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ASPERGILLOSIS IN ARTIFICIALLY BREEDING BLACK SWANS (*CYGNUS ATRATUS*) IN DPR KOREA AND IDENTIFICATION OF CAUSE FUNGUS

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АСПЕРГИЛЛЕЗ ПРИ ИСКУССТВЕННОМ РАЗВЕДЕНИИ ЧЕРНЫХ ЛЕБЕДЕЙ (CYGNUS ATRATUS) В ДНР КОРЕЯ И ИДЕНТИФИКАЦИЯ ПРИЧИННОГО ВИДА ГРИБКА

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Abstract

Black swans (*Cygnus atratus* (Latham) 1790), which was native in Australia, were distributed all over the world and artificially reared. Aspergillosis is non-contagious fungal disease infected in some poultries and wild birds and the important agent are some of *Aspergillus* species.

Infection by Aspergillus species results in respiratory disease in poultry and wild birds. The disease is generally caused by Aspergillus fumigatus and Aspergillus flavus. This study was proceeded to document the clinical signs and lesions of Aspergillosis in artificially breeding black swan in DPR Korea, isolate and identify the causative fungi from lesions. Black swans at Swan Breeding Center in Taedong County, South Pyongan Province, DPR Korea were investigated. The infected swans died suddenly after two or three days when they lost appetite and had symptoms of dyspnea, sudden retraction or deformity of their neck and abnormal walking. The infected black swans had white fecal diarrhea. The fungal hyphae were also observed in the eyes.

Here, we confirmed the clinical cases of Aspergillosis in artificially breeding black swans in DPR Korea and A. flavus was isolated in Potato Dextrose Agar (PDA) and identified morphologically from fungi cultivated in Malt Extract Agar (MEA), Czapek Yeast Extract Agar (CYA) and Czapek Dox Agar (CZ). The morphological characteristics like colony colors were similar with the findings of who isolated *Aspergillus flavus* from maize and soil in Kenya. The microscopic characteristics of fungus isolated from aspergillosis lesions in Black swans were investigated. The morphologically identified fungus was confirmed as A. *flavus* by sequencing of PCR product of 600bp fragment with primers ITS1/ITS4 and Blast analysis.

Keywords: black swan, *Cygnus atratus*, Aspergillosis, *Aspergillus flavus*, respiratory disease.

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Резюме. Черные лебеди (Cygnus atratus (Latham) 1790), обитающие в Австралии, получили распространение по всему миру и выращиваются искусственно. Аспергиллез — это незаразное грибковое заболевание, отмечаемого у некоторых домашних и диких птиц, вызываемого разными видами грибков Aspergillus.

Заражение видами Aspergillus приводит к респираторным заболеваниям у домашних и диких птиц, чаще обусловленного Aspergillus fumigatus и Aspergillus flavus. Настоящее исследование было проведено для документирования клинических признаков и поражений аспергиллеза у искусственно разведенных черных лебедей в КНДР, выделения и идентификации возбудителей. Черные лебеди были обследованы в Центре разведения лебедей в уезде Тэдон, провинции Южный Пхёнан, КНДР. Зараженные лебеди внезапно умерли через два или три дня, при потере аппетита, наличии симптомов одышки, внезапного втягивания или деформации шеи и атипичной походки, белой диареи. Грибковые гифы также отмечены в области глазах.

В настоящей работе были подтвердили клинические случаи аспергиллеза у искусственно выведенных черных лебедей в КНДР, от которых был выделен A. flavus на картофельно-декстрозном агаре (PDA) и идентифицирован морфологически из грибов, культивируемых в агаре с экстрактом солода (MEA), агаре с экстрактом дрожжей Чапека (CYA) и агаре Чапека-Докса (CZ). Морфологические характеристики, такие как цвет колоний, были схожи с данными, полученными исследователями, изолировавшими Aspergillus flavus из кукурузы и почвы в Кении. Были исследованы микроскопические характеристики Aspergillus flavus, выделенного из патологического материала у черных лебедей. Морфологически идентифицированный возбудитель был верифицирован как

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A. flavus путем секвенирования продукта ПЦР фрагмента 600 п.н. с праймерами ITS1/ITS4 и Blast анализа.

