

## DIAGNOSTIC PROCEDURES AND AVAILABLE TECHNIQUES FOR THE DIAGNOSIS OF ASPERGILLOSIS IN BIRDS

Dominik Fischer, DVM, and Michael Lierz, Dip. ECZM (Wildlife Population Health), Dip. ECPVS

### Abstract

Aspergillosis is a common and life-threatening disease in birds affecting single individuals and flocks. Acute and chronic forms of this mycotic disease have been known for centuries; however, antemortem diagnosis remains difficult. Reliable, rapid, and noninvasive diagnostic testing is desirable, especially for chronic aspergillosis cases, when other disease conditions may be present. Therefore, research has focused on the development and improvement of clinical diagnostic procedures and techniques. At present, a definitive diagnosis for aspergillosis is based on cumulative evidence from several diagnostic tests. Potential sources of fungal infection, predisposing factors, and management conditions may be identified during anamnesis. Suspect cases can be confirmed by clinical examination, radiology, or computed tomography. Additionally, results from a blood biochemistry panel, complete blood count, acute phase protein analysis, and protein electrophoresis may be used as ancillary tests to determine a patient's condition. *Aspergillus* antigen, toxin, and *Aspergillus*-specific antibody detections from blood samples may aid in determining a diagnosis, but are currently not considered reliable for all avian species. For most aspergillosis cases pathological and histopathological lesions are diagnostic and molecular biological assays are available to identify the fungal agent from biopsy or necropsy specimens. A definitive diagnosis is based on fungal identification with histopathological evidence demonstrating the fungal agent inside the affected tissue. However, endoscopic or laparoscopic samples submitted for fungal culture and subsequent identification are still regarded as valid for a clinical diagnosis of aspergillosis in birds. The current status, benefits, and disadvantages of diagnostic procedures and techniques for avian aspergillosis are provided to aid the avian practitioner in selecting a test protocol to determine a definitive diagnosis. Copyright 2015 Elsevier Inc. All rights reserved.

**Key words:** respiratory mycosis; mycotic disease; fungal infection; *Aspergillus*; avian; mold

**A**spergillosis is one of the most important and common diseases in captive birds, and is caused by fungal organisms of the genus *Aspergillus* (mainly *Aspergillus fumigatus*). Infections with fungi of other genera such as *Penicillium* and *Mucor* are rare, but the disease caused by these organisms is also called "aspergillosis." Single birds and flocks of different avian species may be affected and suffer from acute or chronic forms of this localized or systemic disease.<sup>1-14</sup> Therefore, acute disease may develop following an overwhelming exposure to fungal spores, whereas chronic cases are typically caused by the fungal agent and other predisposing factors such as immunosuppression, bacterial infections, vitamin A deficiency, and genetic factors.<sup>15,16</sup> Although aspergillosis has been well-known to avian medicine for centuries, antemortem diagnosis remains challenging.<sup>10,17,18</sup> The diagnostic objective is to identify the fungal organism consistent with clinical disease signs or pathological lesions. However, many described diagnostic techniques do not focus on specific identification, but try to indirectly demonstrate the presence of fungi.<sup>19</sup> This review provides information regarding the various diagnostic procedures that differ from invasive to noninvasive

From the Clinic for Birds, Reptiles, Amphibians and Fish, Justus Liebig University, Giessen, Germany.

Address correspondence to: Dominik Fischer, DVM, Clinic for Birds, Reptiles, Amphibians, and Fish, Justus Liebig University Giessen, Frankfurter Str. 91-93, 35392 Giessen, Germany. E-mail: [dominik.fischer@vetmed.uni-giessen.de](mailto:dominik.fischer@vetmed.uni-giessen.de)

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procedures and may provide a tentative diagnosis or definitive diagnosis. However, this information emphasizes that with most cases one test is often not adequate, but a reasonable combination of diagnostic techniques is required to obtain a proper diagnosis of avian aspergillosis.

