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Case Report—

Diagnosis and Successful Treatment of a Presumptive Case of Aspergillosis in a Micronesian Kingfisher (*Halcyon cinnamomina cinnamomina*)

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SUMMARY. A 4-yr-old male Micronesian kingfisher was suspected of having an aspergillus infection. The infection was thought to be related to stress associated with movement to a new enclosure/exhibit and cage-mate aggression. The diagnosis was based on an elevated white cell count, positive antibody and antigen aspergillus titers, and abnormal plasma protein electrophoresis characterized by a moderate elevation of α2 and severe elevation on the β protein fractions. The bird was treated with antifungal medication administered systemically and by nebulization for 10 wk. Response to treatment was monitored by serial white cell counts and plasma electrophoresis. Clinical improvement in this bird was correlated with a return of the white blood cell count to normal levels and what was considered a normal protein electrophoresis distribution.

RESUMEN. *Reporte de Caso*—Diagnóstico y tratamiento exitoso de un caso presuntivo de Aspergilosis en un halcón micronesio (*Halcyon cinnamomina cinnamomina*).

Un halcón micronesio macho de cuatro años de edad fue considerado sospechoso de tener una infección con Aspergilus. Se pensó que la infección podía estar relacionada al estrés asociado con la mudanza a un nuevo recinto de exhibición y a la agresión por compañeros de jaula. El diagnóstico se basó en un recuento elevado de células blancas, títulos positivos de antígeno, anticuerpos contra Aspergilus, y un patrón electroforético de proteínas plasmáticas caracterizado por una elevación moderada de las fracción α2 y una severa elevación de la fracción β. El ave se trató por 10 semanas con antimicóticos administrados sistémicamente y por nebulización. La respuesta al tratamiento fue evaluada por medio de recuentos seriados de células blancas y electroforesis de las proteínas plasmáticas. La mejoría clínica del ave se correlacionó con el regreso del recuento de células blancas a niveles normales y con lo que se considera como una distribución normal en el patrón de electroforesis de las proteínas plasmáticas.

Key words: aspergillosis, itraconazole, electrophoresis, Micronesian kingfisher

Abbreviations: Ab = antibody; Ag = antigen; BID = twice a day; CP = chemistry panel; ELISA = enzyme-linked immunosorbent assay; EPH = electrophoresis; ISIS = International Species Information System; MKF = Micronesian kingfisher; PO = orally; SID = once a day; SQ = subcutaneous; TP = total proteins; WBC = white blood cells

The Micronesian kingfisher (*Halcyon cinnamomina cinnamomina*), endemic to the Pacific Islands of Micronesia, is one of the world's most endangered bird species. The species is now extinct in the wild and the remaining kingfishers, approximately 64 in all, are in U.S. zoos.

Aspergillosis, caused by the fungus *Aspergillus fumigatus* and in less proportion by *Aspergillus niger* and *Aspergillus flavus*, is one of the most important diseases in birds held in captivity (2,3,13,15,16). Micronesian kingfishers (MKF) are considered to be highly susceptible to avian tuberculosis and to chlamydiophilosis to a lesser degree, but no account of this species affected by aspergillosis was found in the literature. This report describes the diagnosis, treatment, monitoring using serum electrophoresis, and resolution of a presumptive case of aspergillosis in a Micronesian kingfisher.

CASE REPORT

A 4-yr-old, male Micronesian kingfisher presented to the hospital with weight loss and lethargy 3 wk after completing an uneventful 4-wk quarantine period. On visual examination, the bird was alert and aware of its surroundings but appeared weak, as evidenced by perching down on its hocks. The physical exam revealed a thin bird with a prominent keel. Body weight had decreased from 74.9 g 4 wk earlier to 56.9 g on the day of presentation. Other abnormalities noted were that both hocks were abraded and moderately swollen.

A blood sample, collected from the jugular while under hand restraint, was submitted for a complete blood count, chemistry panel (CP), and aspergillosis antibody-antigen titer plus electrophoresis (EPH). Radiographies were not taken because the bird was deemed too ill to withstand anesthesia. Blood work revealed a severe leukocytosis (WBC = 68.2 × 10^3/μl, International Species Information System [ISIS] range: 8.19 ± 4.92 × 10^3/μl) with heterophilia (heterophils = 56.6 × 10^3/μl, ISIS range: 2.58 ± 2.00 × 10^3/μl) and monocytosis (cells = 6.8 × 10^3/μl, ISIS range: 0.91 ± 0.66 × 10^3/μl). In addition, moderate hyperproteinemia (TP = 4.8 g/dl, ISIS normal: 3.6 ± 0.8 g/dl) with normal albumin levels (1.6 g/dl, ISIS normal: 1.9 ± 0.6 g/dl) were present. The rest of the CP was within normal limits. Serial fecal examination (fecal ova/parasite, acid-fast and routine cytology and culture) did not reveal any abnormal result. Chlamidophylia screening was negative.

