Part 1. Veterinary medicine

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DOMESTIC PARROTS AS A POTENTIAL SOURCE OF MYCOBACTERIOSIS

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Summary. The article presents the results of bacteriological examination of five samples of feces from grey parrots (*Psittacus*) (n = 3), cockatoo (*Cacatua*) (n = 1), yellow-crowned amazon (*Amazona*) (n = 1). Five cultures of mycobacteria were bacteriologically isolated from the five samples. According to biochemical and cultural-morphological characteristics, mycobacterial cultures are classified as *Mycobacterium scrofulaceum* (n = 1) and *Mycobacterium genavense* (n = 4). Isolated cultures of mycobacteria are important in human pathology. Infected exotic poultry pose a potential risk of mycobacterial infection in their owners, so it is necessary to conduct research on biological material

Keywords: identification, isolation, mycobacteriosis, *Mycobacterium scrofulaceum*, *Mycobacterium genavense*, parrots

Introduction. Avian tuberculosis is one of the most significant diseases of domestic, exotic birds, and poultry, and birds kept as pets are the most frequently infected. It was previously thought that in most cases the infectious agent of avian tuberculosis was *Mycobacterium avium* complex (MAC), namely *M. avium*, belonging to serotypes 1, 2, 3, and 6 (genotype *IS901*+ and *IS1245*+) (Dvorska et al., 2007; Fulton and Sanchez, 2013; OIE, 2018; Aranaz et al., 1997).

However, when using molecular-genetic methods, it was found that most mycobacterial infections (up to 80%) in captive birds, especially in species of birds Passeriformes (Sparrow) and Psittaciformes (Parrot), are caused by *M. genavense*, while MAC identified only in 5–10% of cases (Hoop, 1997). *M. genavense* has also been found in birds of Coraciiformes (Gray-breasted), Piciformes (Woodpeckers), Columbiformes (Doves), Ciconiiformes (Storks) and Galliformes (Chickens) (Tell, Woods and Cromie, 2001; Schmitz et al., 2018a, 2018b).

In studies conducted in Switzerland, *M. enavense* was isolated in 71%, and *M. vium* complex only in 17% of the samples. Other isolates included *M. fortuitum* (4%), *M. tuberculosis* (4%), *M. gordonae* (2%), and *M. nonchromogenicum* (2%) (Hoop, Böttger and Pfyffer, 1996). In a recent study of 170 birds from the Passeriformes and Cittaciformes orders, the infection rate was up to 91% (Schmitz et al., 2018a). In addition, isolated cases of infection have been reported in exotic captive birds caused by *M. scrofulaceum*, *M. kansasii*, and *M. fortuitum* (Dhama et al., 2011).

The geographical distribution of *M. genavense* (mainly in domestic birds, companion birds, birds in zoos and wildlife sanctuaries) includes countries in Europe, the United States and Australia. No cases of *M. genavense* have been detected in large commercial poultry farms. In birds, *M. genavense* causes disseminated disease with clinical and histopathological features that do not differ from infection caused by *M. avium* (Antinoff et al., 1996; Van der Heyden, 1997).

According to some authors, exotic birds and poultry have a species predisposition to mycobacterial infections (Painter, 1997; Quaranta et al., 1996; Morita et al., 1999; Hejlícek and Treml, 1995). Mycobacteriosis is much more common in parrots (Psittacinae) of the genera *Amazona*, *Pionus*, *Brotogeris*, *Melopsittacus undulatus*, as well as canaries (*Serinus canaria domestica*), snipe (*Carduelis spinus*) and toucans (*Ramphastidae*) than in other companion birds. Amazon (*Amazona* sp.) and gray-cheeked parrots (*Brotogeris pyrrophterus)* are most commonly affected among Psittacinae.

Grey parrots are the only birds in which cases of tuberculosis caused by *M. tuberculosis* and *M. bovis* are often detected (Schmidt et al., 2008; Washko et al., 1998; Fulton and Sanchez, 2013). The reason for the high prevalence of mycobacterial infections among parrots, especially caused by *M. genavense*, may be genetic factors, specific susceptibility of species, as well as exogenous causes (housing conditions, congestion, stress, etc.).

