/JVI.00718-17.
7. Li WT, Lee MS, Tseng YC, et al. A case report of reptile-associated nidovirus (serpentovirus) in a ball python (*Python regius*) in Taiwan. J Vet Med Sci 2020; 82(6): 788-792.
8. Tillis SB, Josimovich JM, Miller MA, et al. Divergent serpentoviruses in free-ranging invasive pythons and native colubrids in Southern Florida, United States. Viruses 2022; 14(12): 2726. doi: 10.3390/v14122726.
9. Doneley R, Monks D, Johnson R, et al. Reptile medicine and surgery in clinical practice. New Jersey, USA: John Wiley & Sons Ltd. 2018; 453-471.
10. Uccellini L, Ossiboff RJ, de Matos RE, et al. Identification of a novel nidovirus in an outbreak of fatal respiratory disease in ball pythons (*Python regius*). Virol J 2014; 11: 144. doi: 10.1186/1743-422X-11-144.
11. Tawichasri P, Laopichienpong N, Chanhome L, et al. Using blood and non-invasive shed skin samples to identify sex of caenophidian snakes based on multiplex PCR assay. Zool Anz 2017; 271: 6-14.
12. Hoon-Hanks LL, Layton ML, Ossiboff RJ, et al. Respiratory disease in ball pythons (*Python regius*) experimentally infected with ball python nidovirus. Virology 2018; 517: 77-87.
13. Cox M, Hoover M, Chanhome L, et al. The snakes of Thailand. Bangkok, Thailand: Chulalongkorn University Museum of Natural History 2012; 47-57.
14. Dervas E, Hepojoki J, Smura T, et al. Serpentoviruses: more than respiratory pathogens. J Virol 2020; 94(18): e00649-20. doi: 10.1128/JVI.00649-20.