TRADITION AND MODERNITY IN VETERINARY MEDICINE, 2023, vol. 8, No 2(15): 19–25

MULTIPLE ORGAN MYCOSIS AND NEOPLASIA IN AN INDIAN ELEPHANT (*ELEPHAS MAXIMUS INDICUS*)

Georgi Popov*, Vasil Manov, Teodora Popova

University of Forestry, Faculty of Veterinary Medicine, Sofia, Bulgaria
E-mail: gpopov@ltu.bg

ABSTRACT

A 58–year–old female Indian elephant (*Elephas maximus indicus*), owned by the Zoological Garden in Sofia, was examined postmortem. The pathomorphological, imaging and microbiological studies revealed lobular exudative pneumonia and zonal interstitial fibrosis with supramiliary calcifications and single extensive ossified areas. The uterine musculature was neoplastically transformed, and the endometrium with catarrhal–purulent inflammation. The results of the performed microbiological tests show the probable development of mycosis in the lungs and uterus of the animal, with the causative agent *Penicillium oxalicum*.

The obtained results are not described in the available worldwide literature, for the wild and circus and zoo–bred representatives of the elephant family.

Key words: Indian elephant, mycosis, pneumonia, endometritis, myoma.

Introduction

The Indian elephant is one of three subspecies of the Asian elephant and originates from mainland Asia (Wilson and Reeder, 2005). Since 1986, the Asian elephant has been listed as Endangered on the IUCN Red List as the wild population has declined (Williams *et al.*, 2020). The infectious pathology in this elephant is not well studied, including mycoses.

Species of the genus *Penicillium* are one of the most widespread molds. They are ubiquitous in the environment and, when isolated under laboratory conditions, are generally considered non–pathogenic to humans and animals. One of the reasons is the fact that their conidia are easily spread through the air. Therefore, histological evidence of clinical infection is also necessary when isolating them from clinical materials. However, there are reports of invasive fungal infections, primarily pulmonary, caused by species of this genus. Most often it concerns *P. marneffeis*, but there are also such data for other species such as *P. chrysogenum*, *P. citrinum*, *P. commune*, etc. (Mori *et al.*, 1987; Lyratzopoulos *et al.*, 2002; Ramírez *et al.*, 2018; Beena *et al.*, 2021). As early as 1951, Gilliam and Vest reported on human urinary tract mycosis caused by *Penicillium* spp.

Penicillium oxalicum has been reported as a cause of opportunistic fungal infection in patients with acute myeloid leukemia, diabetes mellitus, and chronic obstructive pulmonary disease (Chowdhary *et al.*, 2014). *P. oxalicum* has also been reported to cause genital infection in water buffalo (Kozakiewicz, 1992).

The aim of the present work was to carry out pathoanatomical, pathohistological and microbiological studies with a view to establishing diseases that contributed to the death of the elephant.

Materials and methods

Materials from the uterus (with leiomyoma) and lung with calcified areas from the female 58 years old elephant Artaida (Indian elephant), bred in "Zoo" Sofia, were pathomorphological and microbiological examined.

Gram– and Pfeiffer–stained microbiological impression samples were prepared and examined from the materials, as well as cultures on selective and elective nutrient media: Endo, Chapman, Columbia blood agar (Biolab Zrt. H–1141, Budapest Ov. utr.), Colorex orientation agar (HiMeida Laboratories Pvt. Ltd. Mumbai India, obtained by Ridadom–Sofia) and Sabouraud with chloramphenicol (BUL BIO NCIPD Ltd. – Bulgaria).

The species identification of the isolated fungi was performed according to Kozakiewicz (1992), and of the bacteria according to the Bergey International Identifier (Holt *et al.*, 1994) according to their morphological, cultural and biochemical characteristics.

For the histopathological examination the samples were fixed in 10% buffered neutral formalin solution. The fixed materials were dehydrated in gradual ethanol row – 50°, 60°, 70°, 80°, 90°, 96° and absolute alcohol. After dehydration, the samples were cleared in xylene and embedded in paraffin blocks. Sections with thickness of 5 μm were performed with a rotary microtome. The sections were attached to slides by histological glue and again processed with xylene, and then rehydrated by a descending alcohol row – absolute alcohol, 96°, 90°, 80°, 70°, 60°, 50°, and finally were stained with Hematoxylin–Eosin. Microscopic examination and photography were performed with a Levenhuk D740T light microscope with integrated camera.

Results

Stages of the performed autopsy and pathological examination are presented in Figures 1 and 2.

