

Diagnosis of pulmonary aspergillosis in geese by histopathological and microbiological methods

ABSTRACT

In this study, we aimed to determine the presence of pulmonary aspergillosis by histopathological and microbiological methods in geese that are economically grown in the Kars region of Turkey. Totally 150 lung tissue samples of geese, average age of 9 weeks, which died between 2013 and 2020 and were brought to our department were included in the study. In order to reveal the presence of *Aspergillus* fungi, Periodic acid-Schiff (PAS) staining was applied to the sections as suggested by manufacturer. The microbiological examination of the tissue samples was carried out by the standard mycological culture technique on Sabouraud Dextrose Agar and by the phenotypical characterization of the emerged cultures. We observed large and small multifocal yellowish-white nodular structures in the lungs and air sacs macroscopically. In the histopathological examination of the lung tissues, we detected granulomatous structures with varying numbers and sizes. We diagnosed the *Aspergillus* agents in 20 (13,33%) of the tissue samples by detecting structures resembling typical tree branches in the middle of granulomatous structures with PAS staining. An identical positivity was obtained by the microbiological method and the emerged agent was solely identified as *Aspergillus fumigatus* with the growth pattern and macroscopic and microscopic morphological features. In conclusion, we found the presence of aspergillosis as 13,33% by histopathological and microbiological methods in geese which were brought to our department between 2013-2020. Based on these data, we concluded that aspergillosis is one of the most important infectious diseases among the goose deaths in the Kars region of Turkey

Keywords: Aspergillosis, goose, histopathology, microbiology

INTRODUCTION

Aspergillosis is the systemic fungal disease caused by some species that belong the saprophytic, ubiquitous genus *Aspergillus* and can affect humans, dogs, cats, horses, marine mammals, wild and domestic birds, as well as invertebrates such as bees or corals (Tell et al., 2019; Della Vedova et al., 2019; Melo et al., 2020). Aspergillosis is mostly caused by *Aspergillus fumigatus* (accounts for approximately 95% of the cases), although rarely, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus glaucus*, *Aspergillus nidulans* and other *Aspergillus* species or mixed infections may play a role in the development of this disease (Tell et al., 2005; Beernaert et al., 2010). Aspergillosis is more common in birds than in mammals. Avian aspergillosis affects all species of domesticated birds, aquatic birds, wild birds and ornamental birds especially turkeys, chickens, white storks, ducks, quails, ostriches, parrots, canaries, pigeons, great rhea, flamingos, penguins, seagulls (Ziołkowska et al., 2014; Latif et al., 2015; Gulcubuk et al., 2018).

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Research Article

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The average age of animals affected by the avian aspergillosis outbreaks is between 3 days and 20 weeks, causing serious economic losses for the poultry industry (Aslan et al., 2015; Timurkaan et al., 2017). Aspergillosis is observed in two forms, acute (epizootic) and chronic (sporadic) disease varying in spectrum from local involvement to systemic dissemination (Leishangthem et al., 2015; Fagbohun et al., 2020). While the acute form of the disease causes high morbidity and mortality in young birds, the chronic form is usually observed in adult and immunocompromised birds, it is sporadic and has a lower mortality rate (Shoukat et al., 2018). The predisposing factors causing the disease can be briefly summarized as follows; species predilection, cage conditions, poor ventilation, malnutrition, toxins, vaccinations, long-term use of antibiotics-corticosteroids, moisture, inappropriate temperature, hygiene, contaminated food or bedding, trauma and transport (Seyedmousavi et al., 2015; Sa'idu et al., 2016; Savelieff et al., 2018). Difficulty in breathing, anorexia, cyanosis, diarrhea, weight loss, anorexia, loss of voice, involvement of the nervous system (lethargy, ataxia, opisthotonos, torticollis, limb paresis) and increased thirst are the main clinical findings of the disease (Tell et al., 2005; Leishangthem et al., 2015; Seyedmousavi et al., 2015). Histopathological lesions are characterized by granulomatous inflammation surrounded by a fibrous capsule associated with fungal hyphae. (Gulcubuk et al., 2018; Hauck et al., 2020). Since the clinical findings are not specific in aspergillosis, antemortem diagnosis is very difficult (Beernaert et al., 2010; Aslan et al., 2015). Methods such as cytology, culture, histopathology or PCR are used for the definitive diagnosis of the disease (Fagbohun et al., 2020). Sabouraud Dextrose Agar (SDA) agar is the preliminary chosen medium for the isolation of fungal species, even selectivity can be achieved with the addition of various

antibiotics such as chloramphenicol (Sahin et al., 1997; Nagano et al. 2008).

