

Osteoarthritis and systemic infection caused by *Candida Albicans* in a common Mynah (*Acridotheres tristis*)

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Received: April 21, 2014

Accepted: January 7, 2015

Abstract

A 2-year-old male Mynah (*Acridotheres tristis*) was referred with history of abdominal hernia surgery, depression, cachexia, lethargy and swelling of foot joints. Hematological and biochemical findings consisted of leukocytosis, heterophilia, monocytosis, elevation of liver enzymes activity, hyperproteinemia and hyperglobulinemia. Fine needle aspiration (FNA) was performed from foot joints, aseptically. Mycological and bacteriological evaluations were shown *Candida albicans* infection. In spite of local and systemic treatments, signs were deteriorated and the animal was euthanized and then necropsy was done. Pathological findings revealed systemic candidiasis and fungal osteoarthritis.

Keywords: *Candida albicans*, osteoarthritis, Mynah (*Acridotheres tristis*), systemic candidiasis

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Introduction

Mycotic infections are relatively common in avian species. Respiratory tract aspergillosis and alimentary tract infections due to *Candida* and other yeasts are the most frequent forms of fungal disease observed. Yeasts are unicellular fungal organisms that reproduce by budding or schizogony (Mancianti 2001). They encompass nearly 500 species in 56 genera belonging to the Ascomycetes, Basidiomycetes or fungi imperfecti. Candidiasis, also called moniliasis or thrush is classically a superficial mycosis of mucous membranes (white pseudomembranes) affecting the upper digestive tract such as mouth, esophagus and crop. These pseudomembranes are easily removed, leaving ulcer like foci (Rupiper 1998; Nouri 2010). In birds this condition is commonly caused by *C. albicans* while other species such as *C. parapsilosis*, *C. krusei*, and *C. tropicalis* have also been implicated. This yeast-like fungus is an opportunistic agent inhabiting the skin as well as the upper-respiratory, alimentary, and genital tracts of humans and ectotherm animals, including birds. It can cause infection in immunosuppressive conditions, such as steroid administration, prolonged antibiotic therapy, subclinical malnutrition (particularly vitamin A deficiency), and viral infections (Rupiper 1998).

Case presentation

A 2-year-old male Mynah (*Acridotheres tristis*) with history of abdominal hernia surgery was referred to the veterinary teaching hospital of Ferdowsi University of Mashhad due to depression, cachexia, lethargy, swelling of foot joints and granuloma-like masses at the surface of foot skin. Blood sample was collected from brachial vein. Hematocrite was determined manually by a standard at 2500 rpm for 5 min. Total WBC counted by the haemocytometer. To differential cell count, blood smear was stained with Giemsa solution

(5%) and examined microscopically under oil immersion lens (100×) (Campbell 1988).

Plasma biochemistry factors including glucose, AST (Aspartate aminotransferase), CK (Creatine kinase), GGT (Gamma-glutamyl transferase) and LDH (Lactate dehydrogenase) were measured by an autoanalyzer (biotechnical targa 3000 Italy). Total protein was determined by a refractometer and the concentration of globulin was calculated by subtracting the albumin from the total protein. Unfortunately biochemical analysis was limited because of small volume of blood sample; therefore the evaluation of kidney function was not possible. After clinical examination, lateral and ventrodorsal whole body plain radiographs were taken using high resolution mammographic films. Articular fluid was obtained with FNA for microbiological analysis. Based on the results of synovial smear staining, pure yeasts were seen. For more evaluation, the synovial fluid sample was submitted to the mycology laboratory. Samples were inoculated onto Sabouraud glucose agar (Merck Co., Darmstadt, Germany) supplemented with chloramphenicol (0.005%) and kept at 30 °C for 7–10 days before being considered negative. Symptomatic and antifungal therapy continued but clinical signs deteriorated and the animal euthanized and necropsy was carried out.

Results and Discussion

Radiography: In lateral radiograph, gaseous distention of proventriculus and intestine was obvious. Also kidneys were seemed enlarged (Fig. 1 A). Loss of the cardiohepatic “hour-glass” shaped silhouette was seen in Ventrodorsal (VD) radiograph and that is due to hepatomegaly. Also, opacity of the cranial thoracic airsacs was increased (Fig. 1 B). Radiographic evaluation of hindlimbs revealed Moth-eaten osteolysis and periosteal reactions of left digits and tarsometatarsal bone, soft

tissue swelling around this regions and bone destruction in intermediate phalanges of 4th digit and lateral trochlea of tarsometatarsal

bone (Fig. 2). These findings were suggestive of digital osteomyelitis and septic arthritis.



