**NOTE** Avian Pathology

**Aspergillus fumigatus** Infection in an Ostrich (*Struthio camelus*)

Takashi YOKOTA1,2, Tomoyuki SHIBAHARA2a, Yoshihiro WADA3, Rina HIRAKI3, Yoshiharu ISHIKAWA2 and Koichi KADOTA2

1Rumoi Livestock Hygiene Service Center, 6–1 Motomachi, Horonobe 098–3217, 2Hokkaido Research Station, National Institute of Animal Health, 4 Hitsujigaoka, Toyohira, Sapporo 062–0045 and 3Oshima Livestock Hygiene Service Center, 555–13 Nishikikyo, Hakodate 041–0824, Japan

(Received 4 June 2003/Accepted 17 September 2003)

**ABSTRACT.** An 11-month-old female ostrich (*Struthio camelus*) had become gradually emaciated over a 2-week period and subsequently died. Necropsy revealed white to green mold growth on the walls of caseous thickened air sac membranes and multiple white necrotic foci in the lungs and liver. Histologically, the multiple exudative, necrotic and granulomatous lesions were compatible with mycotic infection in the air sacs and lungs, and hyphae positively reacted with a monoclonal antibody (Mab-WF-AF-1) to *Aspergillus fumigatus* wall fractions. Multifocal hepatic necrosis was also found, and several spores were observed in the blood vessels. Fungal culture of these lesions yielded pure growth of *A. fumigatus*. This is an established case of fatal *A. fumigatus* infection in an ostrich rearer in Japan.

**KEY WORDS:** airsacculitis, *Aspergillus fumigatus*, ostrich.

The ostrich (*Struthio camelus*) is naturally distributed in semi-arid and desert areas of some African countries. Recently, ostrich farming has been rapidly expanding in many countries to produce usable products such as meat, hide, eggs, and feathers [12]. Over 320 commercial ostrich farms have been established in Japan, and the population of ostriches reached more than 9,300 in 2001 [3]. In spite of such circumstances, little is known about ostrich diseases in Japan [1, 3].

Aspergillosis is the most common mycotic infection of the respiratory tract in avian species [2, 8]. Several *Aspergillus* infections have been reported in the respiratory tract of the ostrich [4, 6, 9], and the identification of the pathogens was performed mainly on the basis of the morphology of fungal hyphae. This short communication describes the clinical, histopathological, and immunohistochemical characteristics of aspergillosis in an ostrich.

On an ostrich farm in Hokkaido, Japan, where it is bitterly cold in the winter, an 11-month-old female ostrich showed developmental retardation, and was much smaller than normal. The bird was housed in a facility to shut out the cold weather, and oil-burning stoves were used in the room without adequate ventilation. Suddenly, the bird showed lethargy, anorexia and emaciation. The clinical signs deteriorated, and 12 days later, respiratory distress with mouth breathing appeared. Despite antibiotic treatment, bloody diarrhea occurred the next day, and then sulfonamide was administered. The bird died 14 days after onset of the clinical signs. No clinical abnormality was found in any of the other ostriches.

The organs were fixed in 20% phosphate-buffered formalin and embedded in paraffin. Tissue sections (approximately 3 μm thick) were obtained using routine histological techniques. The sections were stained with hematoxylin and eosin, periodic acid-Schiff and Grocott’s methenamine silver for histological examination.

Serial histological sections were prepared for immunohistochemical staining with the Universal Immu-no-enzyme Polymer method using a Histofine simple stain MAX-PO Kit (Nichirei Corp., Tokyo, Japan). The sections were pretreated with 0.1% trypsin prior to immunostaining, and endogenous peroxidase activity was blocked by methanol and 3% H2O2. Primary antibodies used in this study were mouse monoclonal antibodies to *Aspergillus fumigatus* wall fractions, Mab-WF-AF-1 (M3564 Dako, Carpinteria, CA, U.S.A.) and to water-soluble somatic antigens from *Rhizopus arrhizus*, Mab-WSSA-RA-1 (M3565 Dako, Carpinteria, CA, U.S.A.), and a rabbit polyclonal antibody to *Candida albicans* (1750–5507 Biogenesis, Poole, Dorset, England). Sections were lightly counterstained with hematoxylin and assessed by light microscopy. Simultaneously, bovine and human tissues infected with aspergillosis, zygomycosis and candidiasis were stained as positive controls (kindly provided Dr. H. Okada, Rakuno Gakuen University, Hokkaido, Japan and Dr. M. Hotchi, Shinshu University School of Medicine, Matsumoto, Japan). Negative controls were prepared by using nonimmune mouse and rabbit sera in place of the primary antibody.