Ключевые слова: черный лебедь, Cygnus atratus, аспергиллез, Aspergillus flavus, респираторное заболевание.

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1 Introduction

Black swans (Cygnus atratus (Latham) 1790), which was native in Australia, were distributed all over the world and artificially reared [9]. Aspergillosis is non-contagious fungal disease infected in some poultries and wild birds and the important agent are some of Aspergillus species. Aspergillus fumigatus is the most common causative agent [2] and the less common causative agents are Aspergillus flavus, Aspergillus nidulans, Aspergillus terreus and Aspergillus niger [8]. Aspergillosis was found in several Swan species including wild black swan [5] and the causative species was Aspergillus fumigatus. The clinical signs of aspergillosis included lethargy, dyspnea, weight loss and twisting of neck [1,7]. Lesions were generally restricted to lungs and air sacs, where white-yellowish granulomatous nodules were formed [6]. Aspergillus species were isolated in Potato Dextrose Agar (PDA) and identified by macro and micro morphological characteristics in differential media such as Malt Extract Agar (MEA), Czapek Yeast Extract Agar (CYA) and Czapek Dox Agar (CZ) [3]. Aspergillus flavus was grown and isolated in PDA at 28~31 °C and formed particular colonies that changed from white to green [3, 14]. The morphological identification of Fungi can be confirmed by molecular techniques like sequencing and blast analysis of the ITS1/ITS4 region [15].

This study was proceeded to document the clinical signs and lesions of Aspergillosis in artificially breeding black swan in DPR Korea, isolate and identify the causative fungi from lesions. Black swans at Swan Breeding Center in Taedong County, South Pyongan Province, DPR Korea were investigated. The infected swans died suddenly after two or three days when they lost appetite and had symptoms of dyspnea, sudden retraction or deformity of their neck and abnormal walking. The infected black swans had white fecal diarrhea. The fungal hyphae were also observed in the eyes. Such those results were consistent with previous observations in poultry and wild birds [11,13]. There was no blue or vomiting of the feces [4], however, the infected black swans had white diarrhea.

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Dead swans were necropsied and pulmonary materials including nodules were collected to be used as inoculum for fungal culture. When the swans were necropsied, yellow nodules were found in peritoneum (Fig. 1a), lungs (Fig. 1b) and air sacs. In severe cases, green fungal hyphae (Fig. 1c) which were typical macroscopic lesions of aspergillosis were observed [8]. The liver was heavily enlarged and yellowish (Fig. 1d). There were also bleeding spots around the arachnoid. Some researchers reported that Aspergillus flavus produce aflatoxins as secondary metabolites [10], leading to weight loss, immune dysfunction, and liver damage in birds including turkeys [12].

The morphological characteristics of the colonies were investigated by inoculating single spore on Malt Extract Agar (MEA), Czapek Yeast Extract Agar (CYA) and Czapek Dox Agar (CZ) from yellowish nodules of lungs in 6 black swans that died with aspergillosis symptoms according to [3].

When incubated at 26°C for 6 days, the colony diameter was 50±2mm in MEA while 53±3mm in CYA and 38±2mm in CZ. On MEA colonies were yellow green with sporulation rings and downside colors were brown. Colonies on CYA were green at the center and white at the edge with radial lines. Downside colors were white-yellow. On CZ the colonies were green at the center with white mycelia at the edge. Downside colors were grayish. The morphological characteristics like colony colors were similar with the findings of [3] who isolated Aspergillus flavus from maize and soil in Kenya.

The microscopic characteristics of fungus isolated from aspergillosis lesions in Black swans were investigated.

Vesicles were biseriate or monoseriate. The conidia heads were radial with phialides. Their diameters were 340~370μm. Conidia size was between 3.9~4.4μm. Conidiaphore walls were rough in appearance with no color. The morphological (Fig 2) and microscopic (Fig 3) features of the cultured colonies showed that the isolates from granulomatous nodules in black swan were similar to those of Aspergillus

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flavus. A comparison of morphological features based on the literatures [3, 14] showed the similar characteristics of A. flavus. In addition, identification by morphological characteristics of strains cultured on Malt Extract Agar (MEA), Czapek Yeast Extract Agar (CYA) and Czapek Dox Agar (CZ) as described in [3] was a very simple, easy and economical method for Aspergillus species.