Figure 1. (A) Lateral radiograph of coelomic cavity of Myna shows renomegaly and distention of proventriculus and intestine. (B) Ventrodorsal view, note hepatomegaly and increased opacity of cranial thoracic airsacs.



Figure 2. (A and B) Lateral and dorsoplantar radiographs of hindlimbes of Myna show soft tissue swelling, osteolysis and periosteal reactions of left digits and tarsometatarsal bone. Note the bone destruction in intermediate phalanges of 4th digit and lateral trochlea of tarsometatarsal bone.

Clinical pathology: Hematological findings included leukocytosis, heterophilia, monocytosis, hyperproteinemia,

hyperglobulinemia, decreased of Alb/G ratio, increased serum activity of AST and LDH (table 1).

Table 1. Clinicopathological results. ND: not determined (reference range for Amazon parrot; GGT: 1-10 u/l and LDH: 46-208 u/l) a.

Parameters	Case	Normal ^b
PCV (%)	38	38-50
WBC ($\times 10^3 / \mu\text{l}$)	18	8-12
Heterophil (%)	76	45-64
Lymphocyte (%)	20	21-55
Monocyte(%)	4	0-1
T pro (g/dl)	6.8	3.0-4.3
Alb (g/dl)	2.2	2.0-2.8
G (g/dl)	4.6	1.6-4.0
Alb/G	0.47	1-2.67
Glucose (g/dl)	279.2	228-372
AST(U/L)	480	200-352
CK(U/L)	260	250-414
GGT(U/L)	8.24	ND
LDH(U/L)	626	ND

^a Kaneko *et al* (1999)

^b Fudge (2000)

Mycology: Visual examinations of the fungal colonies were made and their characteristics including texture, pigment, and rate of growth on medium were recorded. Colonies were examined under a light microscope to determine the morphological structures of the yeasts on slide mounted in lactophenol-cotton blue. The identification of yeasts was confirmed by urease test, germ tube test and CHROM agar. Mycological analysis confirmed the microorganisms sampled from foot joints as *Candida albicans*.

Gross and microscopic pathology: Gross necropsy revealed liver lesions indicate fatty liver symptoms, renal involvement includes focal necrosis, intestine hemorrhage. The spleen and testis were free of any abnormal macroscopic findings. Collected specimens of kidneys, testes, liver, spleen, duodenum, jejunum, ileum and foot joints preserved in 10% neutral buffered formalin and were sent to the Veterinary Pathology Laboratory of Ferdowsi University of Mashhad. Collected organs for microbial examinations were duodenum, jejunum, ileum and foot joints. Tissue sections fixed in neutral buffered formalin 10%, processed by routine histological methods and stained with hematoxylin and Eosin (H & E). On microscopic examination, in addition to severe congestion; many microorganism colonies

were seen in interphalangeal joints, liver and kidney (Figs 3(I, II and III)). Areas of focal necrosis were found in the liver and also presence of urate crystals in kidney was specified. Meanwhile lesions observed in intestine include congestion, hemorrhage and goblet cells hyperplasia. The organism was recognized by masses of entangled pseudohyphae and budding yeast-like organisms in liver and kidney. Severe hemorrhage, vasculitis with invasion of fungal organisms into the vessels' wall associated with infiltration of inflammatory cells was observed. The inflammatory cells were primarily heterophils, macrophages, and lymphocytes.

To definitive diagnosis of the fungi, Period Acid Schiff (PAS) and Acid Fast staining carried out on the liver, kidney and toe skin. The microorganisms were not stained with Acid Fast but were positive for PAS (Figure 3(IV)), however, histopathologic examinations along with special staining were representative of Candidiasis. Histopathologic evaluation of the specimens taken from foot and joint skin showed multiple granulomatous foci which maybe suggestive of a mycotic infection. The results of radiography and microbiology with blood examinations, in combination with staining of specimens, provided evidences of candidiasis. Candidiasis in foot joint

confirmed and other findings including hemorrhagic and necrotic enteritis and liver lipidosis, hemosiderosis and hemochromatosis

assumed to be nutritional and management disorders.

