

# **Pulmonary squamous cell carcinoma in a captive clouded leopard (*Neofelis nebulosa*)**

**Kittikorn Boonsri<sup>1\*</sup> Koravee Pothichai<sup>1</sup> Thewarach Vechmanus<sup>3</sup>**

**Pinich Boonthong<sup>3</sup> Kidsadagon Pringproa<sup>1,2</sup>**

## ***Abstract***

A female, 5-year-old clouded leopard died three days after showing clinical signs of lethargy and loss of appetite. Macroscopically, multiple, firm, nodular masses were found randomly in the caudal lobes of the lungs. There were no metastatic lesions on the skin. Histopathological examination demonstrated multifocal trabeculae and a nest of large, variable sized of squamous epitheliums. Multifocal keratin pearls were also randomly seen in the masses. No metastasis was found in the other internal organs. Immunohistochemically revealed that the neoplastic cells were immunoreactivity positive for cytokeratin, while negative for vimentin. Based on the histopathology and immunohistochemistry, pulmonary squamous cell carcinoma (SCC) was diagnosed.

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**Keywords:** Squamous cell carcinoma, lung, clouded leopard (*Neofelis nebulosa*)

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<sup>1</sup>Veterinary Diagnostic Laboratory, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand

<sup>2</sup>Department of Veterinary Biosciences and Veterinary Public Health, Faculty of Veterinary Medicine, Chiang Mai University, Chiang Mai 50100, Thailand

<sup>3</sup>Chiang Mai Zoo, Chiang Mai 50200, Thailand

\*Correspondence: Kittikorn.boonsri@cmu.ac.th (K. Boonsri)

## Introduction

The clouded leopard (*Neofelis nebulosa*) is a medium-sized felidae native to Southeast Asia, and is one of the world's most enigmatic types of wild cat (Austin *et al.*, 2007). There have been a few reports describing neoplasms in clouded leopards, including intestinal adenoma (Wada *et al.*, 1996), mesothelioma, leiomyoma, pheochromocytoma (Chu *et al.*, 2012) and skin squamous cell carcinoma (SCC) (Kesdaangakonwut *et al.*, 2014). However, to the best of our knowledge, pulmonary SCC in the lungs has not been documented. In the present study, we describe the pathological characteristics of pulmonary SCC in a captive clouded leopard and their related literature reviews.

## Material and Methods

A captive-born, five year old, female clouded leopard from Chiang Mai Zoo, Chiang Mai, Thailand, was fed on pork, beef, the internal organs and ribs of the pig, carnivore supplements, and calcium carbonate. The cat had shown clinical signs of lethargy and loss of appetite for about three days prior to death. The necropsy was performed at the Veterinary Diagnostic Laboratory, Faculty of Veterinary Medicine, Chiang Mai University, Thailand. Samples tissue of the heart, lungs, liver, kidneys, spleen, pancreas and the duodenum were collected and fixed in 10% neutral buffered formalin and processed for hematoxylin and eosin (H&E) histopathologic examination. To clarify the origin of the tumor cells, an avidin-biotin peroxidase complex (Vector Laboratories Inc., Burlingame, California, USA) immunohistochemistry (IHC) of the lung tissue was performed using anti-AE1/AE3 cytokeratin (Diagnostic BioSystems, Pleasanton, California, USA) and anti-vimentin (Diagnostic BioSystems, Pleasanton, California, USA) antibodies, as previously described (Srivivorakul *et al.*, 2017).

## Results and Discussion

Macroscopically, the lung presented a clouded color with fibrosis of the pleura. Multiple white nodules of size 0.5-1.0 cm in diameter were observed in the caudal lobes of the lung (Figure 1A). On the cut surface, multiple variables of necrosis with abscesses were presented in the lung parenchyma. Other organs showed no remarkable lesions. Microscopically, unencapsulated, poorly demarcated masses were scattered in the lung parenchyma, especially in the bronchioalveolar area. There were numerous sheets or islands of large polygonal cells containing keratin and intercellular bridges. The neoplastic cells had large, ovoid nuclei with prominent nucleoli and an abundance of lightly eosinophilic cytoplasm. Mitotic figures were 3-4 cells/10 high power field (HPF). Multifocal keratin pearl formations were found randomly within the neoplastic areas (Figure 2A). Neutrophilic emperipolesis was presented in the neoplastic keratinocytes (Figure 2B). Some areas of bronchioles were found to have squamous epithelial metaplasia (Figure 3A). Metastatic neoplastic cells were not observed in any other internal organs.

