CASE REPORT

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Bacterial co-infections in a captive *Python bivittatus* with septicemia

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Abstract

This case reports bacterial co-infection in a dead albino python (*Python bivittatus*). The snake was brought in dead to the Universiti Veterinary Hospital, Universiti Putra Malaysia. Necropsy was conducted and organ samples were sent for virus and bacterial isolation, as well as histopathology. Gross pathological lesions include congestion of the heart, spleen and liver and presence of superficial whitish circumscribed spots in the lungs. Bacterial culture showed the presence of *Salmonella enterica ssp arizona, Plesiomonas shigelloides* and *Enterobacter cloacae.* Histopathological evaluation showed evidence of necrosis, degeneration and heterophil infiltration in the liver, kidney, heart and lungs. The snake was diagnosed to have died of bacterial septicemia.

Keywords: Bacterial isolation, Captive snake, Histopathology, *Python bivittatus*, Septicemia

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Introduction

Infections with bacterial pathogens are among the most common causes of mortality in reptiles (Jacobson, 2010). Reptiles such as snakes have been reported to host quite a number of bacterial organisms, which were identified as causes of respiratory and systemic infections following bacterial cultures from organ samples (Schumacher, 2006; Jacobson, 2010). Isolation of snakes in captivity predisposes them to harsh ambient temperatures, resulting in heat stress, immune suppression and associated proliferation of bacterial pathogens resulting in disease (Divers & Mader, 2005; Mitchell & Tully, 2008). The most common sources of these pathogens are either from the environment, contaminated feed and water or via transmission from other infected reptiles within the same cage (Ebani & Fratini, 2005). On the other hand, opportunistic bacteria present within the gut and respiratory system may proliferate during stress and cause life threatening infections (Schumacher, 2006; Mitchell & Tully, 2008). In addition, poor sanitation, unbalanced diet, poor lighting and prolonged immobility tend to enable bacteria pathogens to colonize the digestive tract, get shed in the feces or absorbed into the systemic circulation through the intestinal wall, resulting in septicemia or localized abscesses in the skin and visceral organs. This case reports bacterial co-infections in an albino python that had died of septicemia.

Case Presentation

A p*et al*bino python (*Python bivittatus*) aged 2 years was brought in dead (BID) to the University Veterinary Hospital (UVH), Universiti Putra Malaysia. The owner complained that the snake has been off feed for a couple of weeks. The snake carcass was necropsied at the post mortem unit of the UVH (Plate 1a). The gross pathological evaluation showed the presence of a whitish fibrin like material in the tracheal lumen, marked congestion of the cranial 1/3 of the right lung lobe with the presence of multifocal, circumscribed whitish spots which were superficial in nature (Plate 1b).

The heart, spleen and liver were congested, while the gall bladder was distended with bile, which is expected due to the anorexic state of the animal. The stomach and intestines were empty.

Laboratory Diagnostics

Tissue samples of the heart and lung were sent for routine virological isolation through cell culture, while sterile swabs from the same tissues were sent for routine bacteriological culture and

Plate 1: Carcass of the dead snake (a), visceral organs, showing congested lungs (arrows) (b)

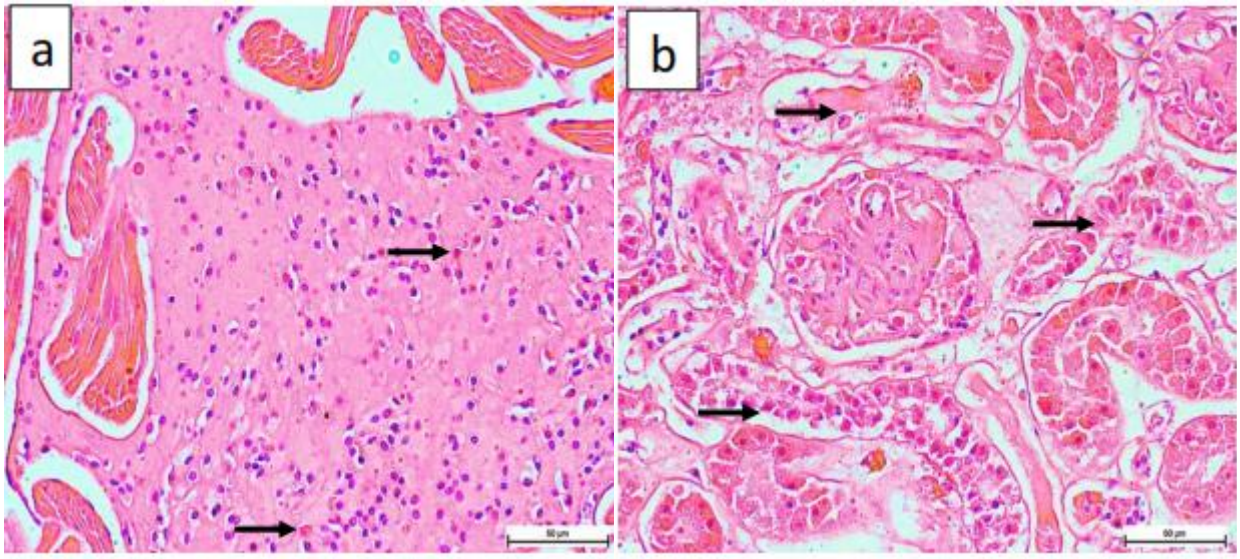

Plate 2: Photomicrograph of the (a) heart showing numerous heterophils in the myocardium (b) kidneys showing necrosis and degeneration of renal tubules (arrows), H&E × 400

isolation using standard bacteriological isolation techniques (Barrow & Feltham, 2004). Tissue samples of the heart, kidney, liver and lung were fixed in 10% buffered formalin, processed, sectioned and stained with H&E for light microscopic examination of pathological lesions.