In addition, concomitant infections (*Macrorhabdus ornithogaster*, circovirus, polyomavirus, avian bornavirus, adenovirus, *Mycobacterium avium* ssp. *avium*, *Mycobacterium avium* ssp. *silvaticum*, *Mycoplasma* sp., Salmonella sp., *Escherichia coli, Aspergillus* sp., etc.), violating the immune status, promote the activation of *M. genavense* in the host (Schmitz et al., 2018a, 2018b; Manarolla et al., 2007).

Diagnosis of mycobacteriosis caused by *M. genavense* is based on the detection of acid-fast rods stained by the method of Ziehl–Neelsen in combination with the culture method on special media for 6–12 weeks (Realini et al., 1999). Detection of *M. genavense* by molecular methods, aimed at a fragment of the 16S rRNA gene with specific primers, can reveal a characteristic signature sequence.

Direct PCR determination of the amplified 16S rRNA sequence is used as an alternative method of bacterial identification. The danger of mycobacterial infections is that many infected birds appear clinically healthy because mycobacteriosis develops slowly and causes a chronic course of the disease, and in most cases in not young birds. Considering the high prevalence of mycobacteriosis among domestic parrots and their high susceptibility to mycobacterial infections, there is a high risk that a bird purchased from an unknown source (from a private owner) will be infected. Occurrence of mycobacteriosis is more probable in places with high population density and poor sanitary conditions.

Due to the fact that the above types of mycobacteria are of great importance in human pathology, domestic parrots can be a potential source of mycobacterial infection for owners, especially for people with weakened immune systems, children and the elderly.

The aim of the study was to conduct a bacteriological investigation of fecal samples for the presence of mycobacterial infection in parrots.

Materials and methods. Studies on the presence of mycobacteria in samples of fecal masses from five parrots, including three grey parrots (genus *Psittacus*, No. 1–3), one cockatoo (genus *Cacatua*, No. 4), and one yellow-crowned amazon (genus *Amazona*, No. 5), were performed by bacteriological (cultural, bacterioscopic) method. Collection of samples of fecal masses in sterile plastic tubes was performed by bird owners. The samples were stored in a refrigerator at a temperature of 4°C for one day, followed by pre-sowing treatment. For decontamination of samples of fecal masses we used a solution of 10% sulfuric acid at exposure for 25 min, followed by washing with sterile distilled water by centrifugation. The precipitate was resuspended in a small amount of sterile saline NaCl and seeded in a volume of 0.3 cm^3 on egg growth medium (pH = 6.0) containing growth factor (alcoholic extract of *M. phlei*) and egg medium for the cultivation of mycobacteria without growth factor (control medium, pH = 7.0). Incubation of crops was performed at a temperature of 37.5–38.0°C. The presence of colony growth was taken into account once a week for four months.

Generic identification, tinctorial and morphological characteristics of the detected microorganisms were determined in smears stained by the method of Ziehl–Neelsen. The species affiliation of the isolated culture of mycobacteria was determined in biochemical (Tween 80 hydrolysis test, amidase and catalase activity, tellurite restoration) and culture (growth rate, growth capacity at 22°C and 45°C, tolerance to 5% sodium chloride in the medium) tests. To do this, a culture suspension at a concentration of 1.0 mg of bacterial mass in 1.0 cm^3 of 0.85% sodium chloride solution was seeded on different for each test medium.

Results and discussion. During the anamnesis it was found that all birds were over 6 years old, all parrots had lethargy and depression, in two grey parrots and one cockatoo (No. 1, 3, 4) — signs of recurrent diarrhea and feather loss, in grey parrot (No. 2) — inflammation of the elbow joint (painful and enlarged), in yellow-crowned amazon (No. 5) in addition to lethargy and depression, other signs of the disease the owner did not observe.

According to the results of cultural analysis of feces from grey parrot No. 2 (with inflamed joint) after 30 days of cultivation on both media were found initially small, round, smooth and shiny bright orange colonies, which eventually merged to form a continuous growth over the entire surface of the medium (Fig. 1). During the microscopy of the isolated culture of microorganisms, acid-fast rods were observed, which were located separately or in clusters (Fig. 2). According to Runyon classification (Runyon, 1959), this culture was classified as slow-growing scotochromogenic atypical mycobacteria (group II). When determining the species affiliation of the isolated culture of mycobacteria, it was found that the optimal temperature for colony growth was 37–38°C, at 22°C the growth of colonies was slower and less intense, at 45°C no growth was observed.