The result of sequencing of 600bp-PCR product with primers ITS1(TCCGTAGGTGAACCTGCGG)/ITS4(TCCTCCGCTTATTGATATGC)[15] and Blast analysis showed that isolated and morphologically identified fungus was Aspergillus flavus.

Finally, the isolated fungi from lesions of black swans were identified as Aspergillus flavus M-Z-M1. Aspergillosis mostly caused by Aspergillus fumigatus in birds including wild swans [2,5] , but data related to Aspergillus flavus were rarely presented. Futher research could be helpful to eliminate potential fungal contaminants such as litter materials and select candidate antifungal agents.

In conclusion, this study suggest that Aspergillus flavus is one of the major agents causing aspergillosis in artificially breeding black swans in DPR Korea. It could be isolated from the nodules of aspergillosis lessions in black swans and identified easily by macroscopic and microscopic characteristics on different media such as MEA, CYA and CZ. The result of sequencing and blast analysis of ITS1/ITS4 region could confirm the morphological identification.

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РИСУНКИ

Fig 1. Clinical signs and Gross lesions of Aspergillosis in artificially breeding black swans

(a). nodules in peritoneum, (b). granulomatous nodules in entire lung, (c). fungal hyphae and green spores in air sacs in severe aspergillosis, (d). yellowish liver by aspergillosis

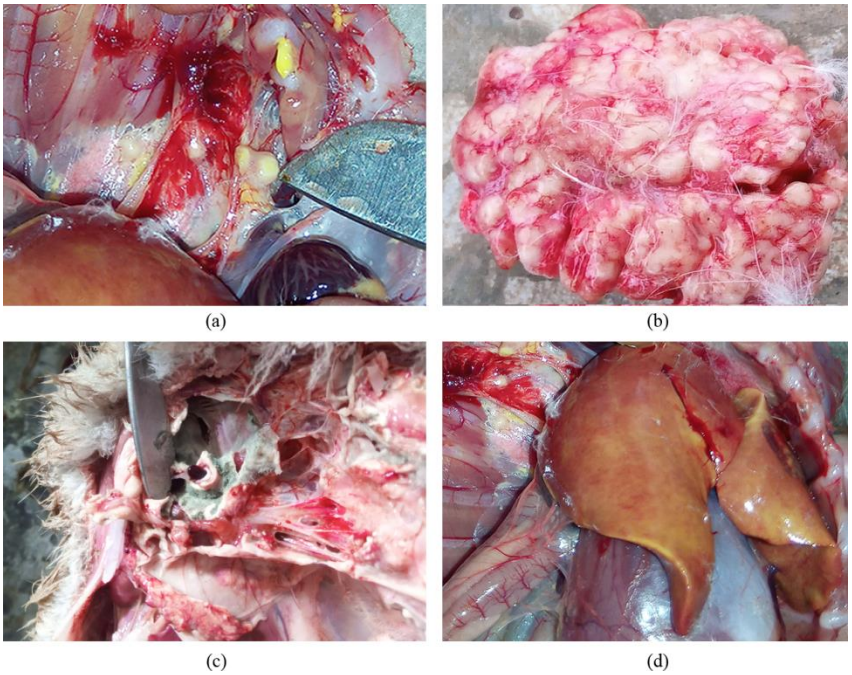

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Fig 2. Growth of agent fungal isolated from yellowish nodules of lungs in black swan with aspergillosis on different fungal media

(a) Malt Extract Agar (MEA): upside, (b) Czapek Yeast Extract Agar (CYA): upside, (c) Czapek Dox Agar (CZ): upside, (d) MEA: downside, (e) CYA: downside, (f) CZ: downside

