

***Mycolicibacterium fortuitum* (*Mycobacterium fortuitum*) complex infection in a captive Soccoro dove (*Zenaida graysoni*)**

✉ MONIKA KRAJEWSKA-WĘDZINA¹, ✉ AGNIESZKA CZUJKOWSKA², ✉ MONIKA KOZIŃSKA³,
✉ MARIOLA BOCHNIARZ¹, ✉ ANNA TRACZ¹, ✉ AGATA HAHAJ-SIEMBIDA¹,
✉ MARCELINA OSIŃSKA¹, ✉ ALEKSANDRA TROŚCIAŃCZYK¹, ✉ ANETA NOWAKIEWICZ¹

¹Sub-Department of Microbiology, Department of Preclinical Veterinary Sciences, Faculty of Veterinary Medicine,
University of Life Sciences, Akademicka 12, 20-033 Lublin, Poland

²Warsaw Zoological Garden, ul. Ratuszowa 1/3, 03-461 Warsaw, Poland

³Department of Microbiology, National Tuberculosis and Lung Diseases Research Institute (NTLD),
Płocka 26, 01-138 Warsaw, Poland

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Krajewska-Wędzina M., Czujkowska A., Kozińska M., Bochniarz M., Tracz A.,
Hahaj-Siembida A., Osińska M., Trościańczyk A., Nowakiewicz A.

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Summary

Mycobacteriosis caused by *Mycolicibacterium fortuitum* (formerly *Mycobacterium fortuitum*) occurs all over the world and has been reported in mammals, reptiles, amphibians, and, most often, in fish. *M. fortuitum* mycobacteriosis in animals is usually fatal, especially in captive species. Cases in companion animals usually do not end in death, especially if the etiological agent has been isolated and the antibiotics susceptibility profile of the strain has been determined. This case study presents mycobacteriosis in a Socorro dove (*Zenaida graysoni*). The bird showed serious chronic signs from the central nervous system, such as balance disorders and difficulties staying on the perch. The necropsy revealed numerous irregularly sized foci of beige-yellow necrosis penetrating the liver, especially in the liver parenchyma and on the surface of this organ. Microbiological examination identified the cultured strain as the *M. fortuitum* complex. The mycobacterial infection with *M. fortuitum* in the Socorro dove at the zoo had primarily a zoonotic aspect. Both the zoo staff and visitors were exposed to potential infection. Secondly, the occurrence of fatal mycobacteriosis in a bird species that no longer occurs in the wild could have ruined the efforts to keep this species alive in captivity.

Keywords: *Mycolicibacterium fortuitum*, *Mycobacterium fortuitum*, mycobacteriosis, Soccoro dove, *Zenaida graysoni*, zoo

The genus *Mycobacterium* currently encompasses 188 species of mycobacteria (10). These include pathogens, such as *Mycobacterium tuberculosis* (responsible for tuberculosis) and *Mycobacterium leprae* (cause of leprosy), which are among the most dangerous for human health (10). Tuberculosis (TB) was the world's second leading cause of death from a single infectious agent in 2022 (8).

Non-tuberculous mycobacteria (NTM) are the largest group of mycobacteria belonging to the *Mycobacterium* genus. NTM are also known by another name mycobacteria other than tuberculosis (MOTT) or atypical mycobacteria (6, 26). The latter name is increasingly rare in scientific reports. MOTT

are acid-fast bacteria widely distributed in the environment: water, soil, dust, etc. (14). They can act as opportunistic pathogens and, under favorable conditions, infect both humans and animals (23, 33).

In 2018, Gupta et al. proposed that some members of the genus *Mycobacterium* be reclassified into new genera, and therefore fast-growing species, such as *Mycobacterium fortuitum*, now belong to the genus *Mycolicibacterium* (10). Based on the results of their comprehensive phylogenomic and comparative genomic studies, Gupta et al. divided the species from the genus *Mycobacterium* into five main monophyletic clades, which are referred as the "Tuberculosis-Simiae" clade, the "Terrae" clade, the "Triviale" clade, the

“Fortuitum-Vaccae” clade, and the “Abscessus-Chelonae” clade (10). In the recommended classification, all major human pathogens remain within the corrected genus *Mycobacterium*, whereas the genus *Mycolicibacterium* consists primarily of environmental species, which may cause clinical signs in both immunocompromised humans and animals (5, 11, 22).

