

# **ASPERGILLUS FUMIGATUS INFECTION IN A PIGEON FLOCK**

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## **Abstract**

An aspergillosis outbreak in a flock of near 350 pigeons was described clinically, microbiologically, and histopathologically. The pigeons showed dyspnoea, depression, rattling, and dyskinesia, and numerous cases of death were noted. Five young moribund pigeons, their bedding, and fodder were examined. The examinations were conducted according to the generally accepted methodologies and recommendations for mycological diagnostics. Paraffin sections of the lungs, trachea, pharynx, and thoracic air sacs were stained with haematoxylin and eosin and periodic acid-Schiff method. The mycological examinations demonstrated the presence of *Aspergillus fumigatus* cells in swabs from the beak cavity of living birds, and in the lungs and air sacs examined post mortem. The presence of *Candida albicans* and single isolates of *Penicillium* sp. and *Scopulariopsis* sp. were also detected in the beak cavity. The mycological examinations of bedding (coniferous shavings) showed its evident contamination. The dominant presence of *A. fumigatus* and some colonies of *Mucor* sp., *Acremonium* sp., and *Trichoderma* sp. were recorded. The feed supplied, regardless of its kind, did not contain any *A. fumigatus* cells. Macroscopically, white-yellowish nodules observed in the lung and air sacs corresponded to acute aspergillosis lesions. Histopathological analysis of the affected organs displayed multifocal areas of necrosis, inflammatory infiltration, and the presence of fungal hyphae, giant cells, and fibrous tissue proliferation at the periphery of the nodules were noted.

**Key words:** pigeons, aspergillosis, *Aspergillus fumigatus*.

Aspergillosis is a respiratory tract infection caused by fungi of the *Aspergillus* genus, in which *Aspergillus fumigatus* is the primary species responsible for infections, both in mammals and birds. Avian aspergillosis, is mainly observed in young turkeys, chickens, and waterfowl (1, 3, 6). It is also reported in other birds including pigeons, quails, ostriches, and penguins (4, 8, 10, 12, 14).

*A. fumigatus* is a ubiquitous and opportunistic fungal pathogen causing severe invasive infections that occur in susceptible organisms showing a breakdown of their defence systems. A specific pathogenicity of *A. fumigatus* is conditioned by its conidium size. Its diameter under 2 µm allows its penetration into small bronchioles deprived of any natural defence systems and thus opened for the lung tissue colonisation (13). Fungus cells capacity to grow and multiply at up to 55°C facilitates the fungus proliferation and its growth invasiveness. The proteolytic enzyme expression as well as serine and aspartic proteases and phospholipases are conducive for the colonisation of lungs and perhaps some other organs, while gliotoxin acts immunosuppressively, cytolytically and inhibits protein synthesis in the host's cells, that in turn induces some disorders in organ functioning and damage of the organs.

Although, the lungs and air sacs are the most often involved organs, the trachea, syrinx, and bronchi may be affected as well (5).

Stress seems to be a major factor predisposing to the development of the disease. It can be caused by transportation, heat, capturing, or changes in the management. Vitamin deficiencies – especially vitamin A, long-term antibiotic use, and age also contribute to the onset of the disease, as well as dusty environments, lead poisoning, and irritants of the airways, like ammonia.

Aspergillosis is divided into two forms: acute and chronic. The acute form primarily occurs in newly hatched birds and has also been found in free-ranging fowls or psittacines under the poor sanitary or ventilation conditions. It results from exposure to a large number of spores. In this form of the disease, the fungus can be isolated from lungs and many other tissues like liver, spleen, or blood. A chronic form is more likely to occur in older birds kept in confinement (9). Chronic aspergillosis is divided into focal: nasal, tracheal, cutaneous, and ophthalmic and generalised form. At generalised aspergillosis, the lungs and air sacs are

chronically infected, resulting in gradual reduction in the respiratory function. Focal aspergillosis shows a better response to treatment, while in the generalised form, the cure is prolonged, usually ineffective, and the prognosis is poor.

This study presents the clinical, microbiological, and histopathological findings observed in the pigeons during an aspergillosis outbreak in a flock.

