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Original article

ASPERGILLOSIS OUTBREAKS IN OSTRICH FLOCKS OF EASTERN IRAN DURING 2010–2012

M. ARAGHI, A. GHANIEI & T. HEIDARI

Department of Clinical Sciences, Faculty of Veterinary Medicine,
Urmia University, Urmia; Iran

Summary

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Aspergillosis is an important infectious disease in ostriches that can be acute or chronic. Because of high morbidity and mortality rate of aspergillosis in ostriches, it can cause serious financial losses to the ostrich production industry. In this study, we discuss some outbreaks of *Aspergillus* infection in ostrich farms of eastern regions of Iran during 2010-2012. Signs of respiratory involvement, anorexia, depression, progressive emaciation and decreased production were the most commonly reported in affected farms. Morbidity rate was 43% and 54.53% in breeding birds and chickens, respectively. Mortality rate was 31.89% in breeding birds and 44.69% in chickens. Necropsy findings were suggestive of fungal infections in respiratory and alimentary tracts. *Aspergillus fumigatus* and *Aspergillus niger* were identified in microbiological and pathological examinations. Management reforms and using some supportive treatments were beneficial for controlling the disease.

Key words: Aspergillosis, Iran, ostrich

INTRODUCTION

In recent years, ostrich farms in Iran are increasing in number and size. Ostrich meat is an important source of protein for human consumption. Also, other valuable products (skin, feathers) are yielded in ostrich farming. Aspergillosis is a common respiratory infection due to mismanagement in avian species (Brown *et al.,* 2008). Management problems especially high density of birds and poor ventilation, excessive use of antibiotics, nutritional deficiencies, and immunosuppressive agents are common factors predisposing to aspergillosis. *A. fumigatus* is the most commonly isolated agent in aspergillosis (Arne *et al.,* 2011). Acute aspergillosis in younger birds causes higher morbidity and mortality rates, while chronic forms in older birds look sporadic (Arne *et al.,* 2011). Weight loss, lethargy and dyspnea are observed in *Aspergillus* infections of ostriches (Kyoung, 2001). Other less common forms of aspergillosis in birds are encephalitis, ophthalmitis, osteomyelitis, dermatitis and systemic form (Fitzgerald & Moisan, 1995). *Aspergillus* spp.

can also involve the proventriculus and gizzard of ostriches leading to gastric stasis (Huchzemeyer, 1999; Sancak & Paracikoulu, 2005). In necropsy, nodular lesions are seen in affected organs, from which fungi can be cultured (Huchzemeyer, 1999).

There are several reports of *Aspergillus* infections in ostriches (Perelman & Kuttin, 1992; Terizich & Vanhooster, 1993; Marks *et al.,* 1994; Yokota *et al.,* 2004; Sancak & Paracikoulu, 2005). Pulmonary aspergillosis is a common finding in aforementioned studies, but in some cases gastric involvement (Sancak & Paracikoulu, 2005) and positive culture of *Aspergillus* from liver and blood vessels are noted (Yokota *et al.,* 2004). Although *A. fumigatus* was the most commonly isolated organism, *A. niger and A. flavus* were also cultured from tissues (Perelman & Kuttin, 1992).

In 2003 was published the first report of aspergillosis due to *A. fumigatus* in Iran (Sasani *et al.,* 2003). Khosravi *et al.* (2008) also reported an outbreak of aspergillosis in an ostrich flock in the country and isolated *A. fumigatus* from different tissues of birds from the affected flock. The morbidity rate was 60%.

The high incidence of aspergillosis in ostrich flocks of eastern Iran during 2010-2012 and huge economic losses to the industry necessitate more comprehensive studies on this infection. In this survey, we discuss 9 *Aspergillus* infection outbreaks in ostrich farms of eastern regions of Iran from 2010-2012. Presumptive diagnosis of cases was based on clinical signs and necropsy. Further characterisation was carried out by culturing of the etiological agent in suitable media. Results of such reports are helpful to understand predisposing factors of infection and to prevent its occurrence.

