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Case Report

Fatal infection by *Acinetobacter baumannii* in *Ramphastos toco* (Muller, 1776)

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Abstract
Acinetobater baumannii is a critically important bacterial pathogen due to its multidrug resistance and association with severe clinical conditions, including septicemia. Its presence in animal hosts and environmental reservoirs underscores the importance of a One Health approach. We describe a fatal case of pneumonia, pericarditis, and aerosaculitis in a juvenile *Ramphastos toco* infected with *A. baumannii*, in Brazil. Postmortem examination revealed multifocal necrotizing lesions in the lungs and air sacs, with Gram-negative coccobacilli confirmed as *A. baumannii* via MALDI-TOF mass spectrometry. This report highlights the emerging threat of *A. baumannii* in wildlife populations and emphasizes the need for enhanced surveillance under a One Health framework.

Keywords: One Health, avian pathology, gram-negative bacteria, septicemia.

Introduction

*Acinetobacter baumannii i*s a Gram-negative, non-motile, strictly aerobic coccobacillus that has emerged as a significant nosocomial pathogen affecting both human and animal populations. While primarily linked with health-care-associated infections, its detection in domestic and wild animals suggests a broader ecological distribution that warrants further investigation. It is considered an emerging opportunistic pathogen in human and veterinary medicine. Although *A. baumannii* exhibits significant genotypic diversity, hospital outbreaks worldwide are predominantly caused by a limited number of clones (1). For example, isolates from cattle and pigs in Scotland contained fewer antibiotic resistance genes and may have fewer virulence genes than the clones isolated from human infections (2).

A. baumannii causes a myriad of clinical manifestations, including infections in multiple tissues, such as the skin, meninges, bladder, and lung, as well as septicemia, especially in hospitalized humans. There are limited descriptions of it causing severe disease in wildlife and some reports in domestic animals, with some animal isolates revealing antibiotic resistance genes (3). The clinical significance of *A. baumanni* stems from its remarkable capacity to develop multidrug resistance, making it a "critical" priority pathogen by the World Health Organization (4–6). This resistance potential, combined with its environmental persistence through desiccation resistance, biofilm formation, and motility, makes it a formidable pathogen in clinical and environmental settings

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(7). The family Ramphastidae comprises 50 species across five genera and is distributed across Central and South America (8). Despite growing recognition of its importance in human medicine, reports of A. baumannii infections in wildlife remain scarce. The current report documents the first known fatal infection in a toco toucan (Ramphastos toco, *Müller, 1776*), providing important insights into the pathogen's potential impacts on wildlife health and conservation.

Case description

A juvenile male toco toucan was found grounded near its nest in São Paulo, Brazil (-23.421479, -46.787048). The initial examination revealed a yellowish oral plaque that resolved after treatment with meloxicam and metronidazole. However, the animal subsequently developed dyspnea and diarrhea and succumbed to illness within four days of presentation. A veterinary pathologist performed a standardized necropsy, with detailed examination of all organs. Necropsy findings included multiple yellow necrotic foci throughout the lung parenchyma, fibrinous exudate covering the pericardial surface, and diffuse thickening with yellow discoloration of the air sacs. (Figure 1). The intestinal mucosa appeared markedly hyperemic.

We sampled the surfaces of the lung and air sac using a swab, collected representative tissue samples of the main organs (lung, air sacs, heart, liver, spleen, brain, kidneys and intestines) in 10% neutral buffered formalin and sent them to the reference