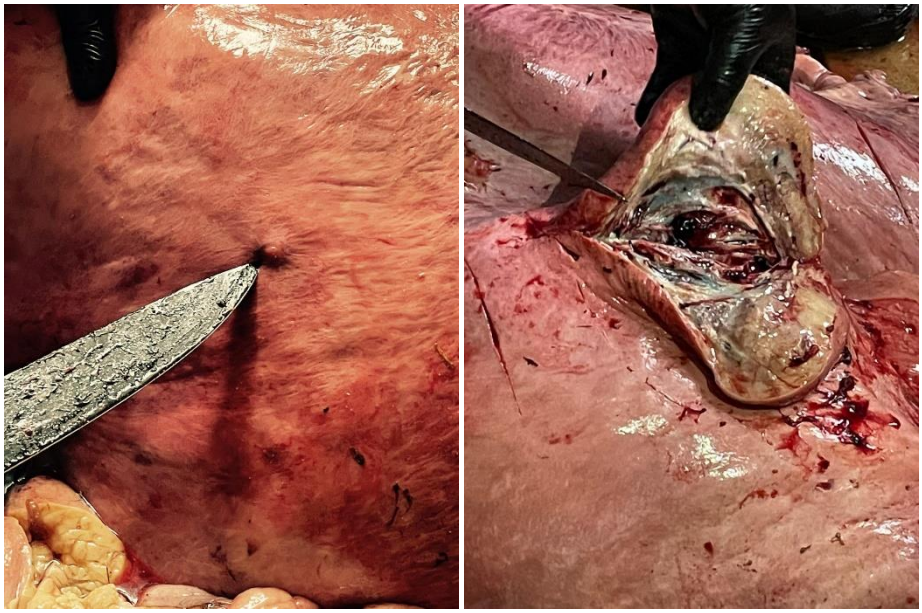
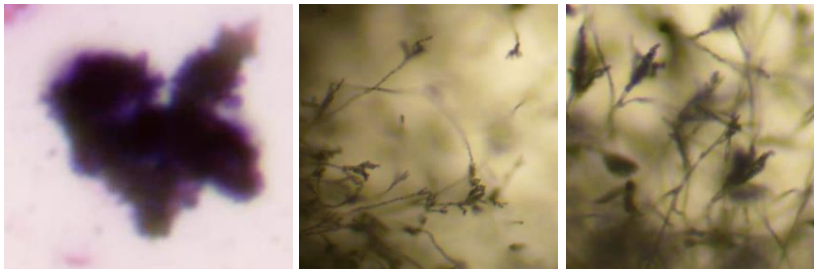


Figure 1: Calcification in a pneumonic region (to the left) and an extensive osseous lesion (to the right) in a pneumonic section of an Indian elephant lung.

Figure 2: Uterus with leiomyoma of an Indian elephant.

Hyphae and spores of filamentous fungi were microbiologically detected in the examined materials (Fig. 3, a).



a **b** **c**

Figure 3: Mycelium and spores of a mold fungus in the lung (a, Gram stain, x 1000) and in culture on Sabouraud agar (b and c), x 100.

At the 4–5th day from the beginning of cultivation, scattered velvety colonies were observed on Sabouraud's agar (Fig. 4), with a blue–green color and white areas in the periphery, and in many of them – in the center, without exudate, pale pink on the reverse. Strong sporulation was observed. The conidiophores were smooth, with 2–4 metules at the tips, and the conidia were large and elliptical (Fig. 3, b and c). According to the obtained results, the isolated mold fungus was identified as *Penicillium oxalicum* (Currie & Thom, 1915).

As a result of the microbiological research, *P. oxalicum* was isolated in large quantities from both materials (Fig. 4), as well as single colonies of *E. coli* and *Citrobacter spp.* with no indicative role as causative agents of infection in current case.

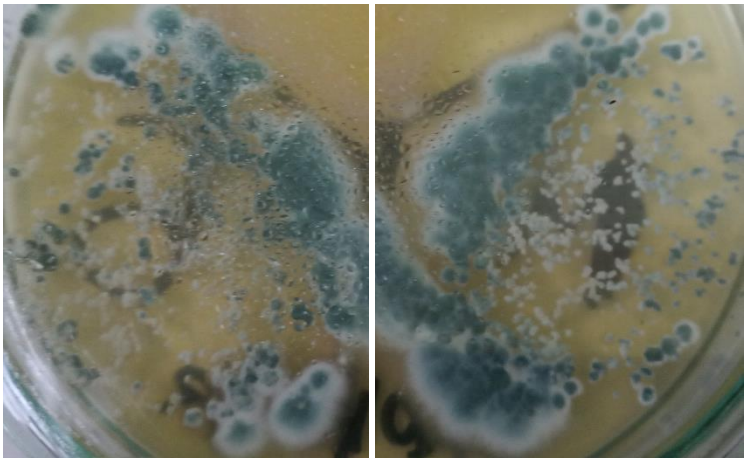


Figure 4: Colonies on Sabouraud agar of *Penicillium oxalicum* on the fifth day after inoculation of material from the lung (left) and from the uterus (right).