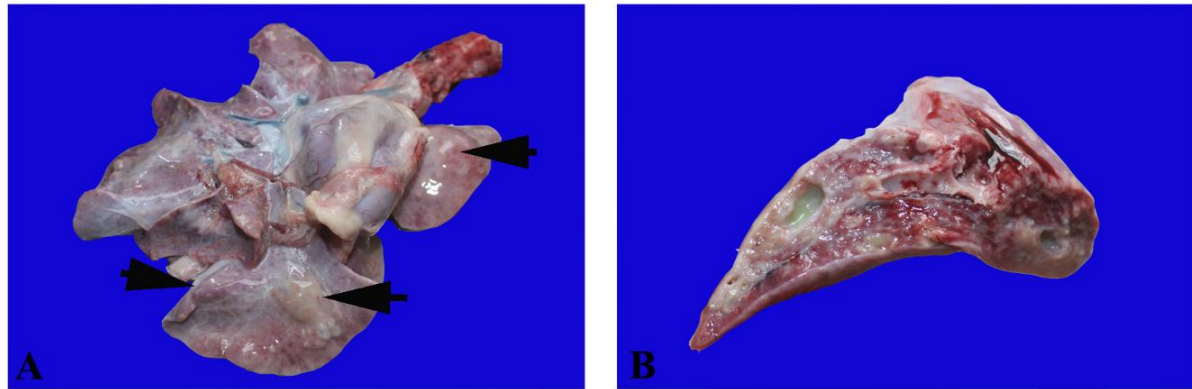
Immunohistochemically, the cytoplasm of the neoplastic cells showed a strong immunoreaction to AE1/AE3 cytokeratin antibodies (Figure 3B), while being negative for anti-vimentin; this suggests that the neoplastic cells were of epithelial origin. Based on the histopathologic characteristics and immunohistochemistry, the mass was diagnosed as pulmonary squamous cell carcinoma.

Squamous cell carcinoma (SCC) is a malignant neoplasm of the keratinocytes. It is a common malignant skin tumor of all domestic animals, including chickens (Goldschmidt & Goldschmidt, 2017). Cutaneous SCC in the skin has been reported in wild animals and reptiles such as the white rhinoceros (Langer *et al.*, 2016), red deer (Ulrich *et al.*, 2014), the clouded leopard (Kesdaangakonwut *et al.*, 2014), the Northern Brown bandicoot (Beck *et al.*, 2017) and the saltwater crocodile (Hill *et al.*, 2016). Kesdaangakonwut *et al.* (2014) reported cutaneous SCC in clouded leopards from Dusit Zoo, in urban Bangkok. They found ulcerative wounds with whitish masses in the skin. However, no tumor metastasis was found elsewhere. In the present case, the clouded leopard had no skin masses and wounds, but the tumor mass was found only in the lungs, and thus, pulmonary SCC was diagnosed. In animals, pulmonary SCC had been reported in dogs (Aydin *et al.*, 1997), cats (Silva *et al.*, 2012) and Atlantic bottlenose dolphins (Ewing & Mignucci-Giannoni, 2003). This is the first report of pulmonary SCC in the clouded leopard. Since, there were areas of the ciliated pseudostratified epithelium transformed to metaplastic squamous epithelium and keratin pearls presented in the neoplasm, is likely that this squamous cell carcinoma was a primary lung tumor. However, some tumors may develop multiple foci of squamous epithelium with keratinization, such as basosquamous carcinoma. Thus, differential diagnosis of this tumor should be included. The important diagnostic feature used to distinguish basosquamous carcinoma from SCC is that it is generally an expansive growth of basaloid cells (Shin *et al.*, 2011). In our report, the immunohistochemical positivity for AE1/AE3 cytokeratin was used to determine the epithelial origin of the tumor, however, it was not sufficient for the diagnosis of pulmonary SCC. The recommended immunohistochemistry in lung squamous cell carcinoma is p40, TP63, and CK5/6 (Inamura, 2018). Among pulmonary SCC antibodies, p40 is the best marker in terms of specificity and sensitivity (Bishop *et al.*, 2012).