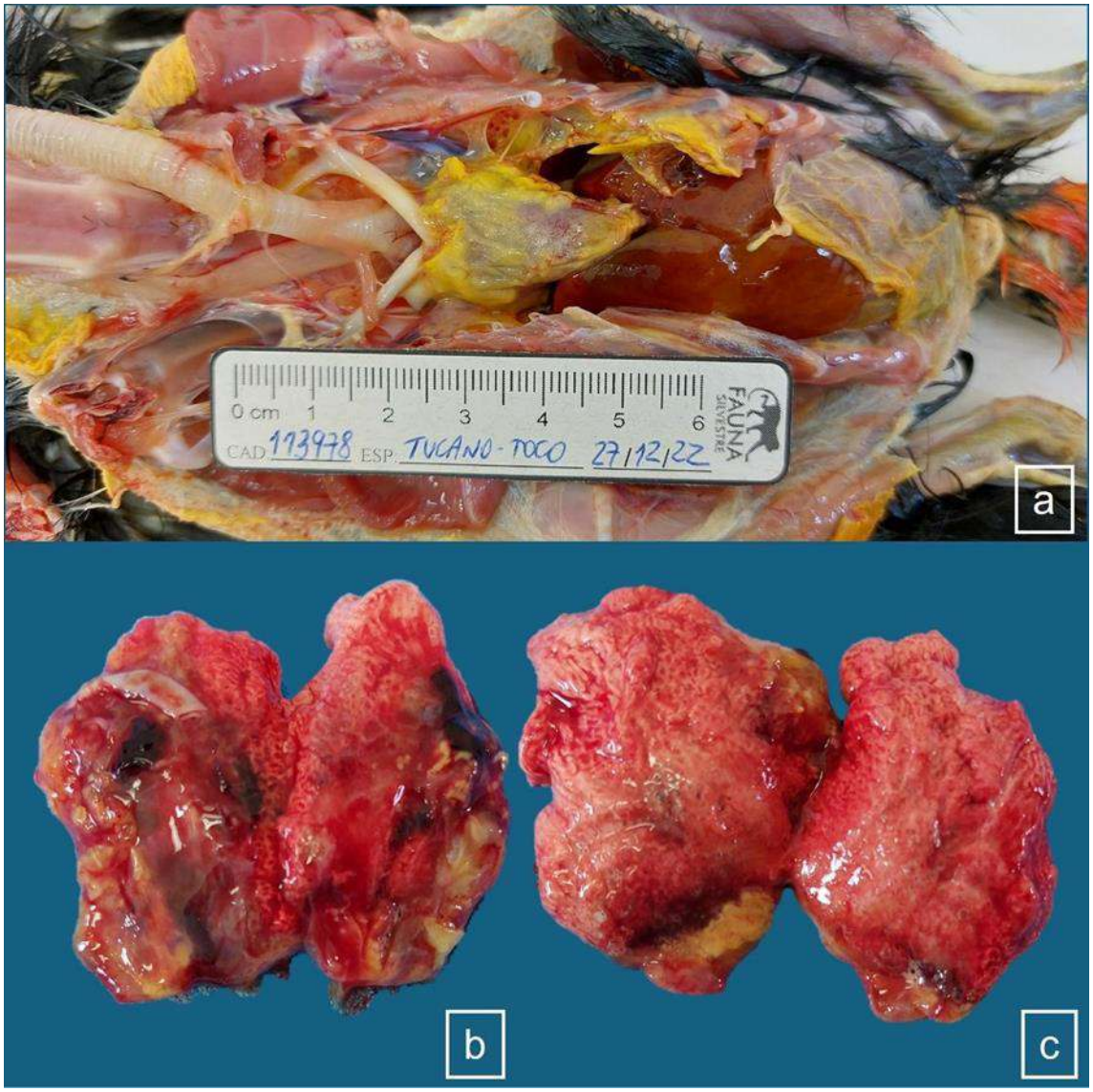

Figure 1. Macroscopic lesions observed at a *Ramphastos toco* necropsy. A. Coelomic cavity, ventral view. Marked thickening of the pericardium and air sacs, with yellowish coloration. B. Lung, ventral surface. C. Lung, dorsal surface. Increased volume, deposit of fibrinous material on the surface, congestion, presence of yellowish caseous plaques in the caudal and lateral regions.

