

Case Report**Outbreak of Respiratory Aspergillosis in Backyard Duck Flock in Tunisia****Khaled Kaboudi^{1*}, Ahmed Rejeb², Moncef Bouzouaia¹, Muhammad Tanveer Munir³ and Sajid Umar⁴**¹Department of Poultry Farming and Pathology, National Veterinary School of Tunisia, 2020 Sidi Thabet (Ariana), TUNISIA²Department of Pathological Anatomy and Histology – National Veterinary School of Tunisia, 2020 Sidi Thabet (Ariana), TUNISIA³Nantes Atlantic National College of Veterinary Medicine, Food Science and Engineering (ONIRIS), 44300 Nantes, FRANCE⁴National Veterinary School of Toulouse, 23 Chemin des Capelles, 31 076, Toulouse Cedex 3, FRANCE***Corresponding author:** kaboudikhaled17@gmail.com

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Abstract

Aspergillosis is one of the most frequent mycosis affecting avian species. Here, we report an outbreak of aspergillosis in a backyard flock of ducks (Anas platyrhynchos), aged between 4 wks to 1 year with respiratory signs as dyspnea, gasping and nasal discharge, and up to 14.28% mortality. At necropsy, congested lungs, exudative trachea-bronchitis, airsacculitis and extensive multifocal granulomatous pneumonia were recorded. Histopathology examination revealed chronic multifocal mycotic granulomatous pneumonia. Aspergillus fumigatus was identified by microbiologic study from air sacs and lungs of all autopsied animals. Farm floor was disinfected and bedding material was changed followed by treatment of ducks with copper sulphate (1 g/5 liters) in drinking water fortnightly for two months. Clinical signs and mortality were abided after two months.

Key words: Aspergillosis, Duck, Histopathology, Tunisia

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Introduction

Aspergillosis is a mycotic disease affecting many species of domestic and wild birds. It is caused by several species of the genus *Aspergillus* spp. *Aspergillus fumigatus* is the most important and frequent one implicated in cases of aspergillosis in poultry (Charlton *et al.*, 2008). Alike most of fungal species, it is an opportunistic pathogen, causing disease in immunocompromised birds or in birds exposed to overwhelming numbers of fungal spores (Arné *et al.*, 2011 ; Umar *et al.*, 2015a & b; Copetti *et al.*, 2015).

In most cases, the primary site of development is the respiratory tract (air sacs and lungs) but blood dissemination frequently occurs, leading to macroscopic lesions in a wide range of organs or tissues. Indeed, airsacculitis is a major reason for carcass condemnation at slaughter inspection. In spontaneous cases, lesions range from miliary to larger granulomatous foci, which are white in color and protrusive to the surface of the internal organ (Perelman and Kuttin, 1992; Femenia *et al.*, 2007). Infection by *Aspergillus* sp. has been reported in almost all domesticated avian species and production types: layer cockerels, pullets in cages, broiler breeders, and growers of chicken or turkey poults, common duck breeders, goslings, great rheas, ostriches, Japanese quails, or pigeons (Beernaert *et al.*, 2010). In spontaneous outbreaks, mortality ranged between 4.5% and 90% depending on age and immune status of birds (Arné *et al.*, 2011). Here is reported the first case of aspergillosis in free range ducks in Tunisia manifesting severe respiratory signs. This paper describes clinical, mycological and pathological findings of a rare case of aspergillosis in a free-range flock of ducks.