Extensive dark hyperemic areas with a dense consistency were observed during the pathomorphological examination of the lungs. A smooth and wet cut surface was found, from which a cloudy and liquid exudate, mixed with gas bubbles, poured (Fig. 5 A). Randomly distributed gritty structure were detected during the incisions in few areas of the lung. In the left caudal lobe, two extensive bony formations and numerous small calcifications with the size of a millet to a pea were found (Fig. 5 B). The largest bony lesion, which is about 10 cm long, 6 cm high and 4 cm thick, was examined radiographically. An X–ray–positive entity with varying intensity of calcification was found.

Catarrhal inflammatory edema in the alveolar and bronchial lumen were observed during the histopathological examination of the lungs. Protein (fibrin) casts, desquamated epithelium, granulocytes, alveolar macrophages, single lymphocytes and numerous erythrocytes were found in the exudate (Fig. 6 A). Dark stained calcifications were also observed in separate zones of the affected areas (Fig. 6 B).

During the pathological examination of the reproductive tract, a significant enlargement of the uterus was found. The uterus mass was around 50 kg. An inflammatory reaction of the endometrium was detected, which was swollen with erosions. Accumulation of cloudy mucus–like exudate was observed in the uterine lumen. Diffuse thickening of the uterine wall, which has a pale pink and dry cut surface, was noted.

A benign neoplastic process of smooth muscle origin was recognized in the histopathological examination of the thickened uterine structure. Chaotically distributed smooth muscle bundles composed of mature leiomyocytes were observed (Fig. 7 A). The cells had elongated rod–shaped nuclei with round ends (Fig. 7 B). No mitoses were detected. The stroma of the tumor was scarce. Growth was diffuse and endophytic.

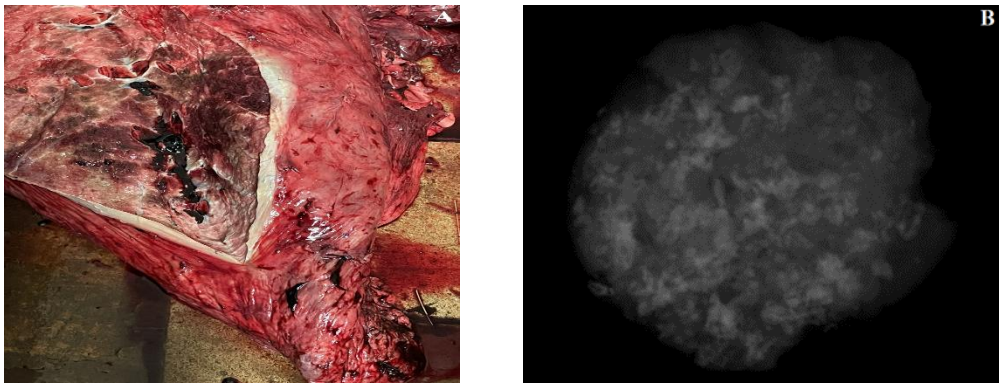


Figure 5: Pneumonic lung of an Indian elephant. A. Smooth and moist cut surface from which oozes cloudy and liquid exudate. B. X–ray examination of a bony lesion in the lung structure with varying intensity of calcification.