Mycobacteriosis caused by members of the *Mycolicibacterium fortuitum* complex occurs all over the world and has mainly been reported in mammals, reptiles, amphibians, and, most often, fish (13, 24, 25). *M. fortuitum* mycobacteriosis in animals is usually fatal, especially in captive species. Cases in companion animals usually do not end in death, especially if the etiological agent has been isolated, and the drug resistance of the strain has been determined (19). This pathogen may also cause abortions in farm animals (5).

The aim of the study was to investigate the cause of neurological signs in a captive Socorro dove (*Zenaida graysoni*), which is classified as extinct in the wild by the International Union for Conservation of Nature (IUCN) (<https://www.iucnredlist.org/species/22690740/178409463>) (32).

The Socorro dove (*Zenaida graysoni*) was originally endemic to Socorro Island, located in the Pacific Ocean 360 miles from mainland Mexico. This slender dove (whose weight is approximately 190 g and size is 26-34 cm) feeds on seeds and small fruits, such as berries. It prefers to walk rather than fly, which increases its exposure to predators (such as feral cats) and may partly explain the extinction of the free-living populations of this species. Other possible causes include intensive grazing by sheep leading to destruction of its natural habitat and changes in that habitat due to locust infestation (*Schistocerca piceifrons*).

Case description

For several weeks, the bird showed severe chronic signs from the central nervous system. It had balance disorders, including difficulties staying on the perch. The veterinary council decided to perform euthanasia. The necropsy revealed numerous irregularly sized foci of beige-yellow necrosis penetrating the liver parenchyma and on the liver surface (Fig. 1). A cytological slide from the liver showed



Fig. 1. Pathological lesions on the liver surface of the Socorro dove

numerous bacteria enclosed in macrophages. Due to the characteristic lesions in the liver and the cytology results, further investigation was carried out to diagnose *Mycobacterium* spp.

Mycobacterial diagnosis. The material for the study was the bird's liver. The organ was examined according to standard laboratory procedures used to isolate acid-fast bacteria of the *Mycobacterium* genus. The obtained sediment in physiological saline (POCH, Gliwice, Poland) was used to prepare a direct microscopic slide and was inoculated on four slants of a Lowenstein-Jensen (L-J) medium base (BioMaxima S.A., Lublin, Poland). The slants were incubated at 37°C, and the culture was observed daily from the fifth day of inoculation.

The remaining part of the sediment was used for bacterioscopy. The smears were stained by the Ziehl-Neelsen method, with carbol fuchsin (BD BBL™ TB Carbofuchsin KF, Becton Dickinson and Company (BD), San Jose, CA, USA) being heated three times in the first stage of staining. Then, the slide was rinsed with acidic alcohol (POCH, Gliwice, Poland) and stained with a contrast dye (BD BBL™ TB Methylene Blue-BD, San Jose, CA, USA). The stained slides were viewed under a light microscope at 100 × magnification.

The isolation of genomic DNA was carried out using the Geno Lyse kit (Hain Lifescience GmbH, Nehren, Germany) according to the manufacturer's recommendations. After the DNA extraction, *Mycobacterium* species were identified according to the protocol of the GenoType® Mycobacterium CM VER 2.0 assay (Hain Lifescience GmbH, Nehren, Germany).

Results and discussion

Reddish acid-fast bacterial cells were visible in the slides heat-stained by the Ziehl-Neelsen method (Fig. 2).



Fig. 2. Acid-fast stain of *M. fortuitum* cells by the Ziehl-Neelsen method (100×)

After 10 days of incubation, creamy colonies began to grow on all four slants with the L-J medium (Fig. 3).