Results and Discussion

Viral isolation in Vero and rat embryonic fibroblast (REF) cells did not show any evidence of viral induced cytopathic effects (CPE) after three successive passages, thus an involvement of a virus was ruled out. Bacteriological culture and identification showed the growths of *Salmonella enterica ssp arizona, Plesiomonas shigelloides* and *Enterobacter cloacae.* The isolation of *S. enterica* in reptiles has been reported in different parts of the world. In one study, high prevalence of *S. enterica* was reported in reptiles of German and Australian origin (Geue & Löschner, 2002). The study also observed a high prevalence of salmonella in reptiles sourced from the pet shops than from those obtained from breeders. In another study, a high prevalence of salmonellosis was observed in captive reptiles, 91 (47%) in comparison to those from the wild, 41 (14%), this was attributed to diet and captivity conditions that limit the movement of the animals within the environment, thus encouraging the propagation of the bacteria (Scheelings *et al.,* 2011). In yet another study, a prevalence of 53.3% for salmonellosis was reported in imported snakes from Brazil (Sá & Solari, 2001). In most of these studies, samples were obtained from the cloaca or oral cavities, and the animals were alive and apparently healthy. In this report, samples were obtained from visceral organs (heart and lung) which have shown evidence of congestion and inflammation, hence

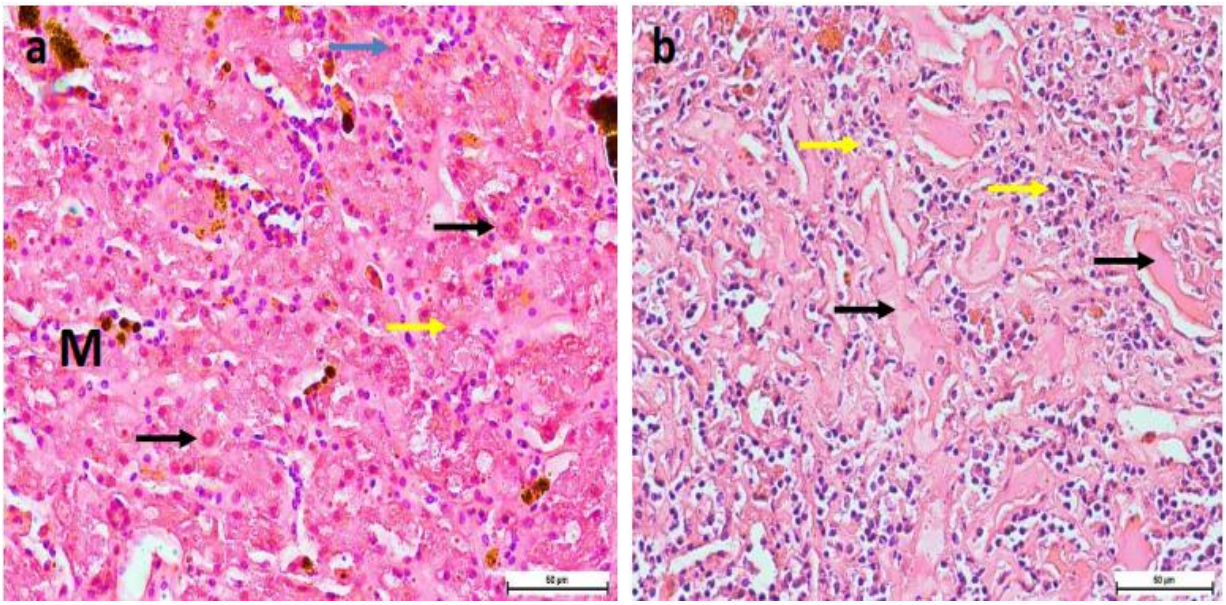

Plate 3: Photomicrograph of the (a) liver showing hepatocyte degeneration (black arrow), necrosis (yellow arrows) and heterophil infiltration (blue arrow), note the golden brown melanomacrophages (M) signifying iron pigmentation from erythrophagocytosis (b) lung showing diffuse suppurative exudation typified by heterophils (yellow arrows) and edema (black arrows) in the air capillaries (arrows), H&E × 400