In this study, we aimed to determine the presence of pulmonary aspergillosis by histopathological and microbiological methods in geese that are economically grown in Kars region.

MATERIALS and METHODS

Animals

The material of this study was composed of lung tissue samples taken from 150 geese with an average age of 9 weeks, brought dead to our department for systemic necropsy between 2013-2020. Information on age and clinical findings of all animals are given in Table 1.

Microbiological investigations

For microbiological examination, the lungs tissue samples were cultured onto Sabouraud Dextrose Agar (Oxoid) plates supplemented with chloramphenicol (0.05 mg/ml). Initially, the surface of the lungs were cauterized with a hot spatula and then the sample was taken from the inner-living part of the tissue with a loop and inoculated onto the Sabouraud Dextrose Agar (SDA) plates. The plates were incubated aerobically at 25 °C and 37 °C for 7 days. Following the incubation, the fungal isolates were identified by growth pattern and macroscopic and microscopic morphological features (Latge, 1999; Gautam and Bhadauria, 2012).

Histopathological investigations

After systemic necropsy of geese, the lung tissue samples were fixed in 10% buffered formalin solution. After routine procedures paraffin blocks were cut to 5 µm thickness and Hematoxylin & Eosin (H&E) staining was applied to the sections in order to detect histopathological changes. In order to reveal the

presence of *Aspergillus* fungi, Periodic acid–Schiff (PAS) staining was applied to the sections as suggested by by manufacturer (Facepath company, barcode number: 8681065133692). Sections were examined and photographed under a light microscope.

RESULTS

Clinical results

Various clinical symptoms such as difficulty breathing, wheezing, weakness, anorexia, depression, diarrhea and nervous signs were recorded in geese.

Microbiological results

Twenty (13,33%) lung tissue samples were found culture positives for mycotic agents. No fungal growth was observed in the remaining 130 tissue samples (Table 1). All fungal isolates were identified as *Aspergillus fumigatus* with some characteristics such as; the rapid growth pattern that the colony size can reach 4 ± 1 cm within the incubation period on SDA agar plates at 37°C; the macroscopical characteristics like entire margined umbonate colonies with green to the dark green surface and colourless to yellow reverse side colours; the microscopical characteristics like branched septated hyphae, dome-shaped vesicle conidiophores, roughened ornamented conidiospores with blue-green head, uniseriate phialides and cleistothecium fruiting body.

Macroscopical results

We detected that the lungs were darker red than their normal pink appearance. We observed

large and small multifocal yellowish-white nodular structures in the lungs and air sacs of 20 (13,33%) of 150 geese examined macroscopically (Figure 1 a-b).



Figure 1. Diffuse yellowish-white granulomas (arrows) in the lung.

Microscopical results

In the histopathological examination of the lung tissues, we detected granulomatous structures with varying numbers and sizes. We observed large areas of necrosis and fungal agents in the center of the granulomas. We also revealed the presence of foreign body giant cells, histiocytes, plasma cells and neutrophils around the necrotic areas. On the outermost layer, there were connective tissue capsules surrounding them (Figure 2a-c). We diagnosed *Aspergillus* agents, which resemble typical tree branches in the middle of granulomatous structures, using PAS stain (Figure 2d).