## Material and Methods

**Animals.** Five young moribund pigeons, their bedding, and fodder were brought by a private bird keeper to the Department of Veterinary Microbiology. The birds (near 350 pigeons) were kept in two dovecotes and supplied with clean water and feed, comprising of barley, maize, sunflower, vetch, rye, and wheat mixture *ad libitum*. Coniferous shavings served as a litter.

For the two-week period, the pigeons showed progressing inappetence, decreasing weight, and at that time, thirty-six young pigeons died. The clinical symptoms of the diseased birds included listlessness, anorexia, dyspnoea, open mouth breathing, coughing, green watery diarrhoea, increased respiration, mucoid discharge from nostrils, wry neck, and lameness. There was no evidence of any trauma, injury, or presence of ectoparasites. All the pigeons prior to the study were treated with doxycyclin and enrofloxacin for a week but no clinical response was observed.

**Mycological examinations.** An autopsy was performed with the aseptic techniques. The tracheal and pharyngeal swabs and samples of the lungs and air sacs were collected from the dead pigeons onto sterile glass Petri plates.

The examinations were conducted according to the generally accepted methodologies and recommendations for the mycological diagnostics. The tissue samples were inoculated on Sabouraud dextrose agar with chloramphenicol (0.05 mg/mL) and incubated at 25°C and 37°C under aerobic conditions.

The samples of litter and feed, *i.e.* 1 g of coniferous shavings and 1 g of grain (wheat, vetch, rye and barley) were suspended separately in 10 ml of Sabouraud liquid medium and incubated for 30 min at 25°C. Afterwards, the samples were shaken; the supernatant was collected after 3-5 min, filtered through the aseptic gauze to be finally sown at volume of 0.2 ml on the Sabouraud dextrose agar with chloramphenicol (0.05 mg/mL) and incubated at 25°C and 37°C under aerobic conditions.

**Histopathological examinations.** The samples of the lungs, trachea, pharynx, and thoracic air sacs were fixed in 10% neutral buffered formalin, routinely processed and embedded in paraffin blocks. The 5 µm sections were stained with haematoxylin and eosin (HE) and periodic acid-Schiff (PAS) method. The morphological evaluation of the samples was performed under the light microscope.

## Results

The pigeons showed the signs of dyspnoea, depression, rattling, and dyskinesia, and numerous cases of death were noted. The mycological examinations demonstrated the presence of *A. fumigatus* cells in the swabs from the beak cavity of living birds and in the lungs and air sacs examined post mortem. The culture examinations revealed an intensive growth of *A. fumigatus* in all cases. The presence of *Candida albicans* and single isolates of *Penicillium* sp. and *Scopulariopsis* sp. were also detected in the beak cavity.

The mycological examinations of bedding showed its evident contamination. The dominant presence of *A. fumigatus* and some colonies of *Mucor* sp., *Acremonium* sp., and *Trichoderma* sp. were recorded.

A feed supplied, regardless of its kind, did not contain any *A. fumigatus* cells. Some other fungi were detected at a low quantity and their genus was dependent on a feed type. The wheat was shown to contain *Mucor* sp., *Alternaria* sp., *Cladosporium* sp., *Penicillium* sp., and *Acremonium* sp., barley – *Fusarium* sp. and *Cladosporium* sp., while triticale – some spores of *Acremonium* sp., *Cladosporium* sp., and *Alternaria* sp., whereas vetch only *Mucor* sp. It is noteworthy that the isolated fungus genera were represented by only single colonies. No homogeneous fungus culture with numerous colonies was demonstrated.

