


## CLINICAL REPORT

## *Mycobacterium avium avium* Causing Conjunctivitis in a Cockatiel (*Nymphicus hollandicus*)

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## ABSTRACT

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Avian mycobacteriosis, caused by *Mycobacterium avium avium*, presents a significant concern in both wild and domestic bird populations, including pet birds and poultry, with zoonotic potential. This article discusses the case of conjunctival mycobacteriosis in a cockatiel, a rare manifestation of the disease. A 5-year-old male cockatiel presented with a protuberance on its right lower eyelid, which upon biopsy revealed necrotic eosinophilic follicles surrounded by multinucleated giant cells and an inflammatory cell infiltrate. Ziehl-Neelsen staining confirmed the presence of acid-fast rod-shaped bacteria within phagocytic cells, indicative of *Mycobacterium avium avium* infection. PCR assay targeting the rpoB gene further confirmed the species identification. Phylogenetic analysis showed high sequence similarity with human isolates. This case underscores the importance of considering mycobacteriosis in birds with granulomatous conjunctivitis and highlights the complexity of diagnosis, emphasizing the utility of histological examination alongside molecular techniques for accurate identification. Detection of mycobacterial infection in avian species is crucial both for veterinary and public health perspectives, given the zoonotic potential of these pathogens.

## Introduction

Mycobacteriosis is a worldwide disease that occurs in different kinds of birds including pet birds, and wild birds as well as poultry. *Mycobacterium avium subsp avium* is a subspecies of the *Mycobacterium avium* complex, a group of bacteria known to cause various infections in animals and humans. It is significant to note that mycobacteriosis can also infect humans and is classified as a zoonotic pathogen.<sup>1,2</sup> Avian mycobacteriosis is a group of infectious diseases caused by several species of *Mycobacterium* genus bacteria. All species of *Mycobacteria* induce a chronic inflammatory response with the granulomatous pattern. Many species of mycobacteria have been isolated from birds so far, among them, *M. avium subsp avium* and *M. genavense* are well-recognized pathogens of pet birds.

*Mycobacterium avium subsp avium*, unlike *M. avium subsp genavense*, is less frequently isolated in psittacinae and songbirds.<sup>2</sup>

Since mycobacterial lesions are frequently isolated from the intestine and liver, the oral route is known to be the primary transmission mode. Moreover, damage to the respiratory system in some cases confirms the airborne route of transmission.<sup>3</sup> Following oral transmission of *M. avium*, it invades the intestinal cells and other organs such as the lungs, spleen, liver, bone, gonads, and air sacs may be infected. In most birds, classical chronic lesions of mycobacteriosis are non-caseated, non-mineralized nodules in different organs.<sup>4</sup> Classic tubercles of mycobacteriosis are not found in birds. However, skin and conjunctival masses have been reported in some cases.<sup>3</sup>

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In contrast to internal organs which are frequently infected with mycobacterium via ingestion or inhalation, cutaneous form and ocular granulomatous lesions are less common.<sup>1,3</sup> Dyspnea and respiratory involvement and neurologic signs have been reported in some cases.<sup>5,6</sup>

Mycobacteriosis can be diagnosed by microscopic evaluation. Mycobacteria spp. are non-spore and non-motile bacilli.<sup>7</sup> These acid-fast organisms are usually identified by Ziehl-Neelsen (carbol-fuchsin) staining of biopsy or fecal specimens.<sup>8</sup> These rod-shaped bacteria are found within tissue macrophages or epithelioid cells or extracellular. The bacteria appear as pink-red color rods in acid-fast staining.<sup>9</sup> In this report, we describe conjunctival mycobacteriosis in a cockatiel (*Nymphicus hollandicus*).