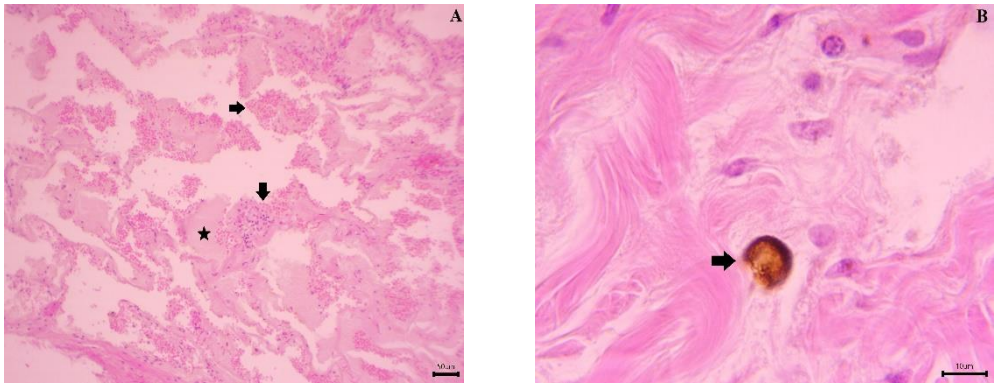


Figure 6: Microscopic examination of an Indian elephant lung (H&E). A. Inflammatory edema in the alveolar lumen with presence of eosinophilic, protein–rich fluid (asterix) mixed with inflammatory cells and erythrocytes (arrows) (100x). B. Fibrotic area of the inflammatory nidus with the presence of darkly stained calcium deposits (arrow) (400x).

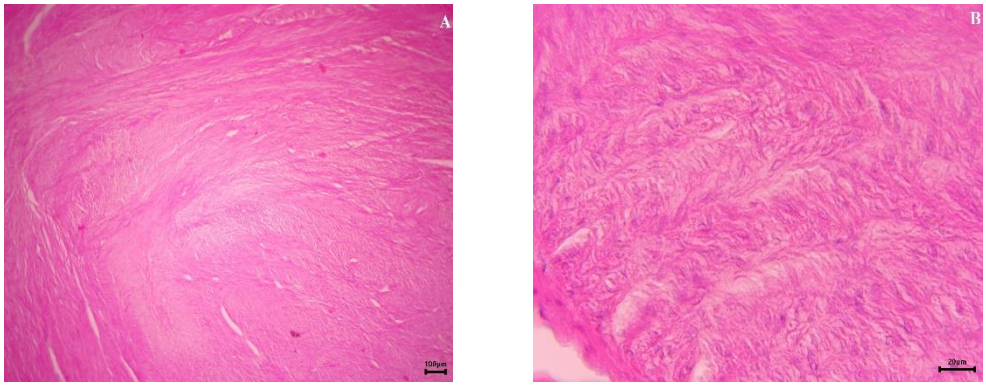


Figure 7: Microscopic examination of a uterine leiomyoma in an Indian elephant (H&E). A. Tumor parenchyma composed of chaotically distributed smooth muscle bundles with diffuse endophytic growth (100x). B. Bundles of mature leiomyocytes with elongated rod–shaped nuclei and round ends (400x).

Discussion

Infections caused by *Penicillium* species, except *P. marneffei*, are rare. Usually, a predisposing factor is a previous mechanical damage to the affected tissues caused by trauma, surgical intervention or prosthetic material, in cases of non–pulmonary diseases involving *Penicillium spp*. This pathogen is described as causative agents of allergic lung disease in humans (Lyratzopoulos *et al.*, 2002). Fungi of the genus *Penicillium* release chemical substances with biological activity such as acids, toxins and antimicrobial substances. When these pathogens developed in the affected tissues of the body, in addition to mechanical local damage, they probably play the role of pathogenicity factors with local and general toxic effects.

Mycotic pathogens often cause a proliferative inflammatory reaction in the affected tissues and organs. In the initial phase of infections, an acute inflammatory response with pronounced exudation is observed. Under the action of fungal causes, the alterative component of inflammation is significant, expressed in necrotization of the affected structures. In the necrotic zones, calcium is often deposited, due to the alkaline pH of the affected area and disturbed calcium metabolism. After the acute phase, a chronic proliferative inflammation develops, with accumulation of mononuclear cells and intense fibrosis.

P. oxalicum produces the highest amount of oxalic acid compared to the other species of the genus, accompanied by a decrease in the pH of the infected tissue (Ikotun, 1984). In addition, *P. oxalicum* exhibits strong antagonistic activity against plant pathogenic fungi. This species produces antifungal substances, with at least two active components proven (Yang, 2008). This type of mold is found in the soil, on corn and other cereals, and is pathogenic for greenhouse cucumbers. *P. oxalicum* also produces exotoxins, mainly hepatotoxins: secalonic acid D and oxalin, as well as roquefortin C. This type of mold is distributed worldwide, with a preference for warmer climates (Kozakiewicz, 1992). The presence of *P. oxalicum* in the lung samples is indicative that it plays a role in the etiopathogenesis of the catarrhal pneumonia that developed. The detected productive lesions, namely the fibrosis as well as the calcifications in the pneumonic areas of the elephant were an indication of lifelong inflammatory and degenerative–necrotic lesions of unclear genesis.