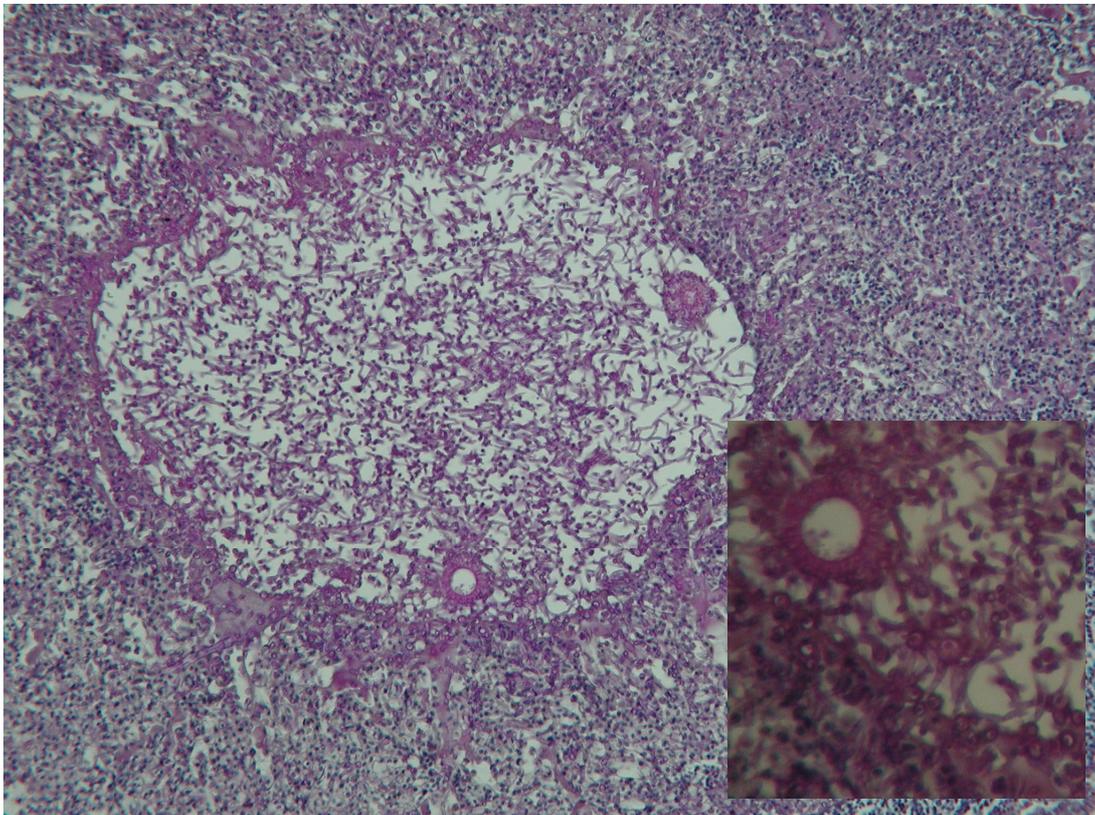
At post-mortem examination of the affected pigeons, white to yellow caseous nodules were observed in the beak cavity, pharynx, lungs, and thoracic air sacs (Fig. 1). Nodules observed in the lungs and air sacs corresponded to acute aspergillosis lesions. Histopathological analysis revealed the multifocal areas with necrosis, inflammatory infiltrates, and the presence of fungal hyphae, giant cells, and fibrous tissue proliferation at the periphery of the nodules. The nodules in the lungs, developed around fungal colonies, consisted of long branching septate hyphae (Fig. 2). In some cases, a tendency to forming conidiophores and spores of *A. fumigatus* was observed (Fig. 3). The foci were surrounded by a zone of neutrophils, macrophages, and tissue debris (Fig. 4). The larger foci were encapsulated with fibrous tissue and infiltrated with lymphocytes, macrophages, and occasionally multinuclear cells. The granulomatous tissue expanded and compressed the adjacent alveoli.