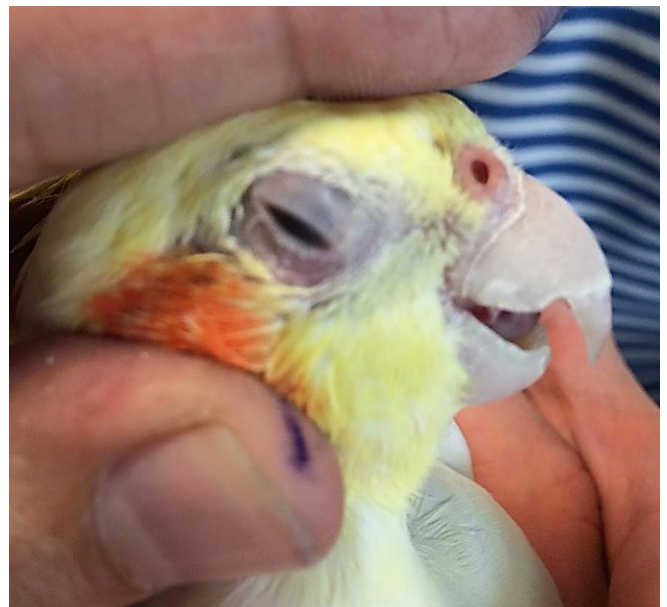
### Case Description

The case involved a 5-year-old male cockatiel that was presented to the veterinary hospital due to a protuberance on its right lower eyelid. Upon closer examination, it was determined that the mass originated from the nictitating membrane, which is a specialized third eyelid found in many bird species. Remarkably, the bird appeared to be in good overall condition and did not display any clinical symptoms (Figure 1). To investigate the nature of the protuberance and its underlying pathology, biopsy specimens of the conjunctiva (the thin, transparent tissue covering the front surface of the eye) were collected. These specimens were preserved in 10% neutral buffered formalin and subsequently sent to the diagnostic pathology service for further analysis. The samples were embedded in paraffin, allowing them to be thinly sliced into sections measuring 5  $\mu$ m. The sections were then stained using hematoxylin and eosin, which are commonly used dyes in histological examination. Upon histological examination, it was observed that a significant portion of the conjunctiva had been replaced by necrotic eosinophilic follicles located at the center of the affected area. These follicles were surrounded by multinucleated giant cells, indicating an immune response to the infection or inflammation. At the periphery of the follicles, there was a dense accumulation of mononuclear inflammatory cells, such as macrophages, epithelioid cells, lymphocytes, and plasma cells. Additionally, extensive collagenous fibers, which are produced by fibroblasts, were present in this region (Figures 2 and 3). Importantly, further examination using the Ziehl-Neelsen staining method revealed the presence of acid-fast rod-shaped bacteria. Acid-fast staining is a diagnostic technique that specifically highlights mycobacteria, allowing them to be visualized under a microscope. The bacteria-stained pink-red within the cytoplasm of phagocytic cells, indicates their presence and association with the observed inflammatory

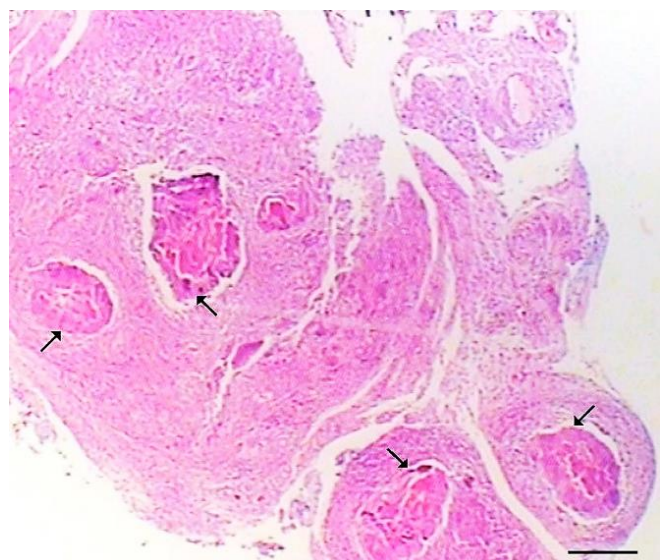
response (Figure 4). These findings suggest that the cockatiel's conjunctival pathology is associated with an infection caused by *Mycobacterium avium* subsp *avium*. The presence of necrotic follicles, multinucleated giant cells, and an inflammatory cell infiltrate indicates an active immune response to the bacteria. Identification of the specific bacterium involved in the infection will aid in determining appropriate treatment options and preventive measures for the affected cockatiel.

### PCR Assay

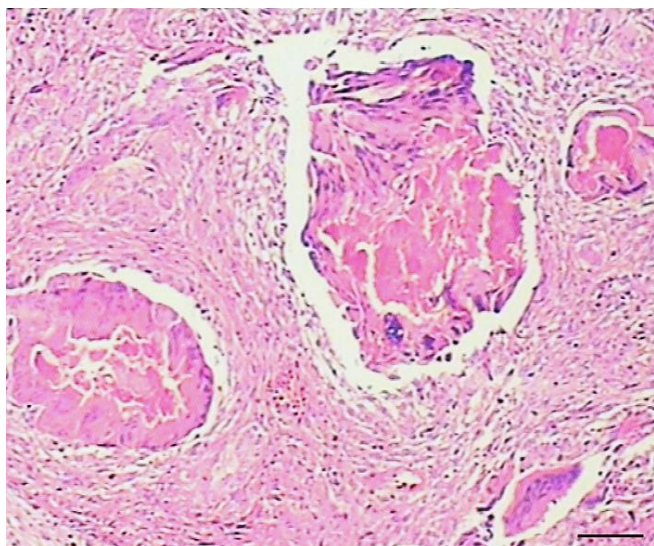
rpoB gene sequencing was performed for definite species identification and construction of the phylogenetic tree of the sample (Figure 5). The rpoB gene of the sample was amplified by conventional polymerase chain reaction (PCR) using the universal primers F: (5'-GGCAAGGTCACCCCGAAGGG-3') and R: (5'-