Initial treatment included lactated Ringer solution (10 ml/kg) subcutaneously (SQ), once a day (SID) (B. Braun Medical Inc., Irvine, CA); cefazolin, 50 mg/kg, SQ twice a day (BID; Cefazolin, 1 g; Bristol-Mayers Squibb Company, Princeton, NJ); amikacin, 15 mg/kg, SQ SID once (Amiglyde-V, 50 mg/ml; Fort Dodge Animal Health, Fort Dodge, IA); itraconazole, 20 mg/kg PO, BID (Sporanox 10mg capsules; Janssen Pharmaceutica, Titusville, NJ); 40 mg clotrimazole diluted in 4 ml water by nebulization during 1 hr

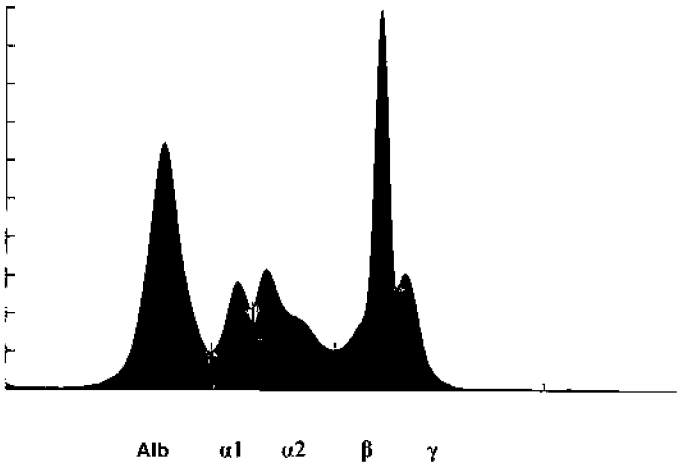

Fig. 1. Plasma protein electrophoresis from a 4-yr-old Micronesian kingfisher showing moderate elevation in the α2 fraction and a single tall peak on the β fraction, indicative of acute inflammation or infection.

a day (Clotrimazole solution 1%; Island Pharmacy, Woodruff, WI); and topical antibiotic ointment on the hock lesions (Triple Antibiotic Ointment; Taro Pharmaceuticals Inc., Bramalea, Ontario, Canada). In the beginning, the bird had to be manually restrained for medication, but within 3 days of treatment, the bird showed improvement in appetite and therefore medication was administered in feed. Fluid administration was discontinued. Initially, the itraconazole was administered via gastric tube with feed. Once the bird started consuming food on its own, the itraconazole was placed in food. The clotrimazole was administered using a DE Villbis Pulmo-Aide nebulizer capable of delivering a particle range of 0.5–5 μm.

The aspergillus test yielded a strong positive result for both antibody (Ab) and antigen (Ag) titers (Ab = 2.3, Ag = 1.9), consistent with active infection rather than exposure (6,22,24). The plasma EPH corroborated that an acute inflammatory process was present, as it showed a marked increase in the α2 and β globulin fractions (Fig. 1) (19,23). The EPH pattern was compared with an EPH performed on banked plasma from this same bird during quarantine exam as well as a different healthy adult MKF. These EPHs showed similar patterns, with no elevation on α2 and/or β globulins (Figs. 2, 3). Once the presumptive diagnosis of aspergillosis was established, the antibiotic was discontinued on day 5 and the treatment consisted only of oral itraconazole and nebulizations with clotrimazole at the above-mentioned dosages.

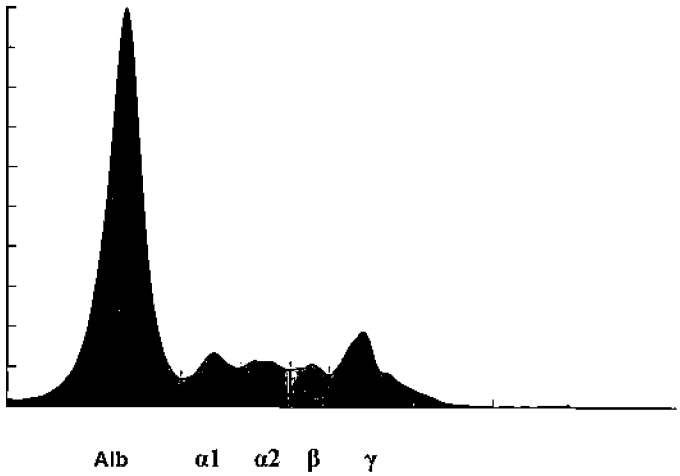

Fig. 2. Plasma protein electrophoresis from the same Micronesian kingfisher in Fig. 1 when the bird was clinically normal. There is no elevation of either α2 or β fractions.

