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Case Report

Burkholderia cepacia and *Aeromonas hydrophila* Septicemia in an African Grey Parrot (*Psittacus erithacus erithacus*)

Ahmet AKKOÇ[1], A. Levent KOCABIYIK[2], M. Özgür ÖZYİĞİT[1,*], I. Taci CANGÜL[1], Rahşan YILMAZ[1], Cüneyt ÖZAKIN[3]

[1]Department of Pathology, Faculty of Veterinary Medicine, Uludağ University, 16059 Görükle, Bursa - TURKEY

[2]Department of Microbiology, Faculty of Veterinary Medicine, Uludağ University, 16059 Görükle, Bursa - TURKEY

[3]Department of Microbiology, Faculty of Medicine, Uludağ University, 16059 Görükle, Bursa - TURKEY

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Abstract: *Burkholderia cepacia* and *Aeromonas hydrophila* infections are described in an African Grey Parrot (*Psittacus erithacus erithacus*) that presented with neurological signs, lassitude, and respiratory distress. At postmortem examination, subperiosteal ecchymotic hemorrhages in the skull, and severe subcutaneous edema in the neck and abdomen were prominent. Round, disseminated, whitish necrotic foci were noted in the congested liver. Microscopic examination revealed chromatolysis in brain neurons. Multifocal coagulation necroses were found in the liver. Non-purulent, subacute myocarditis, thromboembolic nephritis, and interstitial pneumonia were observed. Microbiological examination revealed mixed cultures of *Burkholderia cepacia* and *Aeromonas hydrophila* in brain, lung, liver, kidney, and heart samples.

Key Words: African Grey Parrot, *Psittacus erithacus erithacus, Burkholderia cepacia, Aeromonas hydrophila*

Bir Afrika Gri Papağanında (*Psittacus erithacus erithacus*) *Burkholderia cepacia* ve *Aeromonas hydrophila* Septisemisi

Özet: Bu çalışmada ilk defa, sinirsel bulgular, yorgunluk ve solunum problemi olan bir Afrika gri papağanında *Burkholderia cepacia* ve *Aeromonas hydrophila* enfeksiyonu tanımlandı. Nekropside kafatasında subperiostal kanama, ense ve karın bölgesinde şiddetli ödem vardı. Konjeste karaciğer üzerinde yuvarlak, dağılmış beyaz renkli nekrotik odaklar gözlendi. Mikroskobik incelemede, beyinde nöronlarda kromatolizis gözlendi. Karaciğerde multifokal koagülasyon nekrozu dikkati çekti. Ayrıca, subakut nonpurulent miyokarditis ve tromboembolik nefritis ile akciğerde intersitisyel pnömoni görüldü. Mikrobiyolojik muayenede, beyin, akciğer, karaciğer, böbrek ve kalpten karışık kültür olarak *Burkholderia cepacia* ve *Aeromonas hydrophila* üretildi.

Anahtar Sözcükler: Afrika Gri Papağanı, *Psittacus erithacus erithacus, Burkholderia cepacia, Aeromonas hydrophila*

Introduction

Burkholderia cepacia, formerly known as *Pseudomonas cepacia*, is a gram-negative bacillus commonly found in soil, vegetation, and water (1,2). *P. cepacia* was originally described by Burkholder in 1950 as the causative agent of bacterial root disease in onion bulbs (3). The organism has emerged as an important opportunistic pathogen in immunocompromised patients, including those with cystic fibrosis and chronic granulomatous disease (1). *B. cepacia* has been reported in mixed cultures with other bacteria in clinical specimens obtained from horses with pneumonia (4) and specific-pathogen-free piglets (5). It was also reported as the sole organism involved in a case of vegetative endocarditis in a horse (6) and mastitis in sheep (7). Nonetheless, there is currently no documentation regarding *B. cepacia* infection in birds.

Aeromonas hydrophila, a gram-negative, hemolytic rod-shaped bacteria, is an opportunistic pathogen in fishes and reptiles. Infections in mammals have only been reported rarely (8); however, the organism, either alone or in combination with other organisms, can cause localized and systemic infections in wild birds (9).

* E-mail: ozyigit@uludag.edu.tr

To the best of our knowledge this is the first report of *B. cepacia* and *A. hydrophila* septicemia in a parrot.

Case History

A 10-year-old African parrot, kept as a house pet, was referred to a veterinary clinic with neurological signs (lack of coordination and ataxia), respiratory distress, and lassitude, which began 10 days earlier. During the clinical examination the bird died, and necropsy was performed. Brain, lung, heart, liver, kidney, spleen and intestine tissue samples were taken for histopathological and microbiological examination. For histology, samples were fixed in 10% neutral buffered formalin, processed routinely, embedded in paraffin, and cut into 5-µm sections. Samples from all tissues were stained with hematoxylin and eosin (H&E). After they were examined some selected kidney, liver, and brain tissue samples were stained with Gram, Giemsa, or Ziehl-Neelsen.