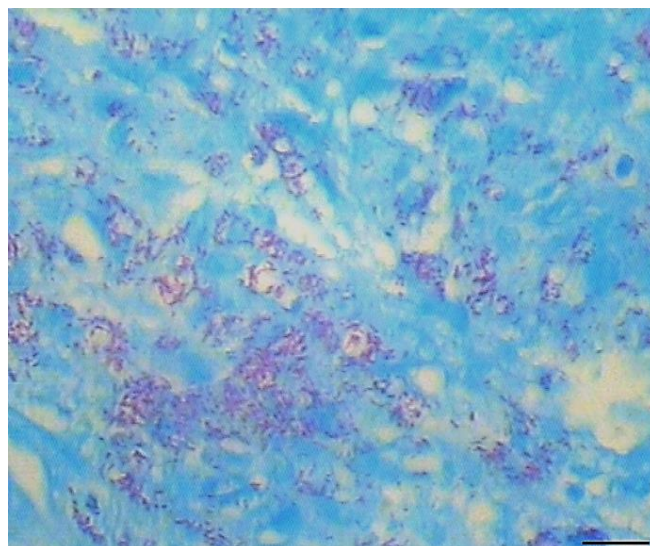
**Figure 1.** Macroscopic appearance of the case suffering from avian mycobacteriosis.



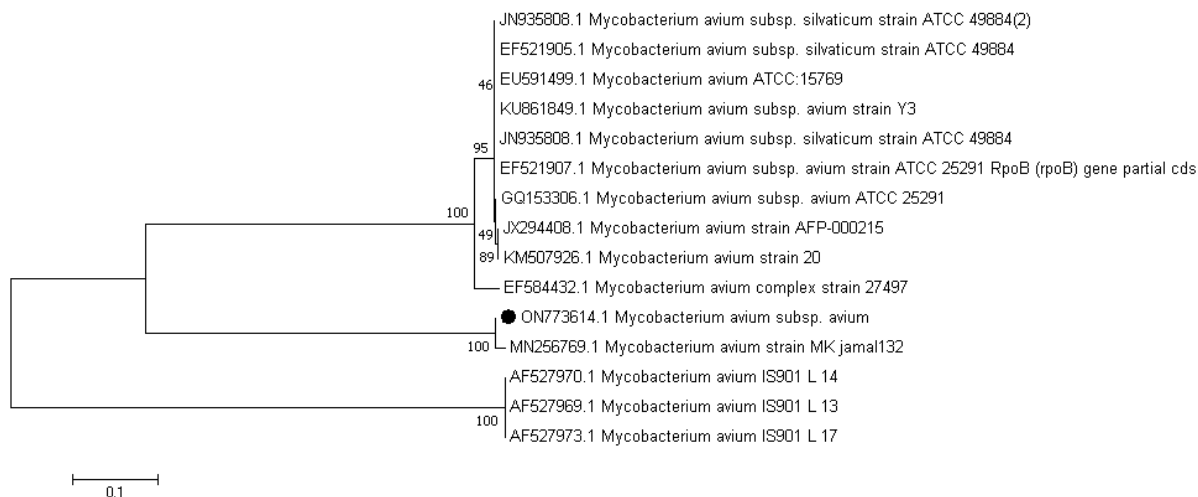
**Figure 2.** Multifocal caseonecrotic materials (arrows) are embedded in a dense fibrous connective tissue. HE Bar=400  $\mu$ m.



**Figure 3.** Caseonecrotic follicles entirely surrounded by palisade pattern of multinucleated giant cells at the margin and mononuclear inflammatory cells associated collagen concentric fibers around the lesion. Bar = 200  $\mu$ m.



**Figure 4.** Mycobacteria microorganisms are packed in the cytoplasm of mononuclear cells by acid-fast stain. Bar = 30  $\mu$ m.



**Figure 5.** Phylogenetic tree based on *rpoB* gene sequencing and neighbor-joining method classification among the sample in this report (NCBI, GenBank accession number: ON773614) and the strains reported by other researchers published in NCBI.