In addition, the growth was not observed on medium containing 5% NaCl. When determining the enzymatic activity in this culture of mycobacteria, positive reactions to nicotinamidase, pyrazinamidase, urea, catalase and negative reactions of Tween 80 hydrolysis and restoration of tellurite from potassium tellurite were established.

Based on cultural-morphological and biochemical characteristics, the culture was classified as *M. scrofulaceum.* Unfortunately, mycobacteriosis caused by *M. scrofulaceum* four months after diagnosis caused the death of the parrot.

In a cultural analysis of the other four samples (No. 1, 3, 4, 5) after 90–120 days of cultivation on medium with growth factor (pH = 6.0) we observed very small, transparent, non-photochromogenic dysgonic colonies smaller than 1.0 mm. Microscopy of scrapings from the surface of this medium revealed a large number of agglomerates of small short acid-fast rods and cocci (Figs. 3–5), in some cases acid-fast rods were detected in the cytoplasm of macrophages (Fig. 6).

Under standard cultivation conditions, that is on a conventional medium for culturing mycobacteria without growth factor and neutral pH, colony growth was not observed, these microorganisms remained nonculturable, but microscopy revealed single small clusters of acid-fast rods and cocci.

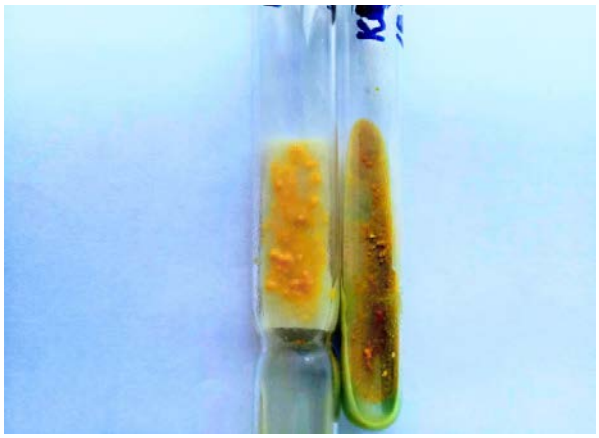

Figure 1. Growth of culture (*M. scrofulaceum*) from grey parrot No. 2 on egg and potato media

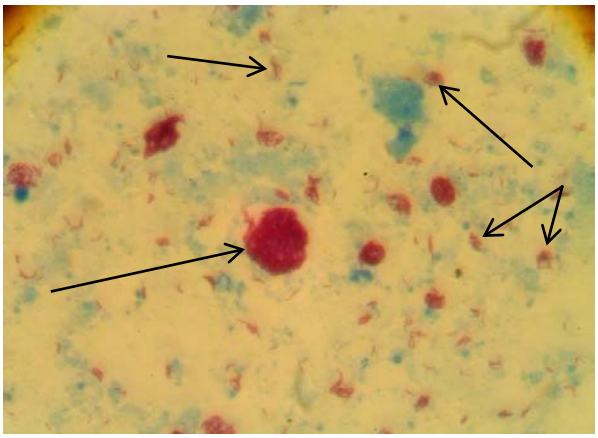

Figure 2. Microscopy of mycobacterial culture *(M. scrofulaceum)* from grey parrot No. 2

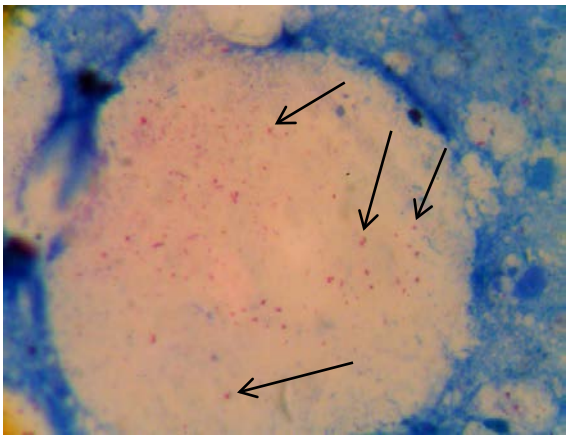

Figure 3. Medium flush (grey parrot No. 1)