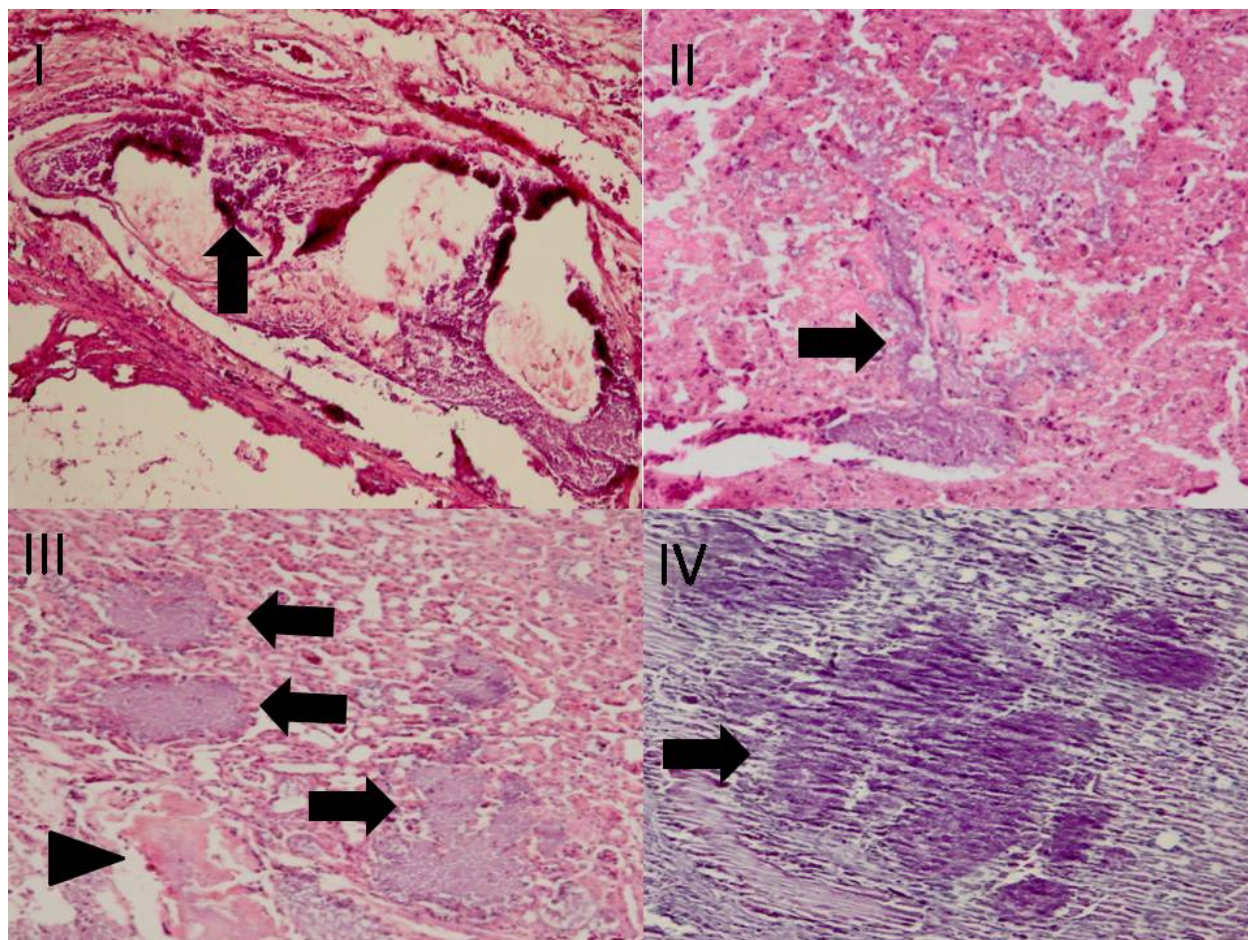


Figure 3. (I) Toe's skin, colonies of pseudohyphae of candida is stained with haematoxylin (arrow) in the dermis layer (H&E staining, 100X).