AGCGGCTGCTGGGTGATCATC-3').<sup>10</sup> PCR was conducted in 25 $\mu$ l reaction mixtures containing 2 mM of MgCl<sub>2</sub> (Fermentas, Lithuania), 0.2 mM of each dNTP (Fermentas, Lithuania), 2.5  $\mu$ l of 10X PCR buffer, 2 U of Taq DNA Polymerase (Fermentas, Lithuania), 10 pmol of each primer (BIONEER, Korea) and 2  $\mu$ l of DNA template. The reaction was carried out in a thermocycler (BIORAD T100- USA) as follows; an initial denaturation at 94 C for 3 min, then 35 cycles of 94 C for 30 s, 59 C for 30 s, 72 C for 45 s and a final extension at 72 C for 10 min. The amplification product (5  $\mu$ l) was resolved by electrophoresis on 1.5% agarose gel in 1X TBE buffer for 1 h at 100 V. Afterwards, the agarose gel was stained with 1  $\mu$ g/ml ethidium bromide (CinnaGen, Iran) and screened using an UV-Trans-illuminator (BIORAD, UK). PCR products were extracted, purified, and sequenced by GATC Company (Cologne, Germany). Sequencing of the *rpoB* gene (NCBI, GenBank accession number:

ON773614) indicated that the isolate was *Mycobacterium avium* subsp. *avium*. CLC software (CLC Genomic WorkBench, Qiagen, Hilden, Germany) was used to generate the cladogram by the neighbor-joining clustering algorithm, bootstrapped 1000 times (using the *rpoB* gene sequence of the isolate and the NCBI GenBank database).

### Clinical Relevance

*Mycobacteria* are types of bacteria that can lead to an infectious disease, typically characterized by a chronic course and the formation of granulomatous lesions in different organs and tissues within the body.<sup>11</sup> The genus of *Mycobacterium* consists of more than 60 species and more than 100 subspecies. There are 20 known species of pathogenic mycobacterium genus in humans and animals, among them three known species such as *M. tuberculosis*, *M. leprae*, and *M. bovis* considered human

pathogens.<sup>12</sup> Mycobacteriosis in companion birds possesses a chronic form and the infection is caused by the combination of *M. avium* and *M. intracellulare*. Birds are susceptible to two species of human mycobacterium including *M. tuberculosis* and *M. genavense*.<sup>13</sup> Consequently, based on the zoonotic potential of this bacterium, the clinical diagnosis of mycobacteriosis in companion birds by vet clinicians is very important.

The clinical presentation of the disease in birds is highly variable. Most affected birds are lethargic and weak. Some infected birds have normal appearance and behavior.<sup>1</sup> Visceral granulomas are the most common lesion. Ocular lesions are rare in birds, but conjunctival mycobacteriosis has been reported in an Amazon parrot (*Amazona* sp.), ostrich, and emu (*Dromaius novaehollandiae*).<sup>14</sup> Instances of swellings in the area around the eyes have been documented, and occasionally, there have been reports of a discharge that ranges from serous to gelatinous in nature.<sup>15,16</sup> This study is the first report of conjunctival mycobacteriosis in a cockatiel. An eye lesion without the obvious loss of body condition is a rare status, which has been seen in this case.

The definitive diagnosis of conjunctival tuberculosis is by confirming the presence of bacteria in the biopsy specimen.<sup>17</sup> The most common and most rapid method for detecting acid-fast bacilli is the evaluation of biopsy samples and conjunctival scrapings under a microscope. Definite diagnosis of conjunctival tuberculosis is performed by observing granulomatous inflammation with caseation in samples. Other methods for diagnosis are culture and polymerase chain reaction (PCR) which can be used for confirmation of histopathology results. However, the sensitivity of PCR in the detection of *Mycobacterium* ranges from 66–80 % and cultures on Löwenstein–Jensen medium are insidious.<sup>18,19</sup>

It concluded that clinicians should consider mycobacteriosis as a differential diagnosis in granulomatous conjunctivitis of cockatiels. Histological examination is recommended in the suspected cases of mycobacteriosis due to the complexity of other diagnostic techniques such as culture-based techniques. Figure 5 represents the phylogenetic relationships among the *M. avium avium* sample isolated in this study and previously reported strains. Comparison of the *rpoB* gene sequence with the strain MKjama132 isolated from humans in Iran indicated high similarity in sequence and phylogenetic relatedness.

### Conflict of Interest

None to declare.