Based on the result obtained in the GenoType® Mycobacterium CM VER 2.0 test, the cultured strain was identified as a member of the *M. fortuitum* complex. The results were interpreted according to the manufacturer's interpretation chart (www.hain-lifescience.de) (Fig. 4).

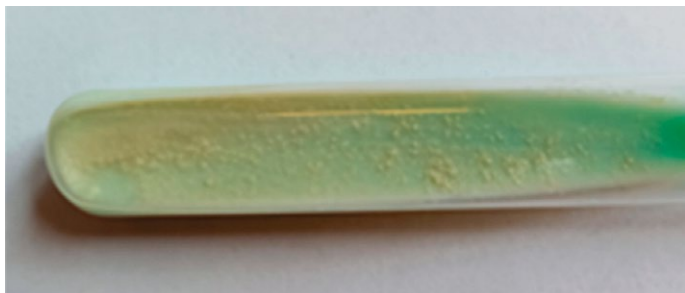


Fig. 3. Creamy colonies of the strain belonging to the *M. fortuitum* complex obtained on the L-J medium after 10 days of incubation at 37°C

The necropsy of the Socorro dove and the result of the microbiological examination proved that the cause of the bird's death was mycobacteriosis due to a member of the *M. fortuitum* complex. Avian mycobacteriosis is a serious disease affecting different species of companion, domestic, and captive exotic and wild birds (15, 28, 38). Mycobacteriosis in birds is most commonly caused by *Mycobacterium avium* and *Mycobacterium genavense*, but infections with other mycobacterial species are also fatal to avians (31, 38).

According to the available literature, avian mycobacteriosis caused by mycobacteria from the *M. fortuitum* complex is rare. Infections are predominantly caused by *Mycobacterium peregrinum* (20, 35). One of the few diagnosed and described cases of *M. fortuitum* in birds was mycobacterial airsacculitis in a Southern Rockhopper Penguin (*Eudyptes chrysocome*) (3). A 21-year-old male southern rockhopper penguin was presented with a chronic history of intermittently decreased appetite, lethargy, and regurgitation. Necropsy examination revealed a severe airsacculitis with thick, yellow granular plaques adhering to the surface of many air sacs, as well as regional contiguous pneumonia (3). It is worth noting that, like the Socorro dove, the Penguin is also a flightless bird. Birds that have lost the ability to fly as a result of evolution are more suscep-

tible to bacterial infections, including environmental mycobacteria (3, 18).

A very important aspect of this research is the protection of valuable animal species that no longer exist in the wild. The Socorro dove has been completely exterminated and was last recorded in the wild in 1972 (4). Rearing in zoos and breeding centers is the only possibility for its survival. According to the species farm report in the Global Animal Database Used by Zoos (ZIMS), three Socorro doves currently live in one institution in Asia, North America has about 70 birds (in 10 institutions), and the greatest number, that is, over 100 birds, live in Europe (in 33 institutions). These numbers add up to less than 200 birds held in captive programs, which are a source for birds used for reintroduction programs. The Warsaw Zoological Garden has a history of breeding this species since 2014. As already mentioned, the Socorro dove prefers a terrestrial lifestyle, which significantly increases the likelihood of contracting diseases, in particular, those caused by environmental pathogens, such as members of the *M. fortuitum* complex.

In 2021, Witte et al. published the results of their research on the occurrence of avian mycobacteriosis in a well-characterized cohort at San Diego Zoo Wildlife Alliance (San Diego, USA). In 1992-2014, 16,430 birds were examined for the presence of mycobacteria from the *Mycobacterium* genus, and the disease was confirmed in 275 individuals (38). The aim of this study was to assess the spread of mycobacteriosis in birds belonging to one cohort, to determine whether patterns of connectivity over time could predict future infections, and to ascertain whether these patterns could be used to distinguish between different sources of infection (37, 38). The researchers' conclusions are not clear, since the ability to transmit the disease is probably variable and sporadic and, therefore, unpredictable (38).