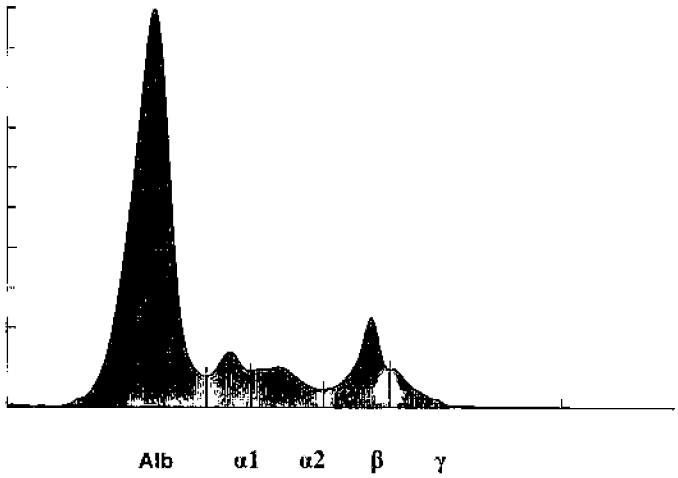

Fig. 3. Plasma protein electrophoresis from a healthy adult Micronesian kingfisher for comparison with the EPH from the sick MKF. The electrophoretic distribution is similar to the one observed in Fig. 2 when the bird was healthy. There is no elevation in either α2 and/or β globulins.

The bird's condition continued to improve and, on day 23, its body weight had increased to 75.0 g. A blood sample taken on this day showed that the leukocytosis was resolving (WBC = $11.0 \times 10^3/\mu l$) and the monocytosis was improving but had not yet returned to normal range for the species (monocytes = $1.87 \times 10^3/\mu l$). The total proteins (TP) were significantly decreased to levels within normal range (TP = 4.0 g/dl), with the albumin remaining at normal levels (1.6 g/dl). Antifungal therapy (oral and by nebulization) was continued for a total of 10 wk. By this time, the bird's appetite, body weight, and attitude had returned to normal and follow-up blood sample revealed a normal white blood count (WBC = $4.7 \times 10^3/\mu l$), with resolved monocytosis (cells = $0.89 \times 10^3/\mu l$). TP and albumin were within normal levels (3.9 g/dl and 2.4 g/dl, respectively). The final EPH showed normal α2 and β globulin

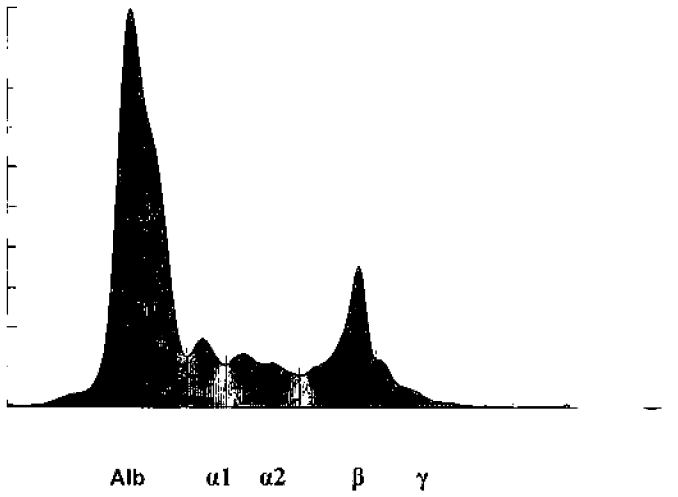

Fig. 4. Plasma protein electrophoresis from the same Micronesian kingfisher in Fig. 1 when the bird was clinically normal after intensive treatment for aspergillosis. The EPH shows minimal elevation in the β fraction but not in the α2 globulin.

patterns (Fig. 4), similar to the initial preclinical EPH and the EPH from the normal MKF. The bird remained nonclinical for 2 1/2 months, then the bird was presented for acute lethargy and dyspnea; despite aggressive shock and antifungal therapy, the bird died the following day. Necropsy revealed no gross or histological evidence of aspergillosis. Pooled samples of different organs were positive for West Nile virus by polymerase chain reaction and immunohistochemical testing, and this was considered the cause of death.

DISCUSSION

Aspergillosis is a common mycotic infection in avian species (12,13,16). All birds are considered susceptible to aspergillosis infections, but it is of particular clinical significance in captive waterfowl, wading birds, penguins, raptors, pheasants, passerines, and some species of psittacines (12,13,16,20). In addition to species susceptibility to aspergillosis, stress, malnutrition, immunocompetency, and concurrent infections may be predisposing factors (1,16). In the case of the MKF, the suspected development of aspergillosis may have been related to three potentially stressful events—transportation from quarantine facility to birdhouse building, introduction to a new environment, and aggressive behavior from the cage mate.