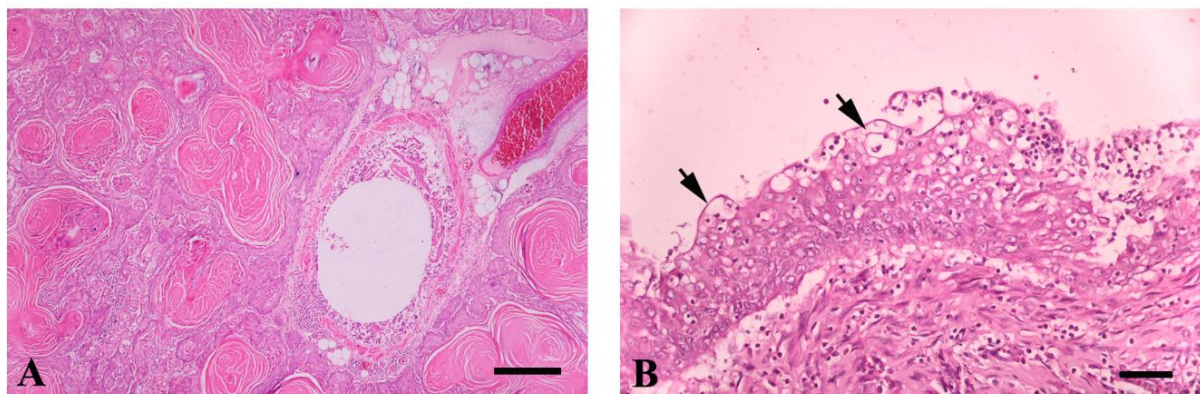
The spontaneous pulmonary SCC was suspected to be associated with air pollution (Husain, 2010). In humans, tumors of large airway origin are associated with tobacco smoking, chronic industrial hazards, air pollution, molecular genetic alteration, and precursor lesions (Husain, 2010). Thus, it is reasonably to speculate that this leopard was exposed to environmental carcinogens because the zoo is situated in the urban center of the city. Though this leopard was 5 years old, it was younger than the cats with neoplasm in the previous reports. In comparison to humans, the risk factor of primary lung cancer in children is associated with a substantial family history of cancer (Wang *et al.*, 2008). Thus, it remains unclear whether pulmonary SCC in the clouded leopard presented in

this study involved genetic alteration. Therefore further studies may include observations of clinical signs, program health checks, identification of risk

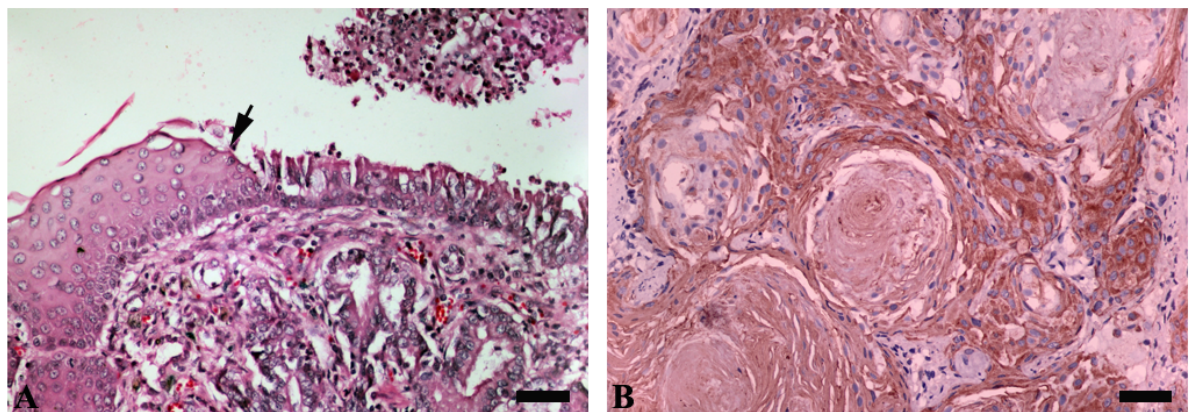
factors and diagnostic methods of SCC in the lungs of wildlife, especially in urban zoos.



**Figure 1** Gross features of the lungs in a female, clouded leopard with squamous cell carcinoma. Multiple nodular masses (arrow) were randomly found in the lung parenchyma (A) and, necrotic masses in the cutting surface of the lung (B)



**Figure 2** Histological features of pulmonary SCC in a female, clouded leopard. Multifocal keratin pearls (A) and neutrophilic emperipolesis (arrow) (B) were presented in the neoplastic areas. Scale bar in A = 200  $\mu$ m, B = 50  $\mu$ m.



**Figure 3** Histological features of pulmonary squamous cell carcinoma from a female, clouded leopard. Epithelial metaplasia (arrow) were present in the bronchus (A). Immunohistochemical features of squamous cell metaplasia with intense cytoplasmic staining for anti-AE1/AE3 cytokeratin (B). Scale bar in A and B = 50  $\mu$ m.

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