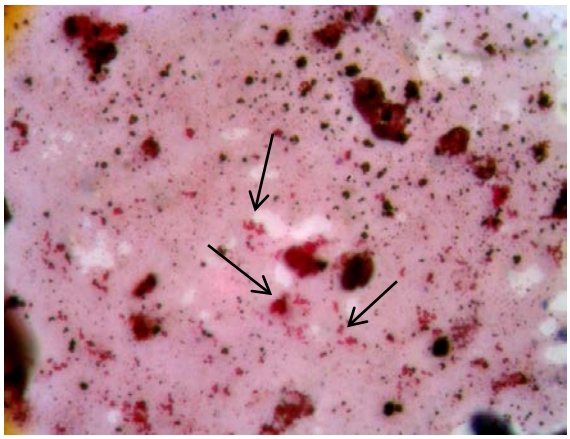

Figure 4. Medium flush (grey parrot No. 3)

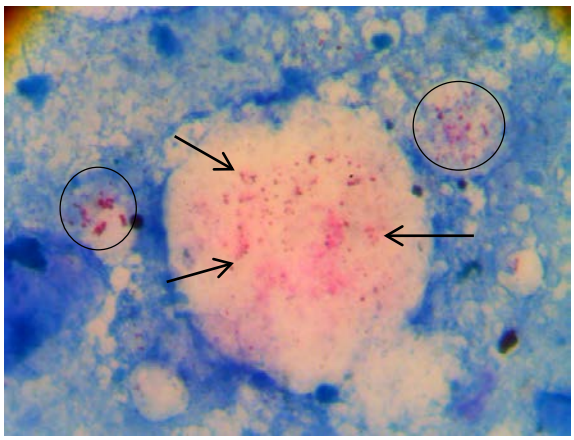

Figure 5. Medium flush (yellow-crowned amazon No. 5)

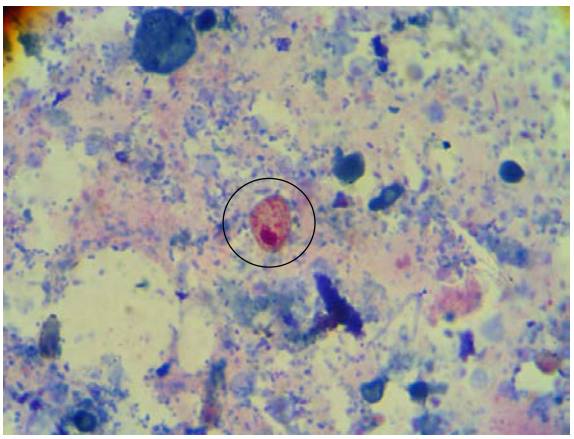

Figure 6. Medium flush (cockatoo No. 4). Macrophage with acid-fast rods

Besides, these mycobacteria did not grow on a medium with 5% NaCl and at a temperature of 25°C and 45°C. Biochemical characteristics were determined only in two cultures (from grey parrot No. 1 and cockatoo). In the other two cultures (from yellow-crowned amazon and grey parrot No. 3) we failed to obtain satisfactory growth of colonies and a sufficient amount of bacterial mass to determine biochemical parameters. Cultures from grey parrot No. 1 and cockatoo hydrolyzed Tween 80 and had positive reactions to pyrazinamidase and urease.

The results obtained, namely: dependence on growth factor, very slow cell replication and colony growth, cell morphology (small cocci), predisposition to acidic environment (pH = 6.0), and high specific sensitivity of *Psittacus* parrots to this species of mycobacteria give reason to believe that the detected microorganisms belong to *M. genavense*. Unfortunately, it was not possible to make a more accurate identification based on molecular-genetic methods. Thus, the isolation of *M. genavense* and *M. scrofulaceum* indicates the circulation and persistence

of these species of mycobacteria among domestic parrots in Ukraine. Given that isolated species of mycobacteria cause lymphadenitis (*M. scrofulaceum*), as well as disseminated infection in immunocompromised individuals, children and the elderly, domestic parrots pose a potential threat to the owners of these birds.

Conclusions. Every parrot with any granulomatous, ocular, sinus, skin lesions, intestinal disorders should be considered as a potential source of mycobacterial infection. To clarify the diagnosis, it is necessary to conduct a bacteriological examination of biological material from such birds.

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