MATERIALS AND METHODS

The survey on aspergillosis was conducted in eastern regions of Iran (2010-2012), where the weather is cold in autumn and winter. Seven breeder flocks (430 birds) and fifteen chicken flocks 2-15 months of age (1280 birds), were included. Among these flocks, three breeder (185 birds) and six chicken flocks (698 birds) were affected by aspergillosis.

Samples from the air sacs, livers and proventriculi were submitted to mycology laboratory for examinations. The samples were cultured aerobically on Sabouraud dextrose agar with chloramphenicol at 37 °C and 45 °C for three days. Isolated fungi were examined microscopically by slide culture based on mycological principles (Quinn *et al.,* 1994).

Tissue samples of the lungs, air sacs and gastrointestinal tract were aseptically collected. The tissue biopsies were fixed in 10% formalin and embedded in paraffin. Sections of tissues were cut (5 µm), placed on slides and stained with haematoxylin and eosin (H&E) and Grocott's methenamine silver nitrate (GMS) for fungi. In addition, direct microscopic examination was performed by 10% KOH/ DMSO (Merck Co., Darmstadt, Germany) for detection of fungal elements.

RESULTS

The clinical signs at affected farms were decreased production, respiratory signs, and alimentary involvement. Birds were raised under semi-intensive conditions, mostly in autumn and winter. Poor ventilation (in the birds that were housed in a facility to shut out the cold weather; 6 flocks), overcrowded populations with dusty bed (6 flocks), unsuitable foodstuffs, nutritional problems and improper

feeding (9 flocks) and long-term antibiotic therapy to cure disease (5 flocks) were the identified farming faults at these ostrich farms that suggested the possibility of inhalation of *Aspergillus* spores and intake of infected feeds. It must be mentioned that more than one problem was observed in these farms. In clinical examinations, anorexia, progressive weight loss and emaciation, gasping (mouth breathing) and respiratory involvement signs, convulsions, sternal recumbency and death were the most important findings in affected birds. In the early stages of disease in breeder flocks, they were losing weight despite pecking food but they stopped pecking in later stages. Convulsion was observed only in two breeder ostriches with very poor body condition scores before death. Decrease in production was observed in breeders. Infected embryos were also observed that caused hatchability losses in breeder flocks.

At the first step, some antibiotics were administered for symptomatic treatment of infection, but none of them caused any improvement. Mortality rates in breeding birds and chickens were 31.89% and 44.69% respectively.

A. fumigatus and *A. niger* were identified after the microbiological examinations (Fig. 1 and 2). Clinical signs and postmortem findings (Fig. 3) were suggestive of *Aspergillus* spp. infection.

Histopathological examinations of affected tissues also confirmed fungal involvement (Fig. 3). In post mortem examinations, lesions were found mainly in respiratory tract particularly air sacs. Yellowish caseous material was noted in the thickened thoracic air sacs, and white to green molds spread to the inner surface. Other organs such as liver and proventriculus were also affected by typical fungal infections in some cases.

To our knowledge, this is first report of *A. niger* infection in ostrich flocks of Iran.

DISCUSSION

A. fumigatus and *A. flavus* are the most important agents causing aspergillosis in poultry (Yokota *et al.,* 2004). In published