For microbiological examination, samples were inoculated onto 7% sheep blood agar (Oxoid CM271) and onto MacConkey agar (Oxoid CM115), then incubated at 37 °C until colonial growth was seen under aerobic conditions (18 h for blood agar and 24 h for MacConkey agar). In addition, the samples were inoculated onto Sabouraud dextrose agar (Oxoid CM41), incubated at 25 °C and 37 °C in the dark for a minimum of 3 weeks, and examined weekly for evidence of fungal growth. Isolates were identified on the basis of colonial and cellular morphology, Gram staining, and biochemical characteristics, using standard methods (8,10) and the Microbact Gram-Negative Identification System (Medvet Diagnostics, Thebarton, South Australia).

Results and Discussion

At postmortem examination severe, diffuse subcutaneous edema, predominantly in the abdomen and neck, was noted. Subperiosteal ecchymotic hemorrhages in the skull and severe hyperemia in the meningeal and parenchymal blood vessels in the brain were observed. The liver was congested, and was disseminated, round, and 0.2-0.8 cm in diameter, with whitish necrotic foci on the serosal and cut surface. No macroscopic lesion, except congestion, was seen in other organs. Microscopically, meningeal and parenchymal vessels were congested. In some areas perivascular cuffing, predominately mononuclear leucocytes, was observed. The nuclei of chromatolytic neurons appeared pyknotic and karyolytic. Diffuse, mild to moderate gliosis was evident in the affected grey matter. Multifocal accumulation of microglial cells around the degenerated and chromatolytic neurons was evident (Figure 1). There were areas showing mild to severe infiltration of heterophils, lymphocytes, and plasma cells in the interstitial part of the lung. Large numbers of basophilic bacteria were seen in and around the blood vessels in the lung. Multifocal coagulation necrosis (Figure 2), sinusoid congestion, and diffuse vacuolization of hepatocytes were found in the liver sections. Non-purulent, subacute myocarditis and thromboembolic nephritis were the other lesions observed.

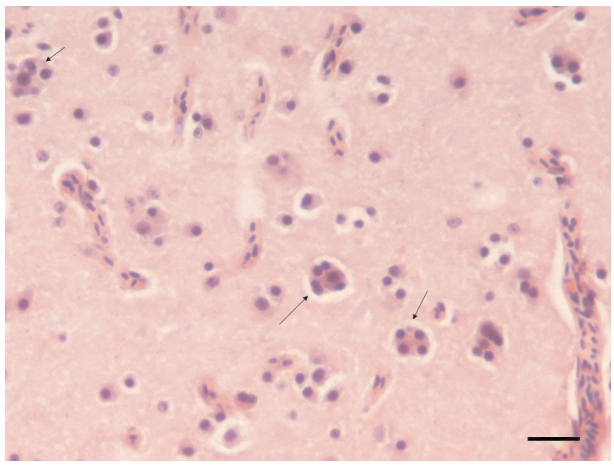

Figure 1. Multifocal accumulation of microglial cells around the degenerated and chromatolytic neurons (satellitosis), (arrows) (H&E, bar = 30 µ).

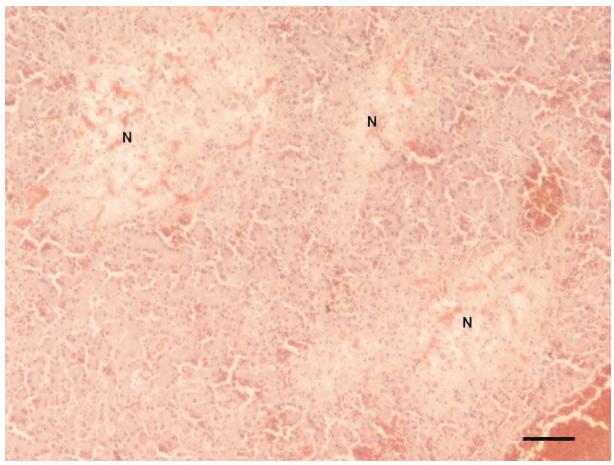

Figure 2. Multifocal coagulation necrosis (N) in the liver (H&E, bar = 170 µ).