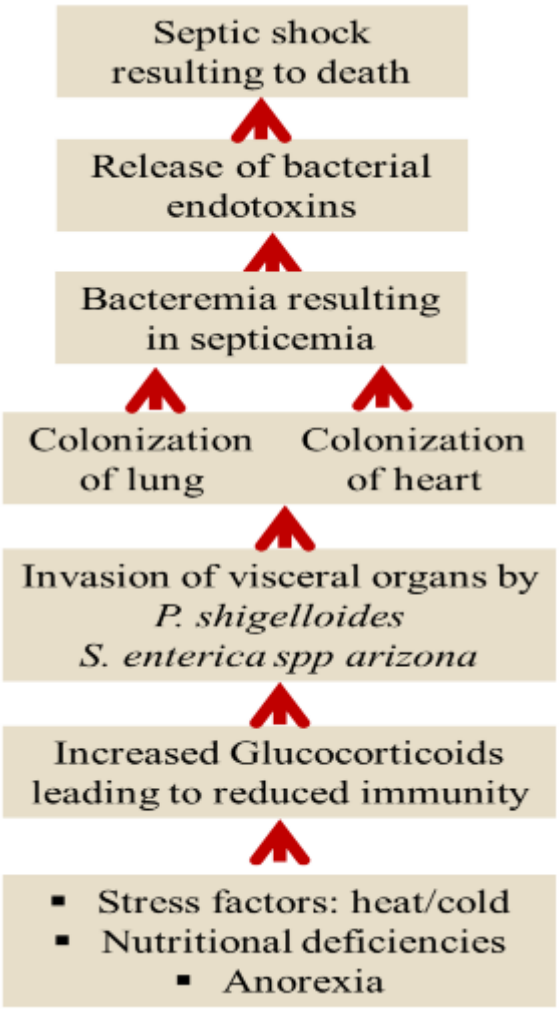

Figure 1: Proposed pathophysiology mechanism that lead to the death of the snake

making it clinically important in the disease association. *S. enterica* group IIIa and IIIb have been shown to be the most important subgroups of *S.enterica arizonae* in snakes and were reported to be clonally associated with isolates obtained from healthy and diseased rattle snakes (Bemis *et al.,* 2007).

Histopathological evaluation showed suppurative myocarditis in the heart (Plate 2a), marked by numerous heterophil infiltration, while there was vacuolar degeneration and necrosis in the kidneys (Plate 2b), mild hepatocyte necrosis and hetrophil infiltration in the liver (Plate 3a) and a diffuse suppurative exudation in the lungs (Plate 3b).

The lesion severity was graded based on mild (+; <25%), moderate (++; <65%) or severe (+++; >70%), depending on the extensiveness and distribution of the lesion in the focal field (Table 1). Exudation was severe in the lung and moderate in the heart, and both organs had numerous heterophils. Degeneration and necrosis were diffuse (severe) in the renal tubules of the kidneys and multifocal (moderate) in the liver. Necrosis was multifocal (moderate) in the liver and lungs and focal (mild) in the heart. From the histopathology evaluation and lesion scoring, the snake showed evidence of hetetrophil infiltration in all the organs examined, with mutifocal to diffuse distribution in the heart and lungs, which is suggestive of suppurative myocarditis and pneumonia. In a recent study, high number of Salmonella was isolated in boid snakes with and without respiratory disease. The authors reported a high prevalence of salmonellosis (33%) in snakes with pneumonia (Schmidt *et al.,* 2013). Even though Salmonella is mostly associated with

Table 1: Summary of lesion severity observed in various organs

Organ/Lesion	Exudation	Inflammatory cells	Degeneration	Necrosis
Heart	++	++	+	+
Kidney	+	+	+++	+++
Liver	+	+	++	++
Lung	+++	+++	+	++

+ = mild, ++ = moderate, +++ = severe

gastrointestinal shedding, other studies have shown proof of infection in the bone and other extra intestinal sites. *S. spp arizonae* has been associated with fatal septicemia and osteomyelitis in a *Coralatus willandi* rattle snake (Grupka *et al.,* 2006; Bemis *et al.,* 2007).

Besides that, *S. spp arizonae* has also been associated with cases of fatal peritonitis, pleuritis, osteomyelitis, meningitis and bacteremia in humans especially in immunocompromised patients (Mahajan *et al.,* 2003). Although two other bacterial species were isolated from the visceral tissues of the snake; *Plesiomonas shigelloides* and *Enterobacter cloacae;* these bacterial species have also been previously reported in snakes and in cases of fatal human infections. *P. shigelloides* has been isolated in cases of septicemia, meningitis, sepsis and splenic abscess in infants and adolescent subjects (Waecker *et al.,* 1988; Ampofo *et al.,* 2001). However, to our knowledge, there is no report of these three organisms being isolated from the viscera of a snake. Based on the information available, a possible pathophysiological process of the disease is shown in Figure 1. It is speculated that the presence of the bacterial co-infection might have resulted to a septicemic condition that led to death of the snake.

This case reports the isolation of three important bacterial species of zoonotic importance in an albino python that had died of septicemia. The association of these bacterial species with infections in humans makes it of public health significance especially to pet snake owners, zoos, aquariums and veterinarians.

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