Case Details

History of Case

The affected farm of ducks (*Anas platyrhynchos*) is located in Kâlaat-El Andalous, Ariana (in the north of Tunisia) at lat. 36.51° N and long. 10.11° E. Severe respiratory signs such as dyspnea, gasping and nasal discharge were observed in a backyard flock of 70 ducks (aged between 4 wks to 1 year) in March 2013. The management of the ducks was carried out under semi-intensive confinement condition having wheat straw as bedding material. The morbidity and the mortality rates were, 31.43% (22 birds) and 14.28% (10 birds), respectively. For case investigation, a total of 10 dead ducks were sent to Department of Poultry Farming and Pathology, National Veterinary School of Tunisia, Sidi Thabet (Ariana), Tunisia Necropsy was performed on six recently dead ducks and gross lesions were recorded. The advanced putrefaction of other dead animals did not allow the necropsy examination. Pieces of lung and air sacs (thoracic and abdominal) were collected aseptically, fixed in 10% buffered formalin and sent to histopathological and mycological analysis. The samples were routinely processed for histopathology, cut into 3- μ m sections, and stained with Haematoxylin and Eosin (HE), and periodic acid-Schiff (PAS) (Bancroft and Gamble 2008). For mycological examination, samples of different organs showing gross lesions were cultured on Sabouraud dextrose agar (Oxoid Ltd., Basingstoke, Hampshire, U.K.) and incubated at 37°C during 48 hours. Some colonies were stained with methylene blue and examined under the microscope for a possible identification of the fungal species. All samples were also cultured in several media: MacConkey agar (Oxoid Ltd., Basingstoke, Hampshire, U.K.), blood agar base (Oxoid Ltd.) and Columbia agar base (Oxoid, Ltd.) supplemented with 5% sterile ovine blood. With the aim of saving the remaining animals, floor was disinfected and bedding was changed and followed with treatment with copper sulphate

(CuSO₄) in drinking water at 1 g per 5 liters fortnightly for two months, according to the therapeutic protocol of Musa *et al.* (2014).

Results

White-grey nodules (0.1-0.5 cm diameter) in lungs and air sacs, associated with lungs congestion lungs and exudative tracheo-bronchitis were described at necropsy of all dead birds (Fig. 1, 2 & 3).



Fig. 1: Sero-exudative trachea-bronchitis in a male duck with aspergillosis.



Fig. 2: Exudative pneumonia with yellow-grey nodules (1-3 mm) (arrows) in a male duck with aspergillosis

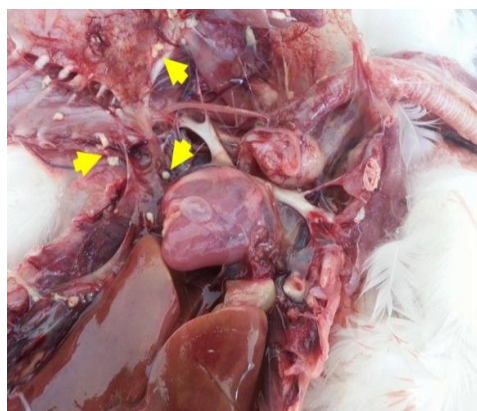


Fig. 3: Yellow-grey plaques (arrows) in the air sacs of male duck suspected

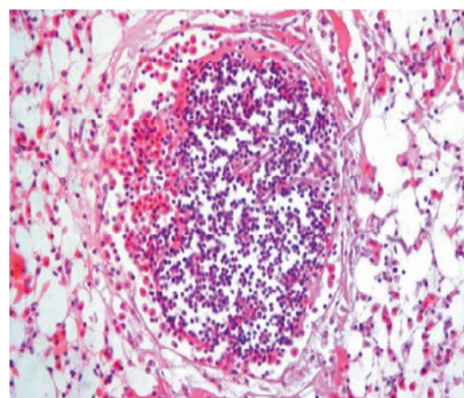


Fig. 4: Lung showing granulomatous lesion with fungal hyphae (arrow) and cellular infiltration (arrowhead) (HEx200)

Histopathological examination revealed nodules of different sizes with small areas of necrosis surrounded by intense granulomatous inflammation consisting of heterophils, lymphocytes, macrophages, numerous

multinucleated giant cells and epithelioid macrophages (Fig. 4). Aspergillitis and mycotic granulomatous pneumonia was observed in 100% (6/6) necropsied birds. Branched septate hyphae, about 10–15 μ m in diameter in PAS stained lung sections (Fig. 5, 6, 7).