Microscopic examination of Gram-stained smears revealed large numbers of gram-negative bacilli. The smears were not positive for modified Ziehl-Neelsen staining and typical bacteria were not observed with Giemsa staining. Colonial growth was noted on both blood agar and MacConkey agar inoculated with all tissues after 18 and 24 h of incubation, respectively. In all, 2 types of colonies grew on blood agar and at similar rates. One colony was 4 mm in diameter, b-hemolytic, round, raised, and opaque-grayish. The other colony was 2-3 mm in diameter, smooth, round, and opaque to tan in appearance. On MacConkey agar, pinkish and bright pink colonies were observed. Both colony types that grew on blood agar were gram-negative, oxidase-positive fermentative bacilli. All the isolates of both examined species exhibited the identical biochemical profile of *A. hydrophila* and *B. cepacia* according to the commercially available identification system used (Microbact Gram-Negative Identification System, Microbact 24E (12E/12A + 12B).

P. fluorescens has been isolated in pure culture from the livers of pet birds affected with necrotizing hepatitis (11). *P. pseudomallei*, the causative agent of melioidosis, was reportedly isolated from a native bird with liver necrosis in Australia (12). In Baltimore, a resident peregrine falcon (*Falco peregrinis*) was reported to have died from a *Pseudomonas* infection involving the pharynx (13). *P. aeruginosa* was reported to be one of the most frequently isolated bacteria grown in pure culture from the livers of free-ranging lesser flamingos (*Phoeniconaias minor*) with discrete necrotic and granulomatous lesions during an epizootic that resulted in the death of more than 18,500 in Kenya (14).

Members of the genus *Aeromonas* are found in aquatic environments and animals. In addition, *A. hydrophila* is reported to be present among the intestinal flora in birds. The intestinal carriage rate of *A. hydrophila* in grey herons (*Ardea cinerea*) was as high as 48% (15). Members of the genus *Burkholderia* are ubiquitous and present in soil, water, and humid environments. In addition to the widespread distribution of both microorganisms, they are considered opportunistic pathogens associated with different infections in animals and humans. *A. hydrophila* was isolated from ducks with salpingitis, septicemia, and airsacculitis (16). The organism was also isolated in pure culture from a pet parrot (*Amazona versicolor*) with bilateral conjunctivitis (17). *A. hydrophila* was reported to be the cause of the acute death of aviary canaries (*Serinus canarius*), and was also isolated in pure culture from a toucan (*Ramphastos toco*) with acute nephritis, and from a cockatiel (*Nymphicus hollandicus*) with chlamydiosis (psittacosis) (18). *A. hydrophila* was isolated in pure culture from a captive ground-hornbill (*Bucorvus abyssinicus*) in Nigeria that had acute hemorrhagic septicemia (19).

The role of *Burkholderia cepacia* as an opportunistic pathogen that causes pulmonary infection in patients with cystic fibrosis and chronic granulomatous disease is well documented, while additional evidence of its etiology in a broader range of infections in both immunocompromised and healthy individuals is accumulating (1). It has also been associated with different infections in several animal species (4-7). In poultry it is known that *Burkholderia spp.* can cause localized or systemic diseases characterized by sinusitis, keratitis and keratoconjunctivitis, and septicemia (16); however, there are no reports of *B. cepacia* infection in parrots.

In the presented case, neurological signs were significant and in accordance with the pathological findings. Additionally, pathological changes seen in the necrotic liver were characteristic of *Burkholderia* spp. infection. In birds *Burkholderia* spp. may cause subcutaneous edema and fibrin (with hemorrhaging in the head and neck), arthritis, inflammation of serous membranes, pneumonia, necrosis in liver, spleen, kidney, and brain, conjunctivitis, sinusitis, and keratitis (16). Intravascular coagulation may be seen in gram-negative bacterial septicemia (20). In such cases, microthrombi seen in lung, liver, and kidney vessels could be related to the effects of endotoxin, because endotoxin is also capable of directly activating both intrinsic and coagulation pathways (20).

Both organisms (*Burkholderia* spp. and *A. hydrophila*) are opportunistic pathogens in birds and are common inhabitants of soil and water, and *A. hydrophila* is found in the normal intestinal flora of poultry (15,16). *A. hydrophila*, either alone or in combination with other organisms, can cause localized and systemic infections in domestic and exotic animals (12,21). Although young birds are more susceptible, *Burkholderia* spp. and *A. hydrophila* have emerged as important opportunistic pathogens in immunocompromised adult birds (1,2). Suppression of the immune system may have caused more severe clinical and pathological lesions in birds

(16,21). In the presented case, there was no information or evidence suggesting immunosuppression in the animal. The pathological changes observed in the body were the result of a mixed infection and were probably more severe than those that would occur in a mono-infection.

This is the first report of a mixed infection caused by *Burkholderia cepacia* and *Aeromonas hydrophila* in an African Grey Parrot (*Psittacus erithacus erithacus*) from Turkey.

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