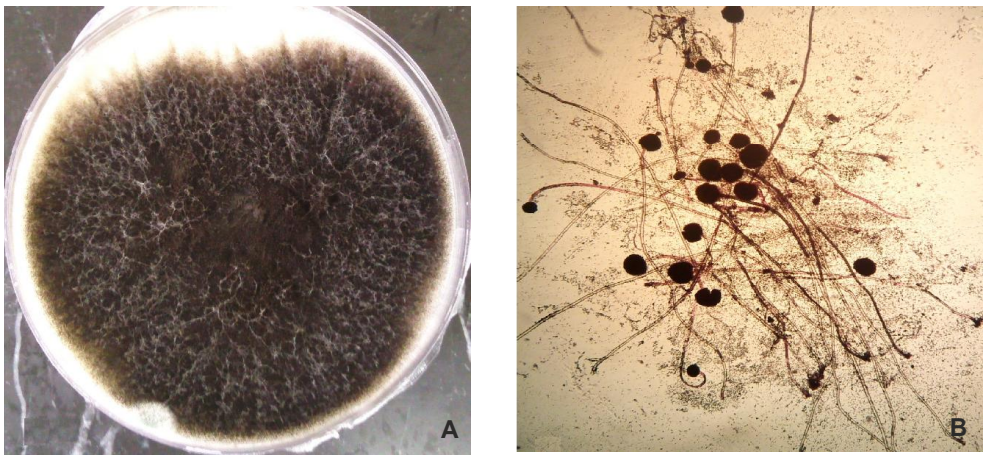

Fig. 1. *Aspergillus niger*. **A.** Cultured on Sabouraud dextrose agar with chloramphenicol at 37 °C; B. Examined microscopically by slide culture (×100).

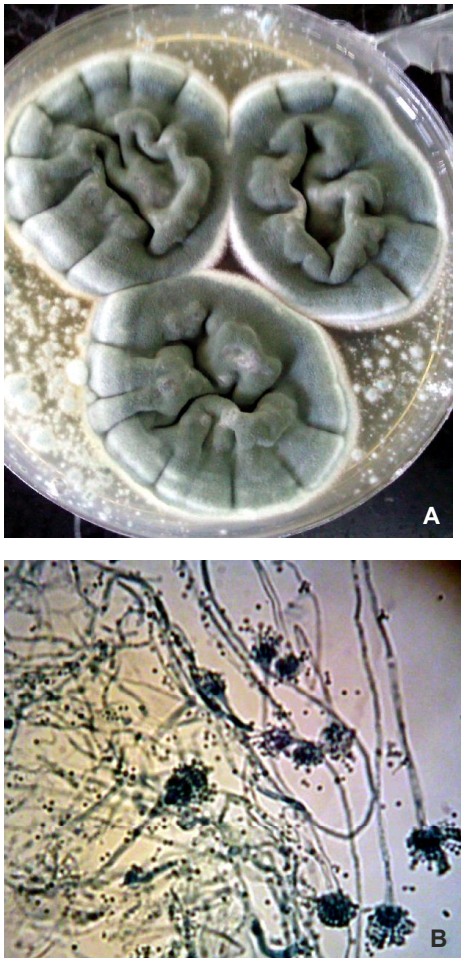

Fig. 2. *Aspergillus fumigatus.* **A.** Cultured on Sabouraud dextrose agar with chloramphenicol at 37 °C; **B.** Examined microscopically by slide culture (×100).

studies of aspergillosis in ostriches, *A. fumigatus* (Sancak & Paracikoulu, 2003; Yokota *et al.,* 2004; Khosravi *et al.,* 2008; Shathele *et al.,* 2009), *A. niger* and *A. flavus* (Perelman & Kuttin, 1992), have been isolated. In the present study, *A. fumigatus* was the most commonly detected organism, but in some cases *A. niger* was also isolated.

Aspergillosis in poultry occurs sporadically in adults, although severe outbreaks of high mortality and morbidity rate have been reported in young birds (Perelman & Kuttin, 1992; Khosravi *et al.,* 2008). Mycotic air sacculitis and pneumonitis (Perelman & Kuttin, 1992; Sancak & Paracikoulu, 2005; Khosravi *et al.,* 2008), proventriculus involvement (Sancak & Paracikoulu, 2005), and mycotic liver infection (Yokota *et al.,* 2004; Khosravi *et al.,* 2008) caused by *Aspergillus* spp. have been previously reported. Progressive weight loss and emaciation involved all affected ostriches of different ages. Gasping or mouth breathing and respiratory involvement signs were observed in most cases with respiratory infection of aspergillosis. Sternal recumbency and death occurred in long-term contaminated birds with aspergillosis. *A. fumigatus* can produce a lot of toxins such as gliotoxin, helvolic acid, fumagillin and aflatoxin- like substances that have immunosuppressive properties. So infected birds may be immunosuppressed (Sancak & Paracikoulu, 2005) and ultimately die due to the acute air succulitis and pneumonia (Yokota *et al.,* 2004).