## Discussion

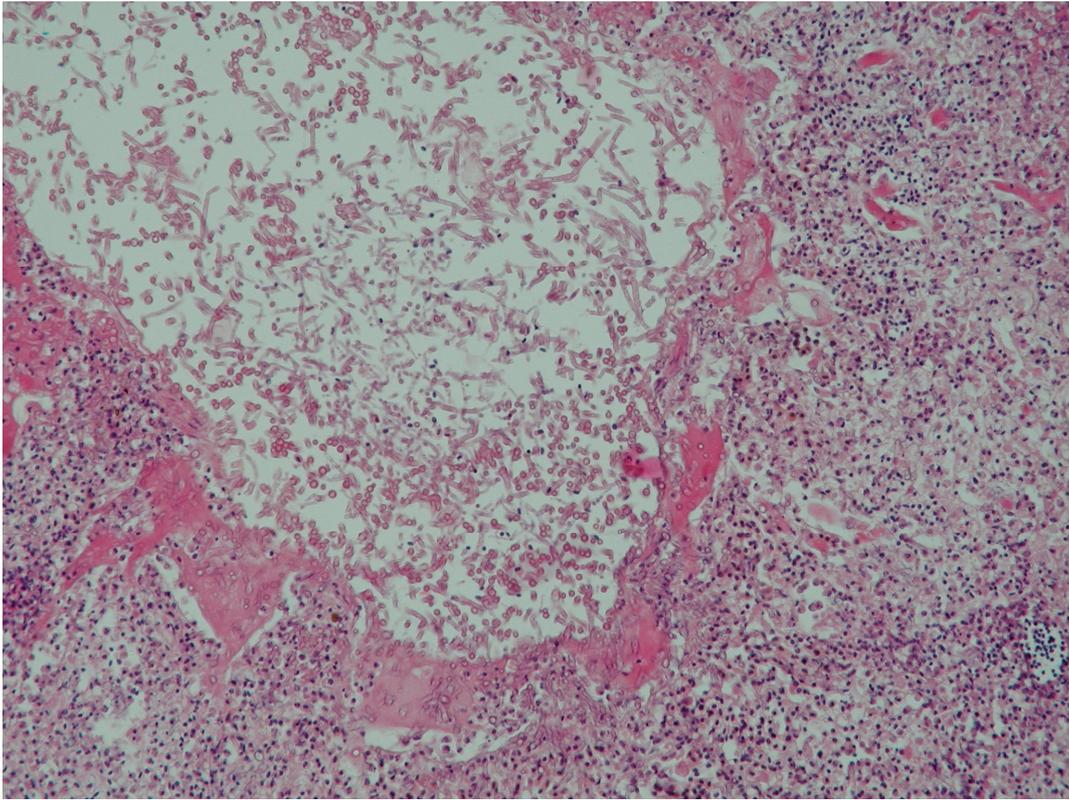
The changes in the environment, its contamination and degradation, contribute to the increasing occurrence of fungi colonisation of the living organisms. The most pathogenic fungi attacking all the species of domestic, ornamental, and wild birds all over the world include *Aspergillus* genus. *Aspergillus fumigatus* and *A. flavus* are most frequently isolated, and then *A. niger*, *A. glaucus*, *A. versicolor*, and *A. terreus* (10, 11).



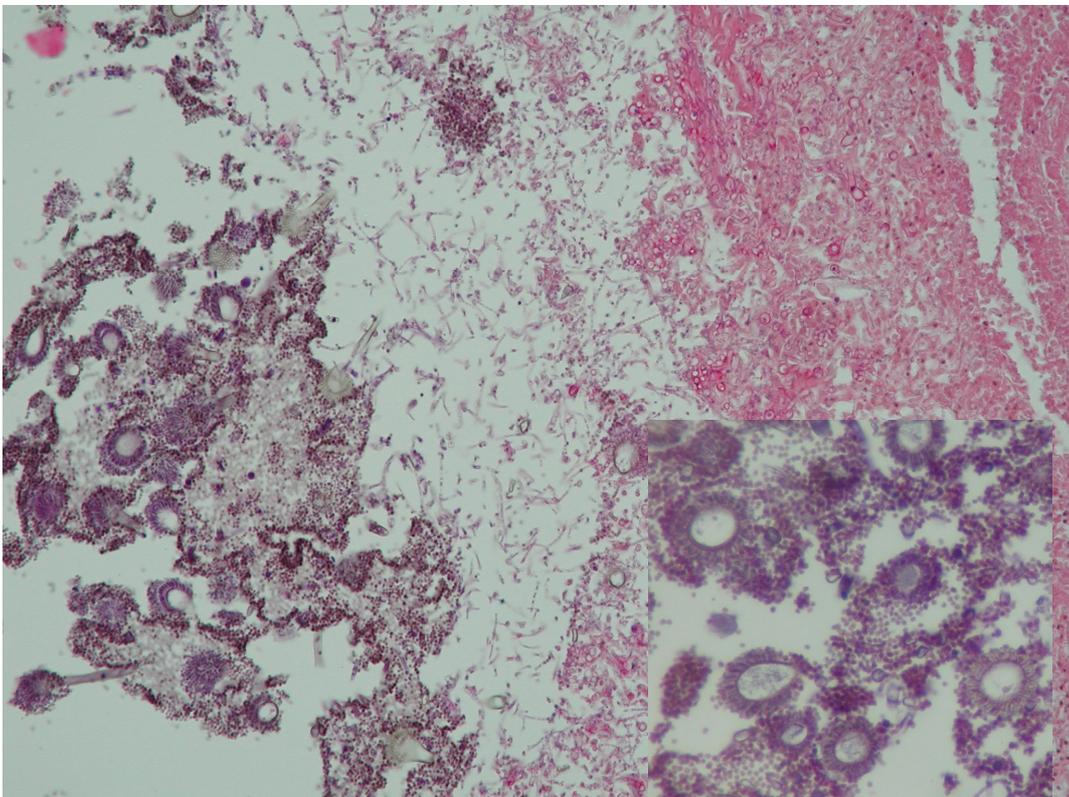
**Fig. 1.** White-yellowish caseous mass in beak cavity.



