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# Surgical Removal and Recurrence of Leiomyosarcoma in a King Cobra, Ophiophagus hannah

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*Short Communication*

# Surgical Removal and Recurrence of Leiomyosarcoma in a King Cobra, *Ophiophagus hannah*

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***Abstract***

An adult male wild-caught king cobra, *Ophiophagus hannah*, of unknown age was raised in captivity for seven years. The snake showed swelling at the caudal third of the body. Physical examination revealed an approximately 15x10 cm firm mass in coelomic cavity. Radiography showed soft tissue density causing complete obstruction of intestinal tract. The mass was surgically removed and diagnosed as leiomyosarcoma. The snake was monitored and the mass recurred two months post-surgery. A second surgical exploration showed severe adhesion of the mass to dorsal aorta, intercostal muscle, ribs and stomach. The snake was euthanized and the recurrent masses were diagnosed as leiomyosarcoma.

**Keywords: k**ing cobra, leiomyosarcoma, *Ophiophagus hannah*, recurrence

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## Introduction

A range of neoplasms have been reported in snakes, of these, tumors in the gastrointestinal tract are most frequently described. In order of greatest incidence, adenoma/adenocarcinoma, fibroma/ fibrosarcoma, squamous cell carcinoma, leiomyoma/ leiomyosarcoma and lymphoma/ lymphosarcoma have been reported in the literature within the gastrointestinal tract of snakes (Mauldin and Done, 2006). Leiomyosarcoma and leiomyoma are tumors of smooth muscle origin with different malignant potential. There are some reports of leiomyosarcoma and leiomyoma in snakes that have been found in the gastrointestinal and reproductive systems (Harshberger, 1974; Ramsey and Fowler, 1992; Martin et al., 1994; Cata˜o-Dias and Nichols, 1999; Hernandez-Divers and Garner, 2002). Most cases had unknown etiology/promotion, and are often considered as spontaneous tumors. The slow clinical progression of many different types of snake neoplasms often make early diagnosis difficult, while late discovery of the tumors limits therapeutic options to supportive therapy and in many cases, the animals require euthanasia due to the advanced nature of the malignancies. The purpose of this report is to document the result of surgical removal of a leiomyosarcoma in a king cobra, *Ophiophagus hannah*, and its recurrence two months post-resection and the pathological diagnosis of the cancer.

## Materials and Methods

***Case history:*** An adult male wild-caught king cobra, *Ophiophagus hannah*, of unknown age was raised for seven years in the snake farm, Queen Saovabha Memorial Institute (QSMI). The snake had a snout to vent length (SVL) of 351 cm and weighed 7 kg. The animal received routine annual health checks at which time it was dewormed. It was reported by the snake farm personnel that the animal had refused to eat for approximately one month and had a slow growing mid-body swelling. The snake was raised in outdoor cage with dimensions of 4x10 m. The cage imitated the natural habitat of the king cobra by using real plants, water pond and shelters provided as a hiding area.

***Clinical and laboratory examination with treatment:*** Physical examination revealed that the snake was alert and responsive to stimulation with normal mucous membrane color. On palpation, the swelling was noted to have an average size of 15x10 cm and was located in the middle of the coelomic cavity (Fig.1a). The slightly movable mass was firm with clear contours and an irregular surface (Fig 1b). Radiographic examination revealed a soft tissue density resulting in complete obstruction of the gastrointestinal tract (Fig. 2). Surgical resection was necessarily performed because of chronic anorexia and gastrointestinal tract obstruction. Pre-operative blood was collected and checked for packed cell volume, uric acid, alkaline phosphatase and aspartate aminotransferase. All clinical chemistry parameters were within normal ranges reported for snakes (Vasaruchapong *et al*., 2013). The snake was anesthetized with 5% isoflurane (Attane®, 250 mL, Piramal Critical Care Inc., Bothlehem, PA, USA) for induction and reduced to 2.5% for maintenance with an oxygen flow rate of 2 L/min in a restraining clear plastic tube. During anesthesia, fluid support was given intravenously with acetate ringer's solution via the right dorsal palatine vein. The incision line was between the first and the second dorsal scale rows on the left lateral side of the body above the midpoint of the mass. Blunt dissection was performed downward to ventral midline then incised coelomic membrane to reach coelomic cavity. A highly vascularized mass was visualized in the coelomic cavity adjacent to the stomach. The blood vessels were ligated and the mass was completely dissected. The coelomic membrane was carefully sutured with 4-0 polyglyconate (Connek, Novatec Healthcare Co.,Ltd., Samutprakan, Thailand) in a simple continuous pattern. The skin was sutured with 2-0 nylon (Nylon, UNIK, Taipei Hsien, R.O.C., Taiwan) in an everting horizontal mattress pattern because reptilian skin has a strong tendency to invert and lead to incision edges that are not in apposition. 20 mg/kg ceftazidime (Cef-4®, 100 mg/mL, Siam Bheasach Co., Ltd., Bangkok, Thailand), IM q 72 h for 5 days (Diethelm, 2005), and 5 mg/kg tramadol (Tramal® 50, 50 mg/mL, Grunenthal GMbH, Aachen, Germany), IM q 24 h for 5 days, were provided.