Yokota *et al.* (2004) reported a 2-year-old ostrich with clinical signs of respiratory distress, cough, anorexia and oedema of the neck as aspergillosis. It had multifocal granulomatous lesions in the lungs and air sacs. Sancak & Paracikoulu (2005) observed a 3-month-old ostrich with progressive weight loss despite of pecking food that had typical fungal lesions in the air sacs. Akkoc (2009) observed respiratory problems and long term inappetence with post mortem findings of fungal lesions in the lungs and thoracic air sacs and AA type amyloid accumulations in the liver and spleen in a female ostrich. Terzich & Vanhooser (1993) observed

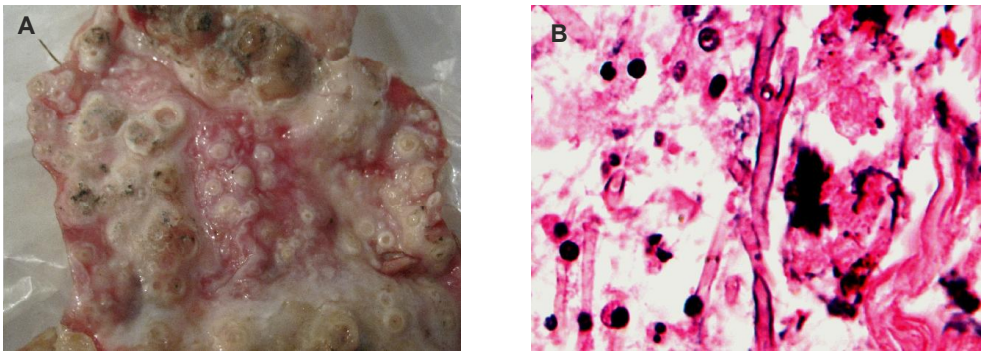

Fig. 3. *Aspergillus infection*. **A.** Postmortem changes in air sac and lung; **B.** Histopathologic slide of infected air sac examined microscopically (×100) showing *Aspergillus* hyphae.

aspergillosis in an adult ostrich, and on postmortem examination, nodules were noted in the lungs and gastrointestinal tract and in the hollow cavities of the bones of the sternum anteriorly. In a 3 to 8 weeks-old ostrich flock, Perelman & Kuttin (1992) reported a brooder borne infection with involvement of lungs. Shathele *et al.* (2009) observed pulmonary haemangioma followed by aspergillosis in an ostrich. In the current study, anorexia was observed in chronic cases and older ones.

There are major difficulties in the diagnosis and treatment of individual infected ostriches. Sancak & Paracikoulu (2005) reported no successful treatments for aspergillosis. In this study, there was no individual successful treatment as well, but we tried to correct problems. Feeding balanced rations to ostrich flocks based on their nutritional requirements was applied in flocks. Furthermore, some vitamin and mineral supplements, such as vitamin A, vitamin D_3, vitamin E and mineral premixes were included in the formulation. Mycotoxin binders as an additive were also added to the diet. With regard to poor ventilation, increased ventilation rates and additional fans were applied. Copper sulfate was used in water treatment to inhibit fungal growth. Via these supportive treatment protocols, the disease was controlled and fatality rates were reduced.

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Correspondence:

M. Araghi
Department of Clinical Sciences,
Faculty of Veterinary Medicine,
Urmia University,
Urmia; Iran
e-mail: araghi77@yahoo.com