Fig. 5: Sabouraud plate exhibiting whitish green color colonies of *Aspergillus fumigatus*.

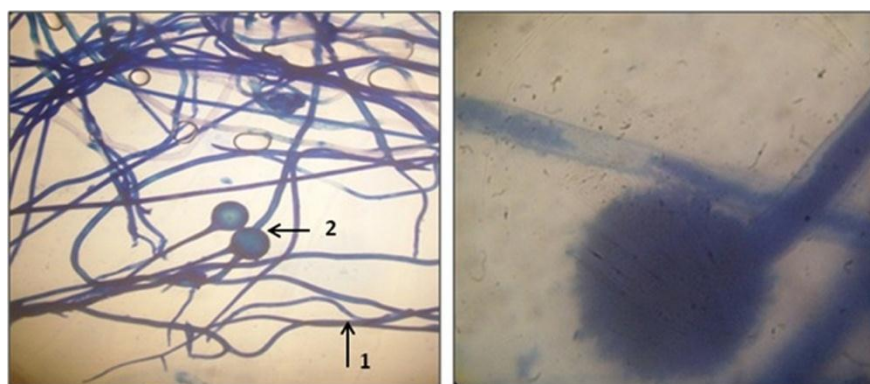


Fig. 6: Methylene blue stain shows (1) fungal heads and (2) hyphae (A: Gx40; B: Gx100).

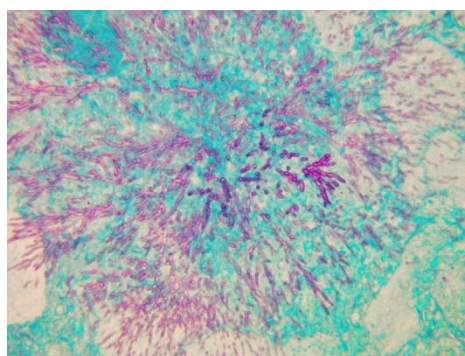


Fig. 7: PAS stained lung section of duck with aspergillosis shows several fungal hyphae in the center of necrotic foci.

The samples of lung showed growth of fungal colonies at mycological analysis. Initially, the colonies were white, acquiring a greenish colour with age. After isolation and proper cultivation of these fungal colonies, the microscopic analysis revealed conidiophores enlarging gradually into pyriform vesicles fertile over half or more of the enlarged area, bearing phialides only, and conidia of 2.5–3.0 μ m, typical of *Aspergillus fumigatus*. The microbiologic study revealed negative results for bacteria isolation.

Discussion

The clinical signs observed in these birds were respiratory distress, dyspnea, gasping and nasal discharge in some birds. Post-mortem examination revealed congested lungs, greyish white nodules and exudates in trachea of some ducks. Nodules, with 1-3 mm of diameter, are localized in the lungs and the air sac. Planel *et al.* (2001), Bhattacharya (2003) and Chu *et al.* (2012) reported similar findings in commercial duck flocks. Many risk factors are discussed in poultry aspergillosis cases. Type and quality of litter are usually considered as important factors. In this study, it is obvious that wet litter is a favorable condition for the development of *Aspergillus* spores, and consequently a potential source of infection, which is also substantiated by Dyar *et al.* (1984) and Rao *et al.* (1982). In the same way, it was speculated in the study of Sajid *et al.* (2006) and Sultana *et al.* (2015) that the sawdust is more favorable for the fungal growth as compared to rice husk because of its higher moisture contents, allowing the fungal growth. This form of aspergillosis was more observed in older turkeys and captive wild birds. Nodules may be also found at the level of the syrinx or adjacent to large bronchi in the lung (Corkish 1982; Sultana *et al.*, 2015). These lesions can explain respiratory symptoms on surviving birds. Kunkle and Rimler (1996) described severe pleuritis, pneumonia and mild interstitial inflammation involving deep to lateral margins in commercial turkeys.

