CASE REPORT

Sokoto Journal of Veterinary Sciences
(P-ISSN 1595-093X/ E-ISSN 2315-6201)
Sa'idu *et al./Sokoto Journal of Veterinary Sciences* (2016) 14(3): 62 – 66.
http://dx.doi.org/10.4314/sokjvs.v14i3.10

Outbreak of aspergillosis in a flock of geese in Zaria, Nigeria

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Abstract

A goose from a flock of twenty five geese, with history of gaping, sternal recumbency, greenish watery diarrhea and inappetence was presented to the Avian and Poultry Health Unit of Veterinary Teaching Hospital, Ahmadu Bello University, Zaria. Ruffled feathers, drooping of the wings, rales, greenish vent and clear greenish diarrhea were observed on physical examination. At necropsy, congested carcass, enlarged and congested liver and spleen, severely hemorrhagic, mucoid and congested trachea, severely congested lungs with multiple and diffused nodular growth all over the lungs were observed. There was a velvety greenish area in the lungs with black spots at the center and nodular growths on the intercostal muscles. Microscopically, the portions of lungs with nodules were composed of necrotic center with intralesional hyphae and conidia typical of *Aspergillus spp*., a peripheral inflammatory cell response composed of mononuclear cells infiltration and obliteration of alveolar cells. The mycologic culture allowed the isolation and identification of *Aspergillus flavus* (*A. flavus*) from lung samples. The gross and microscopic lesions, in combination with the mycologic identification, provided the diagnosis of pulmonary aspergillosis due to *A. flavus* infection. $CuSO_4$ at 1 g per 5 liters of drinking water was used for a period of 7 days with no signs of the infection.

Keywords: *Aspergillus spp*., Geese, Gaping, Intralesional hyphae, Zaria

Received: 30-05- 2016 Accepted: 07-10-2016

Introduction

Aspergillosis is defined as any disease condition caused by a member of the fungal genus *Aspergillus* (Garbino & Lew, 2004). It is a fungal infection caused mainly by *Aspergillus fumigatus* (*A. fumigatus*) and *A. flavus.* The disease was recognized as an avian disease since 1815 and generally involves the respiratory tract (Beytut *et al.*, 2004; Marina *et al.*, 2004). The principal agent causing aspergillosis in poultry is *A. fumigatus*, which accounts for 66 % of the *Aspergillus* species isolated from geese (Ulloa *et al.,* 1987; Ziolkowska & Tokarzewski, 2007). Isolation of *A. versicolour, and A. flavus* is less common (Ziolkowska & Tokarzewski, 2007; Jezdimirovic *et al.*, 2013). Other species rarely isolated include *A. terreus, A. glaucus, A. clavatus, A. nidulans, A. niger, A. amstelodami,* and *A. nigrescens* (Kunkle, 2003; Ziolkowska & Tokarzewski, 2007). Aspergillosis is considered the most common systemic mycosis in birds (Kunkle & Rimler, 1998) Avian aspergillosis involves mainly the lower respiratory tract (Beytut *et al*., 2004; Walter, 2011). These fungi are ubiquitous, but they become pathogenic mainly under stressful conditions, producing opportunistic infections as a result of inhalation of *Aspergillus* spores coupled with compromised immune functions in the host or in association with prolonged diseases (Deem, 2003). Poor ventilation, malnutrition, toxins, vaccinations, long-term use of antibiotics and corticosteroids, hot-humid climate, and stress-associated conditions, such as recent capture, training, and change of ownership, are frequently mentioned as environmental precipitating factors influencing the onset and duration of aspergillosis in falcons (Nardoni *et al*., 2006).

Pulmonary aspergillosis occurs in a wide variety of avian species, and perhaps all birds should be considered as potential hosts susceptible to *Aspergillus* infection (Ainsworth & Austwick, 1973). Outbreaks occur when the organism is present in sufficient quantities to establish disease or when the bird's resistance is impaired by factors such as environmental stress, immunosuppressive compounds, inadequate nutrition, or other infectious diseases. An outbreak of aspergillosis by *A. flavus* that induced systemic aspergillosis in turkey poults with sternal bone involvement has been reported (Ghazikhanian, 1989). This paper describes a case of pulmonary aspergillosis in geese and its management.

Case Presentation

History

A goose was presented to the Poultry Unit of the Veterinary Teaching Hospital, Ahmadu Bello University, Zaria with a chief complaint of dyspnea, greenish watery diarrhea, gaping of the beak and sternal recumbence (Plate I). The problem was noticed in a flock of 25 geese, kept in a semi extensive system of management. They also reported that five days prior to presentation of the case to the hospital, 2 geese manifesting the same clinical signs had died. At the farm, the geese are fed

Plate I: A sick goose from the aspergillosis infected flock. Note: a characteristic of beak gaping (arrow)