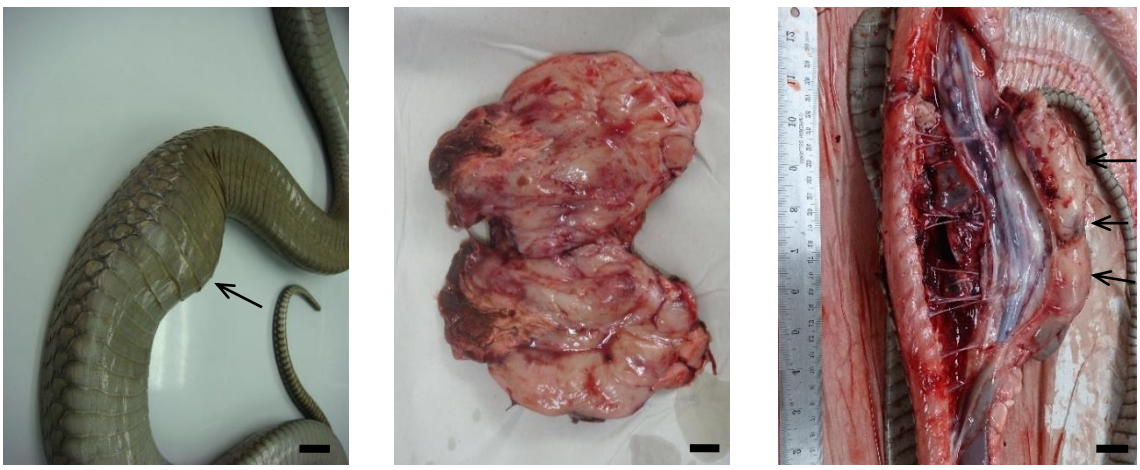

**Figure 1** a) The mid-coelomic cavity contains a mass approximately 15x10 cm (arrow).
b) The cross section of the mass at the first surgery shows creamy, oval, firm, multilobulation.
c) The recurrent multiple masses attach at the dorsal aorta (arrows). (bar= 2 cm)

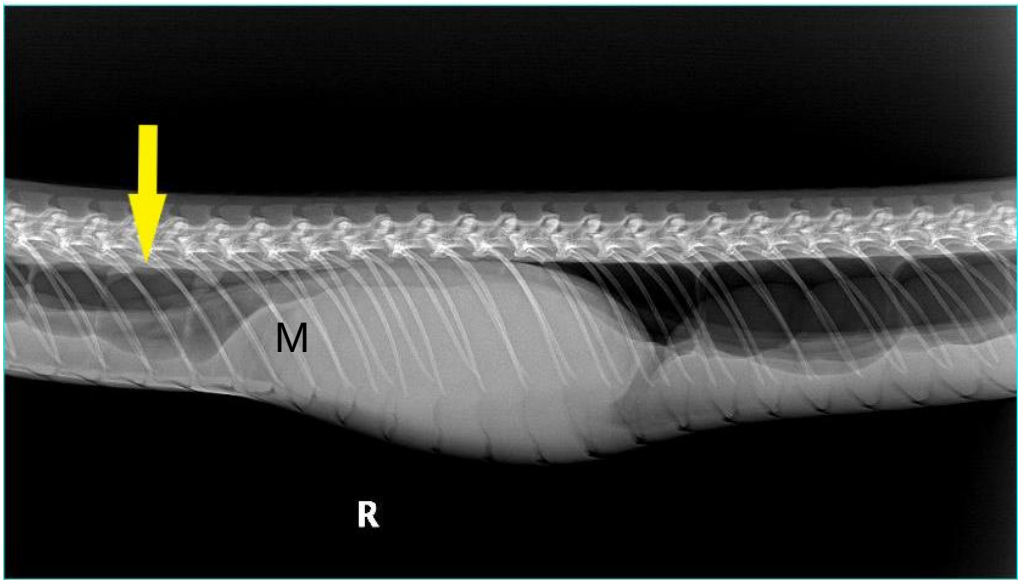


**Figure 2** Radiography of soft tissue density mass (M) shows in the coelomic cavity resulting in gastrointestinal obstruction (arrow).