## ANAMNESIS/HISTORY/ENVIRONMENTAL INVESTIGATION

In cases of acute aspergillosis, clinical signs may develop rapidly over a few days; however, in cases of chronic aspergillosis, signs are recognized late in the disease process by the owner and therefore falsely considered to be acute. A thorough anamnesis may reveal predisposing factors that result in cases of chronic aspergillosis,<sup>20</sup> such as suboptimal husbandry conditions (e.g., insufficient temperature, low ventilation, high dampness, and excessive dryness), poor hygiene, nutritional deficiencies (especially hypovitaminosis A), immunosuppression (e.g., stress and corticosteroid therapy), prolonged antibiotic treatment, bacterial, parasitic or viral infections, intoxication, and exposure to dust or smoke.<sup>4,5,21-26</sup> Other stressors affecting the avian immune system, including trauma, concurrent disease, excessive human traffic throughout exhibits, and recent transportation, should be determined by the veterinarian when questioning the bird's owner.<sup>17,27</sup> Potential fungal contamination should be investigated, including fungal growth on waste, food grain, hay, moldy food, and litter.<sup>28,29</sup> An increased environmental exposure to fungal spores should be expected when a period of high humidity (e.g., flood) is followed by a drying period that allows fungal growth and sporulation.<sup>30</sup> Therefore, exposure to an overwhelming number of spores may cause acute course of aspergillosis in the absence of predisposing factors, even following a single fungal exposure.<sup>15,16,31-35</sup> Air sampling methods and microsatellite typing have been used successfully for fungal identification.<sup>30,33,36-39</sup>

Differences in susceptibility of various avian species to aspergillosis have been reported by different authors based on the prevalence of aspergillosis. Other than psittacines (especially African gray parrots, amazons, and macaws), pheasants, birds of paradise, waterfowl, penguins, merlins, gyrfalcons, red-tailed hawks, rough-legged hawks, golden eagles, and goshawks are thought to be highly susceptible to *Aspergillus* spp.<sup>4,9,10,15,16,40</sup> Individuals belonging to these listed avian species should be thoroughly examined for aspergillosis; however, only a few experimental studies have been performed to determine true differences in

susceptibility between bird species<sup>41</sup> or poultry lineages.<sup>42</sup> Recent infection trials could not confirm a predisposition based on species tested.<sup>32</sup> However, some species are diagnosed more often with clinical aspergillosis than others, which possibly points to a higher relevance of husbandry or management conditions for the development of aspergillosis in those species. In fact, clinical reports and experimental studies indicate that young individuals are more susceptible than older birds.<sup>31,43,44</sup>

## CLINICAL EXAMINATION

Observation of breathing depth and rate from a distance is advised before handling the bird. When holding the patient, its external nares, sinuses, choana, pharynx, and glottis should be evaluated with the lungs, trachea, and air sacs being auscultated, preferably using a pediatric or infant stethoscope relative to the bird's size.<sup>25,45</sup> Audible sounds on expiration are often associated with aspergillosis in the lower respiratory tract, whereas inspiratory stridor may point to tracheal granulomas or similar upper respiratory tract disorders.<sup>20,45</sup> Endurance or stress tests may be performed to evaluate respiratory rate, the respiratory recovery time, and breathing.<sup>45</sup> Therefore, after 30 seconds of induced exercise (e.g., wing motion), the recovery time and the respiratory rate per minute are measured at rest and 2 minutes after the exercise. To achieve wing motion, trained raptors are allowed to grasp the falconer's glove, which is moved up and down with wide and slow movements to force the bird to "flap" its wings to maintain balance.<sup>40</sup> The examiner should look for an increased recovery time and/or a double pump, which means the abdominal wall is interrupting the bird's breathing movement. After resting 2 minutes a fit bird is considered to be able to recover a normal respiratory rate.<sup>45</sup> These respiratory abnormalities are often observed when the animal's breathing is impeded and is influenced by significant disease and other factors.<sup>40</sup>

In general, clinical signs of aspergillosis are often nonspecific and not restricted to the respiratory tract. Depending on the involved organ systems, common clinical conditions associated with avian aspergillosis include performance loss, inability to fly, ruffled feathers, tail bobbing, anorexia,

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