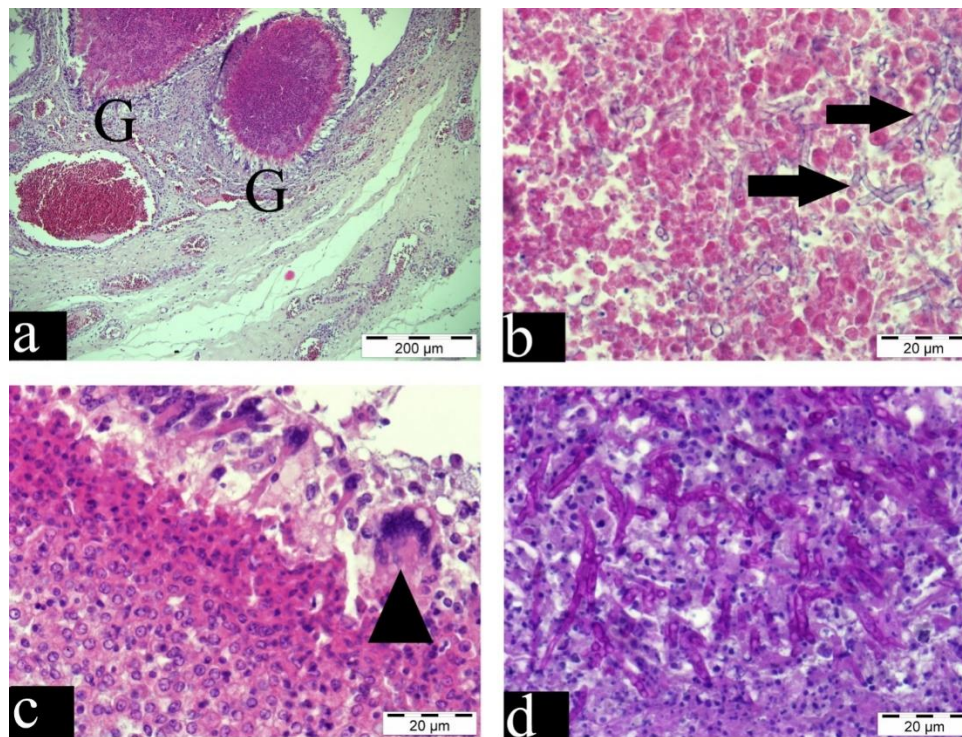


Figure 2. Lung tissue a: Granulomatous structures (G), H&E, Bar= 200µm, b: Aspergillus agents located in the necrotic area in the middle of the granulomas, H&E, Bar= 20µm, c: Lymphohistiocytic cell infiltration and foreign body giant cells (arrowhead) around necrotic area, H&E, Bar= 20µm, d: Hyphae that look like tree branches, PAS stain, Bar= 20µm

Table 1. Information on age, clinical symptoms, histopathological findings, PAS staining and microbiological results of all geese.

Case No	Age	Clinical Symptoms	Histopathological Results	PAS staining	Microbiological Results
Case 1	8 weeks	Difficulty breathing	Granuloma	+	+
Case 2	12 weeks	Diarrhea, nervous signs	Granuloma	+	+
Case 3	6 weeks	Wheezing	Granuloma	+	+
Case 4	6 weeks	Wheezing	Granuloma	+	+
Case 5	6 weeks	Wheezing	Granuloma	+	+
Case 6	6 weeks	Wheezing	Granuloma	+	+
Case 7	16 weeks	Difficulty breathing	Granuloma	+	+
Case 8	8 weeks	Difficulty breathing	Granuloma	+	+
Case 9	4 weeks	Wheezing	Granuloma	+	+
Case 10	16 weeks	Wheezing	Granuloma	+	+
Case 11	8 weeks	Wheezing, diarrhea	Granuloma	+	+
Case 12	10 weeks	Emaciation, difficulty breathing	Granuloma	+	+
Case 13	10 weeks	Anorexia, wheezing	Granuloma	+	+
Case 14	12 weeks	Weakness, difficulty breathing	Granuloma	+	+
Case 15	6 weeks	Wheezing, depression	Granuloma	+	+
Case 16	8 weeks	Wheezing	Granuloma	+	+
Case 17	10 weeks	Difficulty breathing	Granuloma	+	+
Case 18	12 weeks	Wheezing	Granuloma	+	+
Case 19	6 weeks	Wheezing	Granuloma	+	+
Case 20	10 weeks	Difficulty breathing	Granuloma	+	+