Non-tuberculous mycobacterial (NTM) infections are often overlooked because of their rarity and the lack of precisely targeted microbiological diagnostics (36). In many cases, due to non-obvious diagnostics, the waiting time for an accurate diagnosis of the disease in pet animals is very long, which delays proper treatment and reduces the chances for therapeutic success (19, 30, 36). Moreover, it generates a risk for the pet owner because *M. fortuitum* also has zoonotic potential, and the risk group includes primarily humans exposed to contact with the aquatic environment, mainly aquarists and fishery workers (7, 24, 32).

Recently, NTM has been attracting increasing interest from

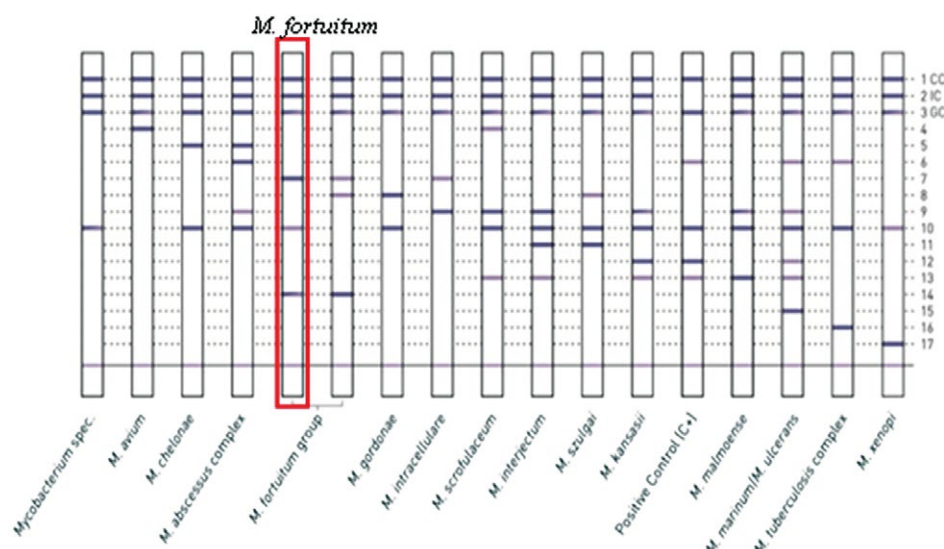


Fig. 4. Interpretation chart of the result obtained in the GenoType® Mycobacterium CM VER 2.0 assay. The box indicates the result for *M. fortuitum* belonging to the *M. fortuitum* complex

scientists. One of the main reasons is an increase in the number of patients without immunosuppression. Human mycobacteriosis due to *M. fortuitum* is increasingly reported in initially healthy patients, customers of beauty salons. Infections were the result of mechanical hair removal, after nipple piercing, after acupuncture treatment, and after breast implantation (1, 9, 12, 27).

A characteristic feature of human *M. fortuitum* infections is their resistance to almost all traditional anti-TB drugs, which usually necessitates combined long-term therapy (36). However, *M. fortuitum* strains isolated from patients with pneumonia showed high susceptibility to amikacin, fluoroquinolones, imipenem, and cefoxitin (17, 21). Although amikacin ototoxicity was confirmed already in the 1980s, it is still the best antibiotic in combination therapy used in the treatment of *M. fortuitum* mycobacteriosis (2, 29).

This is the second case in a Polish zoo where the direct cause of death was euthanasia due to avian mycobacteriosis (19).

The diagnosis of *M. fortuitum* mycobacteriosis in the Socorro dove at the zoo had primarily a zoonotic aspect: both the zoo staff and visitors were exposed to potential infection. It is also worth noting that the emergence of deadly mycobacteriosis in a species that no longer occurs in the wild could have ruined the efforts to keep this species alive in captivity, necessitating greater efforts to conduct diagnostic testing among zoo animals.

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Corresponding author: Monika Krajewska-Wędzina, DVM, PhD, Assoc. Prof., Sub-Department of Microbiology, Department of Preclinical Veterinary Sciences, Faculty of Veterinary Medicine, University of Life Sciences, Akademicka 12, 20-033 Lublin, Poland; e-mail: monika.krajewska@up.lublin.pl