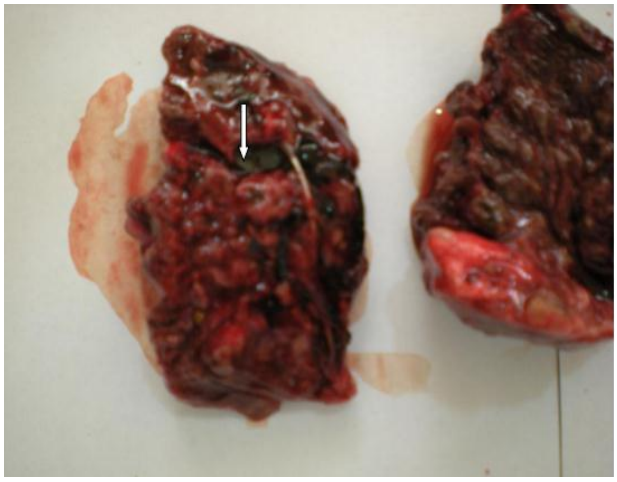

Plate II: Velvety green lung tissue discoloration (arrow) from the dead goose

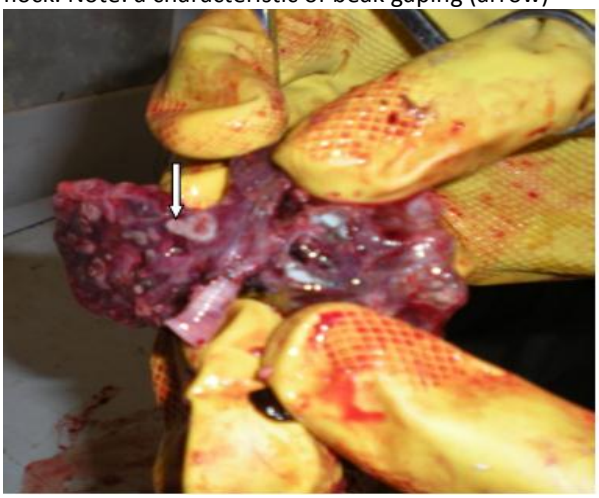

Plate III: A nodular lesion in the segment of the lungs from aspergillosis suspected dead goose

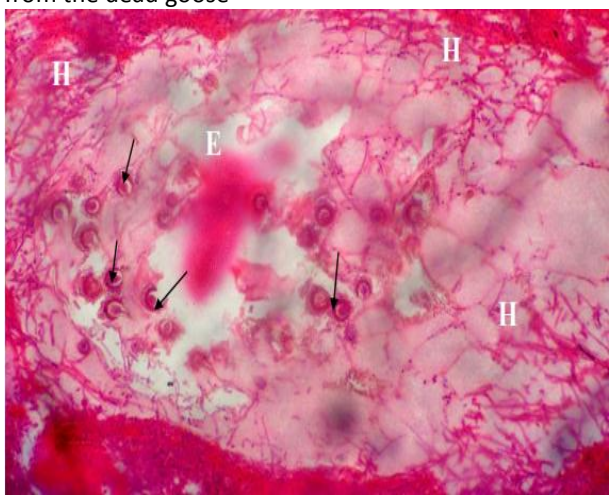

Plate IV: Photomicrograph of lung from the goose with aspergillosis due to *A. flavus*. Note: the hyphae (H) in a zone of tissue necrosis, the conidia (arrows) and edematous area (E). (H&E stain) X 200

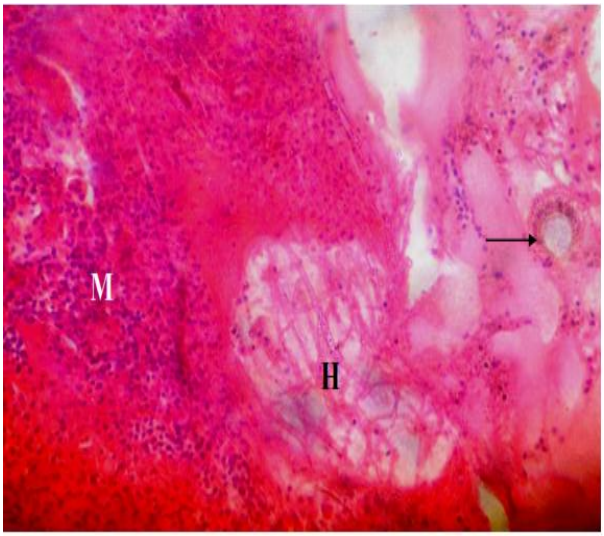

Plate V: Photomicrograph of the lung. Note the infiltration of mononuclear cells (M), hyphae (H) and conidia (arrow) of *A. flavus* in an area of the lung parenchyma with obliterated alveoli. (H&E stain) X 200