### References

1. Tell L, Woods L, Cromie R. Mycobacteriosis in birds. *Revue Scientifique et Technique Office International des Epizooties*. 2001; 20: 180-203. doi: 10.20506/rst.20.1.1273
2. Buur J, Saggese MD. Taking a rational approach in the treatment of avian mycobacteriosis. *Veterinary Clinics: Exotic Animal Practice*. 2012; 15(1): 57-70. doi: 10.1016/j.cvex.2011.12.001
3. Shivaprasad H, Palmieri C. Pathology of mycobacteriosis in birds. *Veterinary Clinics: Exotic Animal Practice*. 2012; 15(1): 41-55. doi: 10.1016/j.cvex.2011.11.004
4. Palmieri C, Roy P, Dhillon A, Shivaprasad H. Avian mycobacteriosis in psittacines: a retrospective study of 123 cases. *Journal of Comparative Pathology*. 2013; 148(2-3): 126-138. doi: 10.1016/j.jcpa.2012.06.005
5. Portaels F, Realini L, Bauwens L, Hirschel B, Meyers W, De Meurichy W. Mycobacteriosis caused by *Mycobacterium genavense* in birds kept in a zoo: 11-year survey. *Journal of Clinical Microbiology*. 1996; 34(2): 319-323. doi: 10.1128/jcm.34.2.319-323.1996
6. Odiawo G, Mukurira J. Avian cerebral tuberculosis. *The Veterinary Record*. 1988; 122(12): 279-280. doi: 10.1136/vr.122.12.279
7. Grange J. The biology of the genus *Mycobacterium*. *Journal of Applied Bacteriology*. 1996; 81: 1S-9S. doi: /10.1111/j.1365-2672.1996.tb04592.x
8. Aranaz A, Liébana E, Mateos A, Dominguez L. Laboratory diagnosis of avian mycobacteriosis. Elsevier; 1997:9-17. doi: 10.1016/S1055-937X(97)80036-9
9. VanDerHeyden N. New strategies in the treatment of avian mycobacteriosis. Elsevier; 1997: 25-33. doi: 10.1016/S1055-937X(97)80038-2
10. Adékambi, T., Colson, P. and Drancourt, M. *rpoB*-based identification of nonpigmented and late-pigmenting rapidly growing mycobacteria. *Journal of Clinical Microbiology*. 2003; 41(12): 5699-5708. doi: 10.1128/JCM.41.12.5699-5708.2003
11. Robbins P, Terrell SP, Bradway D, Wier F. Mycobacterial infection in a fairy bluebird (*Irena puella*): a diagnostic conundrum. *Journal of Zoo and Wildlife Medicine*. 2009; 40(1): 189-192. doi: 10.1638/2007-0011.1
12. Ignatov D, Kondratieva E, Azhikina T, Apt A. *Mycobacterium avium*-triggered diseases: pathogenomics. *Cellular Microbiology*. 2012; 14(6): 808-818. doi: 10.1111/j.1462-5822.2012.01776.x
13. Gelis S, Gill JH, Oldfield T, Jaensch SM, Raidal SR. Mycobacteriosis in gang gang cockatoos (*Callocephalon fimbriatum*). *Veterinary Clinics: Exotic Animal Practice*. 2006; 9(3): 487-494. doi: 10.1016/j.cvex.2006.05.020
14. Pocknell A, Miller B, Neufeld J, Grahn B. Conjunctival mycobacteriosis in two emus (*Dromaius novaehollandiae*). *Veterinary Pathology*. 1996; 33(3): 346-348. doi: 10.1177/030098589603300314
15. Brown R. Sinus, articular and subcutaneous *Mycobacterium tuberculosis* infection in a juvenile red-lored Amazon parrot. 1990: 305-308.
16. Ševčková Z, Ledecký V, Capik I, Levkut M. Unusual manifestation of tuberculosis in an ostrich (*Struthio camelus*). *Veterinary Record*. 1999; 145(24): 708-708. doi: 10.1136/vr.145.24.708
17. Chaurasia S, Ramappa M, Murthy SI, Vemuganti GK, Fernandes M, Sharma S, Sangwan V. Chronic conjunctivitis due to *Mycobacterium tuberculosis*. *International Ophthalmology*. 2014; 34: 655-660. doi: 10.1007/s10792-013-9839-y
18. Marchetti G, Gori A, Catozzi L, Vago L, Nebuloni M, Rossi MC, Esposti AD, Bandera A, Franzetti F. Evaluation of PCR in detection of *Mycobacterium tuberculosis* from formalin-fixed, paraffin-embedded tissues: comparison of four amplification assays. *Journal of Clinical Microbiology*. 1998; 36(6): 1512-1517. doi: 10.1128/jcm.36.6.1512-1517.1998
19. Katoch V. Newer diagnostic techniques for tuberculosis. *Indian Journal of Medical Research*. 2004; 120: 418-428