***Histopathological and immunohistochemical methods:*** A tissue sample from the first surgical resection as well as from the recurrent mass were submitted to the Department of Pathology, Faculty of Veterinary Science, Chulalongkorn University. The tissue sample was fixed in 10% buffered formalin and embedded in paraffin blocks. The 4 μm thickness tissue sections were cut and routinely stained with hematoxylin and eosin (H&E) and special histochemistry of Masson's trichrome (MT) and periodic acid Schiff's reaction (PAS). The sections were immunohistochemically (IHC) stained using an peroxidase anti-peroxidase (PAP) system with a chain polymer kit (Dako EnVision+ System-HRP, anti-mouse and rabbit; Dako, Tokyo, Japan) with the following primary antibodies against cytokeratin (clone AE1/AE3), vimentin (clone V9), desmin, smooth muscle actin (SMA), S-100, neuron-specific enolase (NSE), alpha-fetoprotein and Hepa2 (Dako). Briefly, the tissue sections were endogenous peroxidase blocking by 10% hydrogen peroxide in absolute methanol. The tissue sections were then treated with 8% skim milk in Tris-buffer. Incubation with diluted primary antibodies was carried out and followed by incubation with a chain polymer kit. Finally, the presence of positive cells was visualized by a 3,3′-diaminobenzidine tetrahydrochloride (DAB) reaction. The nuclei were counterstained with hematoxylin.

## *Results and Discussion*

***Post-surgery and tumor recurrence:*** After surgery, the snake was provided with parenteral subcutaneous injection of 20 mL/kg acetate ringer's solution (Acetar, 1,000 mL, Thai Otsuka Pharmaceutical Co., Ltd., Samut Sakhon, Thailand) SC, q 24 h for 5 days. The suture was removed a month after surgery. During this period, the snake shed once and the incision line was completely healed. The snake was alert, had good appetite and defecated normally. Two months after surgery, the snake was cachexic and a new swelling was identified in approximation to the prior surgical site. A second surgical exploration was performed, and a mass with the same characteristics as the previous neoplasm was identified. In this instance, the mass was noted to be invading the gastric wall, dorsal aorta, intercostal musculature and ribs (Fig. 1c), causing complete gastrointestinal tract obstruction. Due to the extremely poor prognosis, the animal was euthanized with 60 mg/kg pentobarbital (Nembutal, 54.7 mg/mL, Ceva Santé Animale, Libourne, France) intravenously (Burns and McMahan, 1995) via dorsal palatine vein, and necropsy was performed.

***Histopathology and immunohistochemistry:*** Histopathologically, the tumor mass revealed a malignant neoplasm. The neoplastic cells were variably spindle, polygonal and rarely stellate, arranged in both dense and loose swathes and occasional densely cellular streams with frequently distinct cell borders. The neoplastic cells were moderate eosinophilic, amorphous faintly granular cytoplasm with central clearing and oval to elongate nuclei with one to three distinct nucleoli. The mitotic rate was very high with occasional single cell death (Fig.3a). The MT special histochemical stain showed positive staining for smooth muscle tumor cells (Fig. 3b). In addition, the IHC stains were negative, with the exception of the SMA (Fig. 3c.) which showed faint immune-positive staining within the neoplastic cells. The SMA protein is a marker of smooth muscle cell origin. The negative result of other IHC might be the affinity of antibodies against the snake tissue, since the antibodies were not derived from reptilian tissues (Atwood, 2001). Both masses were pathologically diagnosed as leiomyosarcoma.

Leiomyosarcoma is a malignant neoplasm of smooth muscle cell origin with a high rate of recurrence and metastasis. It is one of the most frequent soft tissue sarcoma that has poor diagnosis in human medicine (Parvizi and Kim, 2010). Complete surgical resection of the tumor is a standard treatment for primary leiomyosarcoma while additional treatment like radiotherapy and chemotherapy are considered in cases where surgical resection is limited or metastasis (Weaver and Abraham, 2007). There have