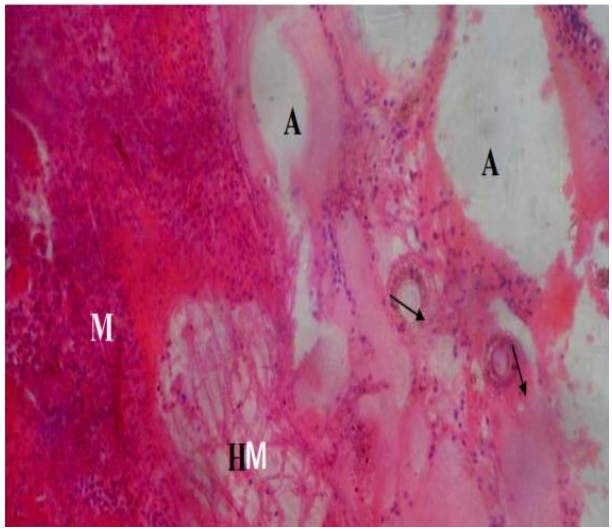

Plate VI: Photomicrograph of the same lung in Plate V. Note the alveoli (A), infiltration of mononuclear cells (M), hyphae (H) and conidia (arrows) of *A. flavus* in an area of the lung parenchyma with obliterated alveoli. (H&E stain) X 200

wheat brand moistened with water; there was a small sized but deep pond, and the geese pen is neighboured by both a chicken and a guinea fowl pen. Upon physical examination, ruffled feathers, drooping of the wings, rales, greenish vent, clear greenish diarrhea and gaping were noticed.

Post mortem examination

The goose died in the hospital and post mortem examination was conducted. The gross lesions observed were congested carcass, enlarged and congested liver and spleen, severely hemorrhagic, mucoid and congested trachea. On incision of the lungs tissue there were velvety greenish areas with a black spot at the center (Plate II), severely congested lungs with multiple diffused nodular growths all over the lungs (Plate III), and there were nodular growths on the intercostal muscles.

Histopathological examinations

Section of the lung was submitted to histopathology laboratory for preparation of hematoxylin and eosin (H&E) stained histopathological slides. Lung sections showed areas of congestion, massive infiltration of mononuclear cells in interalveolar spaces and majority of the alveoli were obliterated (Plates IV and V). Many areas of fungal growth with numerous hyphae and conidia of the organism were observed in parenchyma and alveolar spaces (Plate VI). Tissue necrosis was also observed around some of the affected areas. Evidence of oedema was observed in the alveolar spaces with the mycotic filaments.

Plate VII: *Aspergillus flavus* growth on Sabouraud's dextrose agar 7 days post culture

Microbiological examination

All lung cultures showed growth of fungal colonies after 5 days of incubation in Sabouraud's dextrose agar (SDA, Himedia) with chloramphenicol (0.05 mg/mL) and incubated under aerobic condition at 25°C for 3-5 days (Jung *et al.*, 2009). The colonies had a diameter of approximately 3 to 4 cm in 5 days. The colonies were initially white and then acquired a yellowish-brown pigmentation with age as conidia began to mature, especially near the center of the

colony. In addition, conidial masses became gray-brown, and were transferred to clean microscopic slides containing few drops of Lactophenol cotton blue stain using Roth flag technique (Quinn *et al*., 1994). Microscopically, mycelia were composed of tubular septate hyphae.

Treatment

$CuSO_4$ at 1 g per 5 liters of drinking water was used for a period of 7 days; the client was adviced to avoid the use of moistened bran in feeding birds. The birds recovered after the 7 day treatment of with no signs of the re-infection.

Discussion

The gross lesions observed in this study were similar to those earlier reported earlier (Ulloa *et al.*, 1987; Beytut *et al.*, 2004; Beernaert *et al.*, 2010; Leishangthem *et al.*, 2015). Histopathological analysis of lung tissue stained with hematoxylin and eosin revealed multifocal areas of necrosis, obliteration of alveolar and mononuclear cells infiltration. Fungal hyphae, conidia, edema and giant cells were visualized. This presentation is typical in the progression of pulmonary aspergillosis as described by Chute & Richard (1997). Several factors are involved in the pathogenicity of *Aspergillus* infection of the animal host and it has been demonstrated that some environmental strains are less virulent than the corresponding clinical strains (Aufauvre-Brown *et al*., 1998). Perturbation of the mucociliary system may be an important factor in facilitating airway infection (Amitani *et al*., 1995). Furthermore, various fungal products may interfere with the barrier function of the epithelium. The disease is usually diagnosed at post mortem examination, often based upon the observation of white caseous nodules in the lungs or air sacs of affected birds (Chute & Richard, 1997). The diagnosis was confirmed by laboratory culture and isolation of Aspergillus flavus from the portions of the lungs with nodules and by histopathological findings. Aspergillosis appears to b e more significant in confinement where redisposing factors such as infected hatcheries, heavy contamination of the air or feed, stress and immunosuppression are usually involved (Chute & Richard, 1997). Outbreaks of aspergillosis occur when the organism is present in sufficient quantities to establish disease or when the bird resistance is impaired by factors such as environmental stress, immunosuppressive compounds or inadequate nutrition (Chute & Richard, 1997; Beernaert *et al.*, 2010).

The gross and microscopic lesions, in combination with the mycologic identification provided the diagnosis of pulmonary aspergillosis due to *A. flavus* infection. Geese farming is relatively new practice in Nigeria, many farmers do not have much knowledge about how to manage this avian specie. In most cases, the geese are maintained in small confinement and on some farms the management, sanitation and nutrition of these birds are deficient. This lack of proper care may be the main factor responsible for the introduction of many infectious diseases in geese farms. Biosecurity measures should be adopted on geese farms, eliminating pathogens in the hatchery as well as in every stage of the raising process.

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