**Fig. 2.** The fungal colony in the lung. PAS staining, 100x.



**Fig. 3.** Branching septate hyphae of *A. fumigatus* in the lung. Inflammatory reaction at the periphery. HE, 200x.



**Fig. 4.** Hyphae, conidiophores, and many conidia in the lung. HE, 200x.

According to the researches carried out in 1986-1995 by the Department of Veterinary Hygiene in Poznań (11), out of the total number of 1 461 died birds, 98 cases of mycosis were recognised and among them *Aspergillus* was isolated in 87 cases, which made 5.9% of the examined bird population. Among the isolated fungi, *A. fumigatus* was recovered in 77.5%, *A. flavus* in 19.1%, and *A. glaucus* in 1.1%.

*Aspergillus* sp. infection affects mainly the respiratory system; however, the *per os* infection is possible as well. The highest susceptibility is exhibited by embryos and chicks, while extreme sensitivity of chickens to the mycotic infection results from a higher body temperature (*ca* 42°C) and the presence of air sacks in which the conditions conducive for fungal development occur (7). Some other factors predisposing to infection incidence is confinement of birds (cages, aviaries), poor sanitary conditions, mouldy litter, deficiency of vitamin A and some elements in feed, and litter or sand coming from coniferous woods, especially pines. In each pigeon flock studied, it was the litter of coniferous shavings that were largely contaminated with *A. fumigatus* organisms, and this was likely to constitute a source of bird infection. The factors predisposing to an increased risk of aspergillosis outbreaks include a prolonged and uncontrolled antibiotic therapy applied by the pigeons' owners, as well as the coincidence of viral, bacterial, or parasitic diseases.

In free-living birds, including pigeons, aspergillosis is mainly linked with poor weather conditions and climate, especially high air moisture and low temperature in winter, as well as high temperature in spring. Raczyński and Kempski (11) reported far more frequent cases of aspergillosis in the winter period in dead birds delivered from the zoological garden, individual owners, and foresters. The presented outbreak of aspergillosis in the pigeon flock was also recorded in the winter season.

Acute aspergillosis in the examined flock was characterised by necrotic lesions and the presence of mycelium and *A. fumigatus* conidiophores in the histological preparations of the lung tissues. Similar results were reported by Yokota *et al.* (14) in ostriches, Atasever and Gumussoy (2) in starlings, and Carrasco *et al.* (4) in penguins.

Taking into account the fact that mycotic infections (in most cases) are rated among the contagious but not transmissible diseases, and the major source of flock infection is its surrounding environment and feeds. Therefore, a crucial role in the control of mycoses proves to play the recognition and elimination of the infection source by the disinfection with specific antifungal preparations (15). If chemotherapy is applied, owing to a high and varied resistance level typical of

most fungi, it seems important to ensure appropriate identification (at genus level) of the etiological agent as well as to determine *in vitro* its sensitivity to the drugs administered.

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