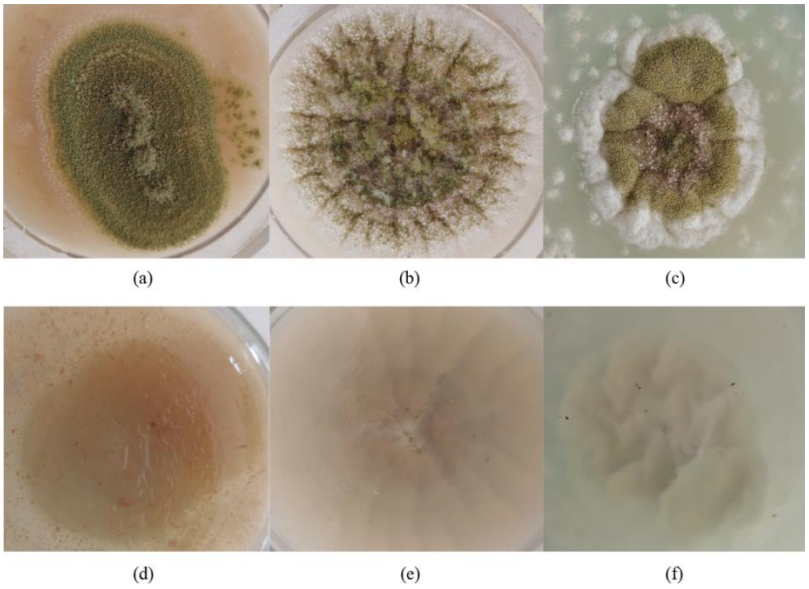

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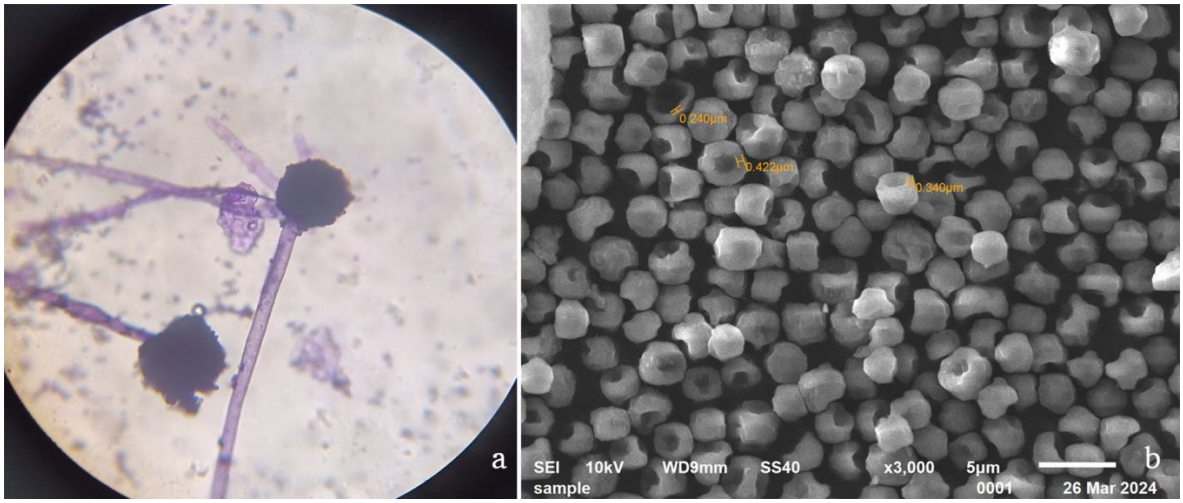
Fig 3. Microscopy of *Aspergillus flavus* isolated from Aspergillosis lesions in black swan.

(a) optical microscopic photograph, (b) scanning electron microscopic photograph



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ТИТУЛЬНЫЙ ЛИСТ_МЕТАДАННЫЕ

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Блок 3. Метаданные статьи

ASPERGILLOSIS IN ARTIFICIALLY BREEDING BLACK SWANS (*CYGNUS ATRATUS*) IN DPR KOREA AND IDENTIFICATION OF CAUSE FUNGUS

АСПЕРГИЛЛЕЗ ПРИ ИСКУССТВЕННОМ РАЗВЕДЕНИИ ЧЕРНЫХ ЛЕБЕДЕЙ (CYGNUS ATRATUS) В ДНР КОРЕЯ И ИДЕНТИФИКАЦИЯ ПРИЧИННОГО ВИДА ГРИБКА

Сокращенное название статьи для верхнего колонтитула:

Aspergillosis in Artificially Breeding Black Swans

Аспергиллез при искусственном разведении черных лебедей

Keywords: black swan, *Cygnus atratus*, Aspergillosis, *Aspergillus flavus*, respiratory disease.

Ключевые слова: черный лебедь, Cygnus atratus, Aspergillosis, Aspergillus flavus, респираторные заболевания.

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