In aspergillosis, the clinical signs vary and depend on the anatomic site of infection, number of spores to which exposed, immune status of the animal, and chronicity of the infection (13,15). In this case, the clinical signs were more similar to the signs seen in waterfowl and wading birds, with diffuse lower respiratory tract disease in which sometimes the only clinical signs are not specific (weight loss and depression) (13,15). In contrast, psittacines, passerines, and raptors commonly have more evident clinical signs, like dyspnea, bobbing motion of tail, increased respiratory effort and rate at rest, and, occasionally, change in pitch or quality of voice if the trachea is affected (1,11,16). It was not possible to determine the site of infection in the MKF, as anesthesia to perform radiographic or laparoscopic examination was considered to be of high risk in this individual. Furthermore, it was not possible to confirm aspergillosis, as the organism was never isolated.

Antemortem diagnosis of aspergillosis has been traditionally considered difficult and a combination of methods is necessary to establish a diagnosis. Clinical signs, clinical history, blood work, laparoscopic examination of air sacs, tracheoscopy, X-rays, serology, electrophoresis, and, more recently, computer tomography and magnetic resonance have been all used for the accurate diagnosis of aspergillosis in different species with different presentations of the disease (1,2,3,5,7,8,11,13,15,18,21). In our MKF, it was decided not to anesthetize the bird due to its already compromised condition. The presumptive diagnosis was based on the clinical history, a marked leukocytosis characterized by heterophilia and monocytosis, the use of more novel diagnostic techniques, such as Ab/Ag titers, EPH, and negative results for other common conditions in this species.

Although the severe elevation of WBC can occur with other pathologic processes, such as mycobacteriosis or chlamydiophilosis, in this case, the elevated Ab/Ag titers, along with positive response to therapy, supported the presumptive diagnosis of aspergillosis. Moreover, improvement on the WBC, TP, and plasma electrophoretic distribution correlated with clinical improvement on the MKF. In this case, both the WBC and the EPH appeared to be adequate to support our diagnosis as well as to monitor clinical progress.

The aspergillus antibody and antigen ELISA have been reported to be of value in aspergillus diagnosis in some avian species, such as alcids, raptors, and Peking ducks (7,8,17,18,24). Inconclusive results in other species have been reported (9,17). At the time of writing, no information about the potential of this test as a diagnostic tool for aspergillosis was available for members of the Coraciiform order. Although initial strong positive Ab and Ag titers were indicative of active infection, a follow-up sample was not sent for ELISA testing; therefore, it was not possible to monitor serologically the resolution of this case. Instead, the resolution of the case was based on clinical, blood work, and EPH improvement. The lack of significant lesions during necropsy suggest full resolution on this case.

Protein electrophoresis has been used as a diagnostic and prognostic tool in aspergillosis in different avian species when used in conjunction with complete blood counts and aspergillosis titers (4,7,18,19,21,23). Distinct variation in protein patterns and indices exist between avian groups and sometimes within groups (21). Due to the low number of MKF in captivity, there are no species-specific electrophoretic patterns for this species. To evaluate the EPH pattern in the index case, plasma from a clinically normal individual and from the affected individual prior to development of clinical signs were used. A marked increase on the $\alpha 2$ and β globulins was observed, which indicated acute infection or inflammation (19,21). It is generally accepted that an increased β globulin fraction, along with suggestive clinical data, supports the diagnosis of aspergillosis in birds (4,5,6,9,18,23). The significance of the elevation of the $\alpha 2$ globulin in MKF is unknown, but, in psittacines species, the elevation of both α and β portions may represent acute, severe inflammation or infection (19), which corresponds with our case.

Treatment of aspergillosis commonly includes the use of one or more systemic antifungal drugs with or without the use of topical (intratracheally, nebulization) agents. A variety of drugs have been used systemically to treat successfully this condition (1,2,3,11,17,23). Itraconazole was selected on this case due to its fewer side effects, oral administration, and once-a-day dosage (14). Based on previous reports of successful nebulization treatment with clotrimazole (1,10) and the noninvasive nature of this treatment, we decided to complement systemic therapy with this modality of treatment. The bird did not show any discomfort or side effect during the nebulization sessions and, subjectively, the clotrimazole therapy had a significant positive effect. Moreover, the long-term treatment, carried out minimizing stress to the patient, was key for the successful outcome of this case.

Based in this report, Micronesian kingfishers are possibly susceptible to aspergillosis, and stressful events should be kept at minimum. If considered that the bird has been subject to excessive stress, the prophylactic use of antifungals might be considered. Institutions with MKF should perform EPH and aspergillus titers (Ab, Ag) in sick individuals with elevated WBC to rule out aspergillosis. Furthermore, electrophoresis tests should be conducted in healthy-captive MKF in order to document its electrophoretic pattern and further evaluate this technique for health screening.

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