Due to the fact that *P. oxalicum* has been reported to cause genital diseases in water buffalo, the isolation this species from affected areas of the endometrium of the studied female elephant suggests a possible tropism of this species to develop in the reproductive system when entering the body other than the lung. In this case, a predisposing factor, in addition to the age of the animal, is probably the reduced protective response of the organ due to the developed neoplastic process of the smooth musculature.

Infections involving *Penicillium* spp. usually develop in patients with reduced general resistance due to various reasons, mainly non–communicable diseases (Chowdhary *et al.*, 2014). Probably, breeding in captivity and in solitude, under conditions different from the natural habitat, the susceptibility of the organism to such opportunistic infections increases. Even with compliance to the maximum extent with all the necessary requirements of the respective species of animals kept in a zoo, as well as providing excellent hygiene, complete nutrition and veterinary medical care, as was this case with the elephant, the organism is more susceptible to such infections than in their natural wild environment. Of course, advanced age also matters in this aspect.

Conclusion

The results of the microbiological studies of the materials from the lung and uterus of the elephant indicate the possibility of the development of mycosis in these organs with the causative agent *Penicillium oxalicum*. In combination with predisposing factors, such as senile weakness of the organism and concomitant diseases, including neoplastic ones, the causative agent induces an inflammatory reaction in the affected organs.

References

1. Beena, H., Gupta M., Kindo A. J. (2021). *Pulmonary infection with Penicillium citrinum in a patient with multiple myeloma*, Indian Journal of Medical Microbiology, 39(2), 259–261,
2. Chowdhary, A., Kathuria S., Agarwal K., Sachdeva N., Singh P. K., Jain S., Meis J. F. (2014). *Voriconazole–Resistant* Penicillium oxalicum*: An Emerging Pathogen in Immunocompromised Hosts*. Open Forum Infectious Diseases, 1(2), ofu029,https://doi.org/10.1093/ofid/ofu029
3. Gilliam J. S. and Vest S. A. (1951). *Penicillium Infection of the Urinary Tract.* The Journal of Urology, 65(3), 484–489.
4. Holt, J. G., Krieg N. R., Snear+t P. H. A., Staley J. T., Williams S. T. (1994). *Bergey's manual of determinative bacteriology*. Ninth edition, Williams & Wilkins.
5. Ikotun, T. (1984). *Production of oxalic acid by Penicillium oxalicum in culture and in infected yam tissue and interaction with macerating enzyme*. Mycopathologia 88, 9–14. https://doi.org/10.1007/BF00439288
6. Kozakiewicz, Z. (1992). *Penicillium oxalicum*. [Descriptions of Fungi and Bacteria]. IMI Descriptions of Fungi and Bacteria, 111, Sheet 1107.
7. Lyratzopoulos, G., M. Ellis, R. Nerringer, D. W. (2002). *Denning. Invasive Infection due to Penicillium Species other than P. marneffei,* Journal of Infection, 45(3), 184–195.
8. Mori, T., Matsumura M., Kohara T., Watanabe Y., Ishiyama T., Wakabayashi Y., Ikemoto H., Watanabe A., Tanno M., Shirai T. and Ichinoe M. (1987). *A Fatal Case of Pulmonary Penicilliosis*. Jpn. J. Med. Mycol., 28, 341–348.
9. Ramírez I., Hidrón A., Cardona R. (2018). *Successful treatment of pulmonary invasive fungal infection by Penicillium non–marneffeiin lymphoblastic lymphoma: case report and literature review*. Clin Case Rep., 6(6), 1153–1157.
10. Williams, C., Tiwari S.K., Goswami V.R., de Silva S., Kumar A., Baskaran N., Yoganand K. and Menon, V. (2020). *Elephas maximus, Asian Elephant*. The IUCN Red List of Threatened Species™ ISSN 2307–8235 (online) IUCN 2020: T7140A45818198 Scope(s): Global Language: English.
11. Wilson, D. E., and Reeder D. M. (2005). *Mammal Species of the World: A Taxonomic and Geographic Reference*. JHU Press. ISBN 978–0–8018–8221–0.
12. Yang, L., Xie J., Jiang D., Fu Y., Li G. and Lin F. (2008). *Antifungal substances produced by Penicillium oxalicum strain PY–1—potential antibiotics against plant pathogenic fungi*. World J Microbiol Biotechnol, 24, 909–915.