(II) Liver, Colonies of candida (arrow). Multifocal necrosis is apparent (H&E staining, 200X).

(III) Kidney, fungal pseudohyphae observed in the interstitial spaces (arrows) along with urate crystals (arrow head) (H&E staining, 200X).

(IV) Kidney, fungal colonies (arrow) (Periodic Acid Schiff Staining, 200X).

Hematology tests revealed leukocytosis, heterophilia and monocytosis that may represent an inflammatory leukogram in response to systemic fungal disease (Fudge 2000). Hyperproteinemia along with hyperglobulinemia and decreased of Alb/G ratio may indicate immunoglobulin and/or acute phase protein production following antigenic stimulation that were reported previously by researchers (Caliendo and Bull 2011). Elevated activity of AST and LDH and

normal activity of CK most likely suggest hepatocellular injury (Fudge 2000) that is confirmed by pathologic findings.

Mycotic infections frequently involve bones in a nonsuppurative type of osteomyelitis, which may be multifocal, in dog and cat. This type of osteomyelitis is usually spread haematogenously, and is therefore usually polyostotic. These lesions can also be monostotic and are metaphyseal in location. Fungal infection is more common in dogs than

it is in cats (Barr and Kirberger 2006) Blastomyces may produce aggressively destructive lesions in the early stages of infection. Coccidioidomycosis and blastomycosis produce proliferative, sclerotic bone changes with small lytic areas. Histoplasmosis produces a more destructive type of lesion. Mycotic infections frequently produce a mottled appearance in bone. Polyostotic osteomyelitis is more likely to be fungal than bacterial in origin (Kealy et al., 2011). It is difficult to distinguish radiographically between a fungal osteomyelitis and a neoplasm; therefore a one biopsy is always warranted (Barr and Kirberger 2006).

Candida associated arthritis may involve one or several joints and may be associated with osteomyelitis. In this case both feet were affected and osteomyelitis confirmed by radiography.

Compared with mycoses in humans, mycotic diseases in animals have received much less attention. Different methods like fungal culturing, histopathology, Polymerase Chain Reaction (PCR) and etc, can be used for diagnosing candida species. There are risk factors for developing this kind of candidiasis which include intravascular cannulation, especially central venous catheterisation associated with total parenteral nutrition, artificial ventilation and administration of broad spectrum antibiotics. These data somehow agrees with this case in which it has undergone surgery and anesthesia for repairing abdominal hernia plus antibiotic therapy (Velasco 2000; Tsuagr 1986). Mycotic infections should be put in the list of differential diagnosis in such cases in order to design an effective therapeutic protocol. To the authors' knowledge this is a rare case of candidiasis in a Mynah (*Acridotheres tristis*).

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استئو آرتريت و عفونت سیستمیک در یک مینای معمولی ناشی از کاندیدا آلبیکنس

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دریافت مقاله: ۱۳۹۳/۰۲/۰۱ پذیرش نهایی: ۱۳۹۳/۱۰/۱۷

چکیده

یک قطعه مینای معمولی نر ۲ ساله با تاریخچه عمل جراحی فتق شکمی و علائم افسردگی، لاغری، ضعف و بی حالی و تورم مفاصل پا و توده های شبه گرانولومایی در سطح مفاصل و پوست پا ارجاع داده شد. در آزمایشات بیوشیمیایی و خونشناسی، تغییراتی چون لوکوسیتوز، هتروفیلیا، مونوسیتوز و افزایش فعالیت آنزیم های کبدی به همراه هیپر پروتئینمی و هیپر گلوبینمی مشاهده شد. پس از اخذ مایع مفصلی از مفاصل انگشتان پا، بررسی باکتری و قارچ شناسی، کاندیدا آلبیکنس بعنوان عامل این شرایط شناخته شد اما علی رغم درمان های صورت گرفته موضعی و کلی، بدلیل رو به وخامت گذاشتن شرایط پرنده، مرگ آرام تجویز و کالبد گشایی صورت گرفت. یافته های پاتولوژی نشان از عفونت سیستمیک کاندیدیازیس با استئوآرتريت قارچی داشت.

واژگان کلیدی: کاندیدا آلبیکنس، استئوآرتريت، مینا، کاندیدیازیس سیستمیک