DISCUSSION

Important fungal diseases that threaten poultry production are Aspergillosis, Candidiasis, Dactylariosis, Favus, Mucormycosis, Histoplasmosis and Cryptococcosis (Fagbohun et al., 2020). In comparison to humans and many mammals, *Aspergillus* species play a role as a primary pathogen for birds (Tell et al., 2019). Birds are more susceptible to aspergillosis due to anatomical and physiological differences (Melo et al., 2020). These differences can be summarized as the birds do not have diaphragm, lack of epiglottis, poor vascularization and limited mucociliary function of the air sacs and heterophiles replacing neutrophils are less effective against hyphae invasion (Tell et al., 2005; Tell et al., 2019; Melo et al., 2020). *Aspergillus* spores are abundant in soil, rotten meat, forage, hay and all kinds of food, and as a result of inhalation of these spores, the lower respiratory system of birds is affected (Beytut, 2007; Gulcubuk et al., 2018). For this reason, it is called mycotic pneumonia (Özmen et al., 2007). The systemic form of the disease can affect the liver, kidneys, brain, bones, skin and eyes (Cacciuttolo et al., 2009). Since the findings of aspergillosis are not specific, the diagnosis of the disease is very difficult (Beernaert et al., 2010; Savelieff et al., 2018). Special stains such as culture, cytology, histopathology or PAS can be used for definitive diagnosis of the agent (Beyaz et al., 2008; Aslan et al., 2015; Timurkaan et al., 2017; Shoukat et al., 2018).

In this study, *Aspergillus* sp. was isolated in twenty (13,33%) of the lung tissue samples. These findings are similar to those reported by Abbas et al. (2017), who reported 15% of all types of *Aspergillus* spp. from pigeon, pet birds and chickens and those reported by Şahin et al. (1997), who reported an outbreak rate of 6,8% in geese in the identical region with this study. The sole and/or predominant agent causing aspergillosis in geese was identified as *A. fumigatus*, which is in great agreement with

those reported from different locations of the world (Şahin et al., 1997; Ziolkowska and Tokarzewski, 2007).

The mean age range of the geese with pulmonary aspergillosis was 9 weeks in this study. This finding is in accordance with the literatures (Okoye et al., 1989; Türkütanıt, 1999; Beytut et al., 2004). Various clinical symptoms such as difficulty in breathing, diarrhea, dyspnea, anorexia, emaciation, increased thirst and nervous signs have been reported in previous studies in geese (Ziolkowska et al., 2014; Sa'idu et al., 2016; Fagbohun et al., 2020). In our study, we obtained similar anamnestic informations from the owners of geese in accordance with the previous literature data (Ziolkowska et al., 2014; Sa'idu et al., 2016; Fagbohun et al., 2020).

We observed that the lung was a darker red than their normal pink appearance in the macroscopical examination of this organ, as reported by McDougale and Vaught (1968). We also detected large and small multifocal yellowish-white nodular structures in the lungs and air sacs, similar to those reported by different researchers (Okoye et al., 1989; Türkütanıt et al., 1999; Beytut et al., 2004; Yhee et al., 2007; Sa'idu et al., 2016).

In previous studies, granulomas (Ulloa et al., 1987; Türkütanıt et al., 1999; Beytut et al., 2004), large area of eosinophilic necrosis in the middle part of the granuloma (Türkütanıt et al., 1999; Beytut et al., 2004), histiocytes, lymphocytes, plasma cells and multinucleated foreign body giant cells around the necrotic area (McDougale and Vaught, 1968; Okoye et al., 1989; Türkütanıt et al., 1999; Beytut et al., 2004; Yhee et al., 2007; Sa'idu et al., 2016) were have been observed. Similar to the literature data (McDougale and Vaught, 1968; Ulloa et al., 1987; Okoye et al., 1989; Türkütanıt et al., 1999; Beytut et al., 2004; Yhee et al., 2007; Sa'idu et al., 2016), we also

observed necrotic granulomatous structures in the lung tissue of geese surrounded by mononuclear cells and multinucleated foreign body giant cells and connective tissue. In addition to these, we revealed the presence of typical tree branching or V-shaped hyphae in the PAS staining. We observed the *Aspergillus* agents localized within necrotic centers correlated with the findings of some researchers (Okoye et al., 1989; Yhee et al., 2007; Sa'idu et al., 2016).

CONCLUSION

In conclusion, we found the presence of aspergillosis as 13,33% by histopathological and microbiological methods in geese which were brought to our department between 2013-2020 and underwent systemic necropsies. Based on these data, we concluded that aspergillosis is one of the most important infectious factors among goose deaths in Kars Region (Turkey). We think that it is important to inform the animal owners with confirmative diagnosis in order to prevent the disease and to eliminate economic losses in poultry.

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Ethical approval: The ethics committee report of this study was obtained from Kafkas University Animal Experimental Local Ethics Committee (Authorization number: KAU-HADYEK-2020/155).

Conflict of interest: Authors have no conflicts of interest to declare.

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