been some reports of leiomyosarcoma in snakes but most of these did not report the management approach (Harshberger, 1974; Ramsey and Fowler, 1992; Cata˜o-Dias and Nichols, 1999.). There was only a report (Martin et al., 1994) in which the surgical excision of a gastric leiomyosarcoma was conducted in a Texas indigo snake (*Drymarchon melanurus erebennus*) (Crother et al., 2012) however the snakes in that study died subsequent to the recurrence of the neoplasm and following a second attempt at surgical removal of the mass. The recurrence of the neoplasm can occur if the first treatment did not fully remove or destroy cancer cells which allows them to survived and continue to grow over time (National Cancer Institute, 2014). In this report, surgical resection was also considered because of the gastrointestinal obstruction and no gross evidence of tumor metastasis on nearby organs. However, there was a local recurrence of the tumor which possibly by incomplete removal could lead to recurrence and allow tumor metastasis. Therefore, other adjuvant therapy such as radiation or chemotherapy should have been considered to control the tumor after surgical resection (Mauldin and Done, 2006). Systemic chemotherapy is considered in the case of metastatic disease or with a tumor with a high metastatic rate. In human medicine, uterine leiomyosarcoma is well documented and treatment also is founded on early surgical resection, and, in advanced cases, chemotherapy (Dinh et al., 2004; Ricci et al., 2017). Doxorubicin, gemcitabine, and gemcitabine plus docetaxel are treatment options for systemic chemotherapy in uterine leiomyosarcoma (Gupta et al., 2013). Doxorubicin has already been used in reptile medicine, however there is a lack of available documentation on potential adverse and toxic effects. Therefore, other adjuvant therapy should be performed with a complete hemogram prior to and during chemotherapy being conducted due to the possibility of immunosuppression and myelosuppression from the chemotherapeutic agent (Mauldin and Done, 2006). This can gather more information on leiomyosarcoma treatment, which remains poorly documented in the reptile medicine literature.

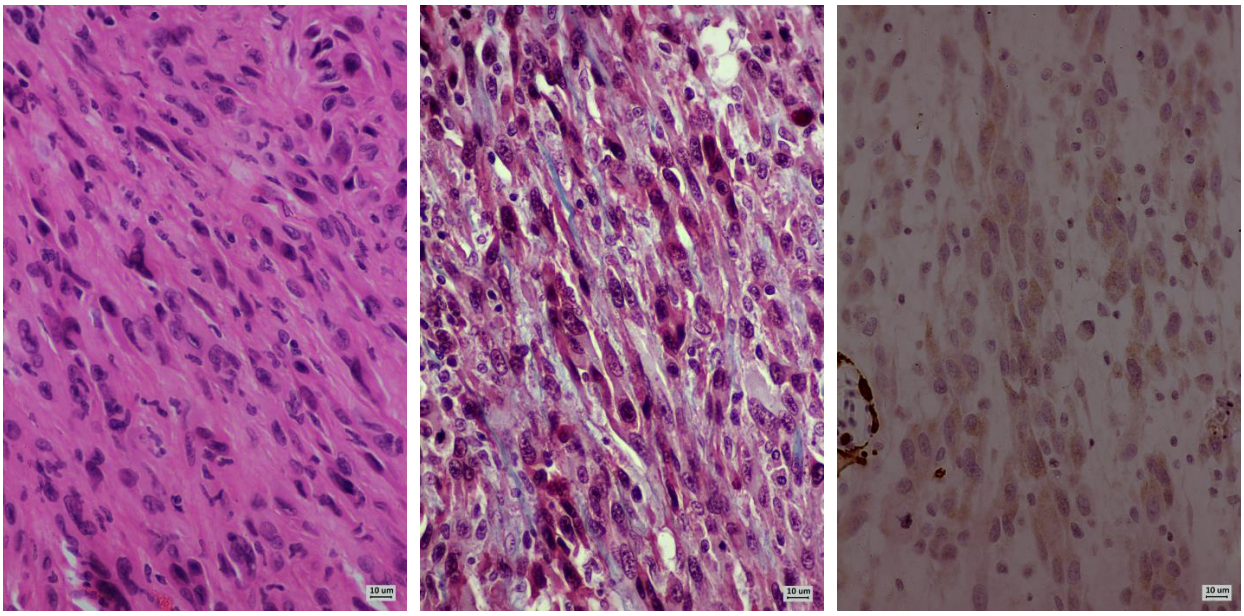


**Figure 3** a) Histopathology of the neoplastic cells are variably spindle, polygonal and rarely stellate. They arrange in both dense and loose swaths, and occasional densely cellular streams with frequently distinct cell borders. The mitotic rate is very high with occasional single cell death (H&E, bar= 10 µm)
b) The positive red stain in the cytoplasm of the spindle tumor cells. (MT stain, bar= 10 µm)
c) The positive immunostain of SMA antibody (brown color) shows in the cytoplasm of spindle tumor cells. (IHC, DAB, bar= 10 µm)

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