Occasionally, organisms, spread from the lung, localize in the eye, causing ophtalmitis, or the brain causing encephalitis (Hubben, 1958). No gross abnormal changes were noted in any other viscera. *Aspergillus fumigatus*, isolated from the affected lungs and air sacs of the dead ducks, was the most important cause of aspergillosis in birds (Campbell, 1986). Identification of this species was made based on colony characteristics (velvety white colonies, at first, which turned dark green to greyish later) and numerous branched septate hyphae under microscope staining with methylene blue. Microscopic observations showed the morphology and the organization of hyphae, which appeared with smooth and colorless walls. These hyphae were organized in a network, each of them have at its top a hemispherical vesicle, and surrounded by conidia. These elements (hyphae, vesicle and conidia) form a fungal head. Species of *Aspergillus* can be identified by observing the characteristic conidial head and colony (Fowler, 1993) (*in* Girma *et al.*, 2016). In this way, the microscopic structures, associated to the cultural properties (growth, colonies aspect), corresponding to the genus *Aspergillus* and are considered as a diagnosis

criteria of the fungal specie (Hungerford *et al.*, 1998). Our findings were in agreement with the results of Yokota *et al.* (2004) and Sajid *et al.* (2006) who reported that white to green mold growth on the walls of caseous thickened air sacs of infected ostrich and commercial poultry flocks, respectively. Ustimenko (1982) also isolated *Aspergillus fumigatus* from lung tissue of dead chicken.

Histology of the lungs showed in HE stain, a subacute to chronic granulomatous pneumonia. Nodules or plaques are characterized by centers of eosinophilic cellular debris surrounded by multinucleated giant cells and epithelioid macrophages, which in turn are surrounded by fibrous tissue and lymphocytes. Our findings are in concordance with the results of Richard *et al.* (1984), Chu *et al.* (2012) and Sultana *et al.* (2015) in commercial turkeys, ducks and chickens, respectively. Hubben (1958) described the same lesions found in meninges and cerebral sections in ducks and turkeys manifesting nervous symptoms caused by *Aspergillus* spp. Infection. Mycelia may be visible in necrotic centers in haematoxylin-and-eosin stained sections either as unstained or faintly basophilic staining mycelia but they are best demonstrated with fungal stains (Ficken, 1996). In fact, hyphae were observed in PAS stained sections of lungs.

In order to limit the contamination of other subjects and control the disease in this flock, floor was disinfected and litter was removed and replaced by new dry hay. Birds were treated by copper sulphate in drinking water. Clinical improvement was observed after the end of the treatment. Indeed, no mortality and respiratory signs were detected in the flock. The efficacy of aspergillosis treatment is disputable, which explains the guarded prognosis. It is not usually done because it is expensive, long-lasting and associated with frequent relapses. Drugs, like itraconazole and amphotericin B have been successfully used in valued birds. However, these substances are not authorized for the poultry use in Tunisia. The choice of copper sulphate, used by other authors (Musa *et al.*, 2014) to treat aspergillosis in broiler breeders, was justified by its availability and cost.

Conclusion

Aspergillosis is a potentially fatal infection caused by the most important and common, fungus, *Aspergillus fumigatus*. All domestic and wild birds are susceptible to infection, which is spread by fungal spores. Acute respiratory aspergillosis outbreak was described in a backyard flock of ducks. The presence of exudative trachea-bronchitis and white-grey nodules in lungs and air sacs are key elements of clinical diagnosis. *Aspergillus fumigatus* was isolated and identified from all samples. Microscopic lesions were characterized by a necrotic inflammation with the presence of fungal hyphae, observed in PAS stained sections of lungs. Moldy litter was considered as the most important risk factor in this case. Treatment, based on copper sulphate, was effective to reduce morbidity and mortality in the studied flock.

Conflict of Interest

The authors declare that there is no conflict of interest.

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