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laboratory, Adolfo Lutz Institute, as part of a wildlife surveillance initiative (VIGIFAUNA). Fragments were then processed, paraffin-embedded, and slides were produced for staining. H&E, Brown & Brenn gram, Grocott-Gomori methenamine silver (GMS), Giemsa, and Ziehl-Neelsen stainings were performed according to the protocols standardized in the laboratory.

On microscopy, the epicardium was infiltrated by lymphocytes, plasma cells, and heterophils, with a mild presence of Gram-negative coccobacilli. The lung had multiple extensive foci of fibrinoheterophilic infiltrate, with cellular debris and a large amount of Gram-negative coccobacilli and rare Gram-positive cocci, which were also present in the air sac, associated with an intense exudate of heterophils and fibrin. Some coccobacilli were positive on GMS staining. A portion of the small intestine presented moderate enteritis, characterized by an inflammatory infiltrate of lymphocytes, macrophages, and heterophils, accompanied by villus atrophy. No microscopic lesions were detected in other organs. Bacterial culture was carried out using nutrient agar, and *A. baumannii* was identified through proteomic analysis employing Matrix Assisted Laser Desorption Ionization – Time of Flight using the MALDI Biotyper 3.0 (Bruker Daltonics, USA), according to the manufacturer's instructions. Pure bacterial colonies were cultured for 24 hours. A small sample of these colonies was collected and placed on the MALDI-TOF plate. Then, 1 µL of the matrix solution (CHCA - α-cyano-4-hydroxycinnamic acid) was added to assist in the ionization of the bacterial proteins.

Discussion

This case is an important addition to the limited literature documenting *A. baumanni* infection in wildlife species. Bacterial infections are a significant cause of mortality

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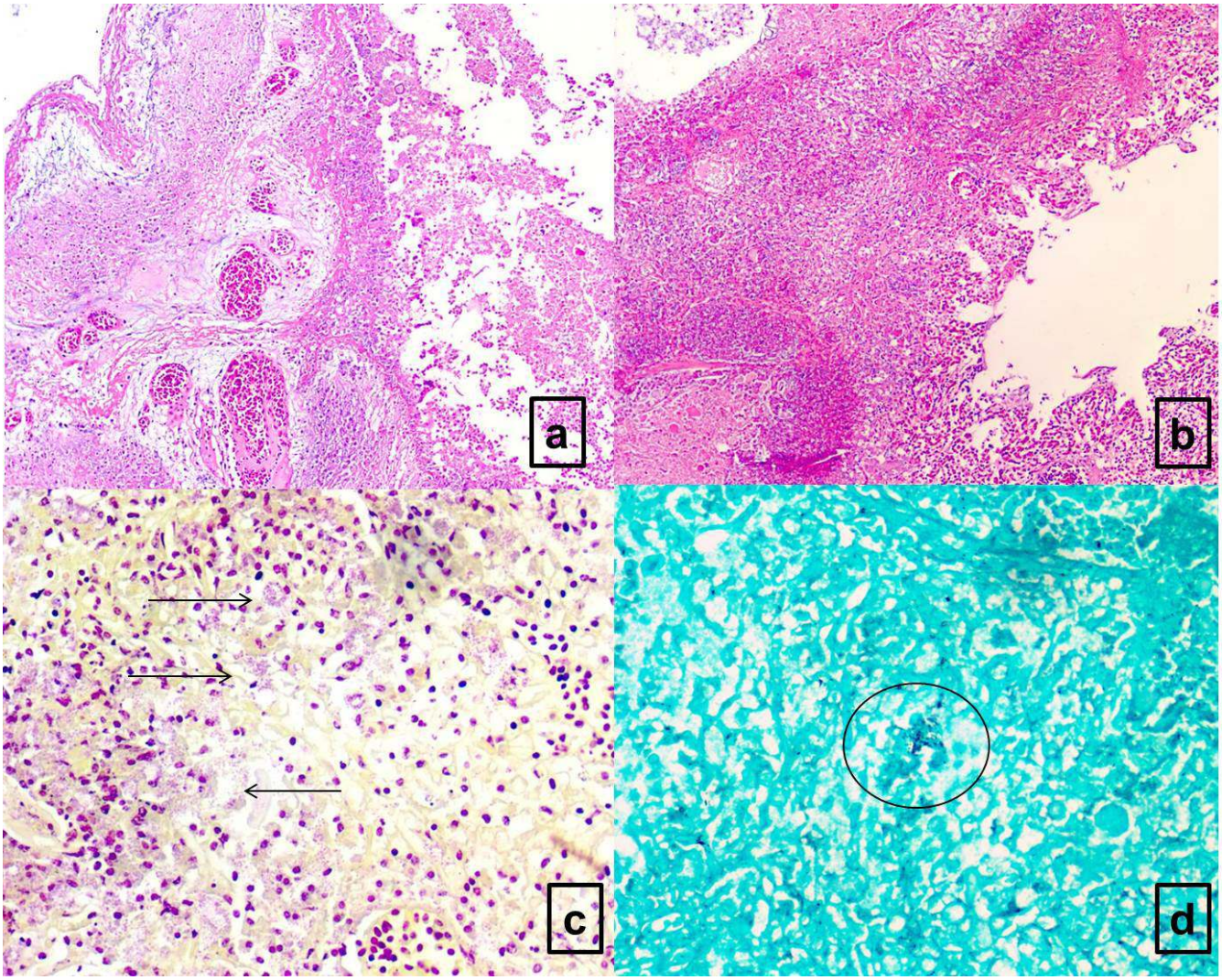