For mycotic examination, samples from the air sacs, lungs and liver were cultured aerobically on Sabouraud dextrose agar at 25°C. The isolated fungus was suspended in lactophenol and examined microscopically by slide culture. The walls of the thoracic air sacs were thickened with caseous material, and white to green mold was distributed heavily on the inner surface (Fig. 1). There were numerous yellow lesions, 0.5 to 5 cm in diameter, on the inner surfaces of the abdominal air sacs. The lungs and liver had many white necrotic foci, 0.1 to 3 cm in diameter, with prominent congestion.
Grossly, fungal organisms spread rapidly on Sabouraud dextrose agar with samples from all organs examined, and the colonies were velvet white with a bluish-green center. Microscopically, the conidiophores were short, smooth, and hyaline. They gradually enlarged, imperceptibly forming expanded flask-shaped vesicles. The vesicles were 15–22 µm in diameter, and produced a single series of phialides on the upper half only. The conidia were spherical to broadly ellipsoidal, 2.3 µm in diameter, and surfaces were finely roughened. From its cultural characteristics, the fungus was identified as *A. fumigatus*.

The walls of the thoracic air sacs were covered with a large amount of cellular debris composed of closely packed necrotic inflammatory cells. The debris was also coated with a layer of serofibrinous exudate in which there were numerous fungal hyphae with a great number of conidia and formation of conidial heads on the inner surface (Fig. 2). Similar but milder lesions were detected in the abdominal air sacs.

In the lungs, the lumina of the secondary bronchus and parabronchus were frequently filled with large masses of cellular debris, where many fungal hyphae existed (Fig. 3). Some masses were encircled by reactive multinuclear giant cells, macrophages and heterophils, but lymphocyte infiltration and development of fibrous tissue were minimal. Small granulomas containing a few hyphae were rarely seen. The hyphae reacted with a monoclonal antibody to *A. fumigatus* wall fractions (Fig. 4).

In the liver, there were multiple necrotic areas of various
sizes, most of which were located in the portal area (Fig. 5). Proliferation of bile ducts was detected within hepatic lobules. Several spores containing *A. fumigatus* antigen were present in blood vessels (Fig. 6). Despite immunohistochemical examination, fungal hyphae were not detected in the necrotic lesions. Free spores were detected within the lumina of systemic blood vessels. Neither water-soluble somatic antigens from *R. arrhizus* nor *C. albicans* antigens were detected in any tissue examined.

The two major agents causing aspergillosis of poultry are *A. fumigatus* and *A. flavus* [2,8]. In previous studies of ostrich aspergillosis, mycotic lesions existed in the lungs and air sacs [4, 6, 9], and *A. fumigatus* could be isolated in one case [9]. In the present case, severe mycotic lesions were also seen in the lungs and air sacs. Fungal hyphae in tissue sections resembled those of *Aspergillus* sp., and stained positively with Mab-WF-AF-1. The widespread distribution of immunohistochemically positive hyphae suggested that the present airsacculitis and pneumonitis were induced by *Aspergillus* sp. alone. In addition, *A. fumigatus* could be isolated bacteriologically, and a diagnosis of *A. fumigatus* infection was established.

There is diffuse centrilobular necrosis in the liver in animals and birds affected with acute aflatoxicosis [2]. In chronic aflatoxicosis, in contrast, hepatic cell necrosis is less pronounced but there is bile duct proliferation and periportal fibrosis [5]. In the liver of the present case, there were extensive areas of multifocal necrosis. Although mycotic hyphae could not be demonstrated, fungal culture of the necrotic lesions yielded pure growth of *A. fumigatus*. Since *A. fumigatus* produces a number of toxins or putative virulence factors, including gliotoxin, helvolic acid, fumagillin
and aflatoxin-like substances [7,10,11], the hepatic lesions may have been caused by some products of *A. fumigatus*. Similar hepatic lesions have been observed in rabbits experimentally infected with *A. fumigatus* [13].

Aspergillosis in poultry occurs as either severe outbreaks of high morbidity and mortality in young birds or sporadically in adults [2, 8]. A 2-year-old ostrich affected with aspergillosis developed respiratory distress, cough, anorexia and edema of the neck, and had multifocal granulomatous lesions in the lungs and air sacs [9]. Similar granulomatous lesions were observed in a 4-month-old ostrich, which was weak, breathing with its mouth open, and pumping its wings [4]. These cases of aspergillosis can be classified into the chronic type [8]. In contrast to these chronic cases, respiratory distress appeared just before death in the present bird, and the most outstanding histological feature of the respiratory system, especially of air sacs, was severe exudative inflammation. Taking into account the hepatic lesions and growth retardation, the present bird may have been in an immunocompromised condition, presumably induced by products of *A. fumigatus* existing in the body or environment, because this fungus can produce toxins such as gliotoxin, which has immunosuppressive properties [10]. The weakened bird, susceptible to subsequent diseases, ultimately died due to the acute airsacculitis and pneumonia.

ACKNOWLEDGMENTS. We would like to thank Dr. H. Okada, Rakuno Gakuen University, Hokkaido, Japan and Dr. M. Hotchi, Shinshu University School of Medicine, Matsumoto, Japan for their advice, and we also acknowledge Dr. Y. Ando and Mr. T. Fujisawa for preparation of the photomicrographs.

REFERENCES