Figure 2. *Ramphastos toco*, photomicrographs. A. Air sac: Severe fibrinoheterophilic aerosaculitis (H&E, 100x). B. Lung: Severe fibrinoheterophilic pneumonia (H&E, 100x). C. Lung: Numerous gram-negative coccobacilli indicated by arrows. (Brown & Brenn, 200x). D. Lung: Aggregate of argentophilic coccobacilli, positive with GMS staining (GMS, 200x).

in avian species. *A. baumannii* is a pathogen of increasing concern, mainly due to the emergence of antibiotic-resistant clones in human and veterinary hospitals and the environment (1). This bacterium's natural host and potential reservoirs are poorly defined, despite its importance in human health (9). The literature on infections in animals is scarce. Some isolates from domestic animals were acquired during hospitalization as a nosocomial agent (10,11). *A. baumannii* has caused fatal pneumonia in European minks, including in a critically endangered species, highlighting the threat also posed to wildlife conservation (12,13). In Brazil, the bacterium was detected in a wild European hare with granulomatous pneumonia, found dead after a collision (14). In avian species, *A. baumannii* was found associated with *Mycobacterium* in falcons presenting multiple cutaneous lesions, highlighting the diagnostic challenges posed by polymicrobial infections in wildlife (15).

In our case, rare Gram-positive cocci were also found in the lung on microscopy, demonstrating a possible co-infection, although their identification was impossible. *A. baumanni* is part of a group of bacteria referred to by the acronym ESKAPE, which are of concern due to their potential to develop multidrug resistance, limiting treatment options. From a One Health perspective, this case raises several important considerations. The wild origin of the affected toucan suggests environmental exposure rather than healthcare-associated transmission. This finding emphasizes the need for expanded environmental surveillance to identify potential reservoirs of this pathogen in natural ecosystems. Furthermore, the potential for antibiotic resistance gene exchange between wildlife and human/livestock strains underscores the interconnected nature of microbial threats across species boundaries, reinforcing the importance of a One Heath surveillance approach, as outlined in the One Health Joint Plan of Action (16).

In conclusion, this case raises several important considerations from a One Health perspective. The wild origin of the affected toucan suggests environmental exposure rather than healthcare-associated transmission. This finding emphasizes the need for expanded environmental surveillance to identify potential reservoirs of this pathogen in natural ecosystems. Furthermore, the potential for antibiotic resistance gene exchange between wildlife and human/livestock strains underscores the interconnected nature of microbial threats across species boundaries.

Conflict of Interest

The authors declare no competing interests.

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