MYCOBACTERIOSIS CAUSED BY MYCOBACTERIUM GENAVENSE IN LINEOLATED PARAKEETS (BOLBORHYNCHUS LINEOLA). A CASE REPORT

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Abstract

Systemic mycobacteriosis caused by Mycobacterium genavense was diagnosed in a 7-year-old captive lineolated parakeet (Bolborhynchus lineola). About a year before death, proventricular dilatation syndrome (PDS) was suggested, because of persistent regurgitations and intermittent diarrhoea. Necropsy examination did not show any signs typical of PDS and mycobacterioses. No caseous necrosis, but focal ulcerated overgrowth in the proventriculus, hypertrophy of intestinal mucosa and splenomegaly, was found. Primary neoplasia was suspected. The crucial examination was histopathology, which revealed changes typical of mycobacteriosis and the presence of numerous acid-fast bacilli. A real-time SYBR® Green PCR test was used and Mycobacterium genavense infection was diagnosed. The mycobacterium was also cultured on BD BACTEC™ 460TB 12B Middlebrook 7H12 medium.

Key words: parrots, Mycobacterium genavense, pathology, real-time PCR.

Mycobacterium genavense is a fastidious microorganism with special growth requirements and was first detected in 1990 in a human patient with acquired immunodeficiency syndrome (1). Since then, it has been identified as a cause of mycobacteriosis in different avian species (7, 8, 10, 14, 13). Different data suggest that 10%–15% of disseminated mycobacterial infections in AIDS patients may be due to this bacterium (4). In two surveys conducted on birds, M. genavense was considered as about 30% to 95% (8, 13) of all Mycobacterium sp. infections. It indicates a high significance of that bacterium as an avian pathogen. In other pet animals, disseminated or local M. genavense infections were occasionally described (9, 10, 12). In human AIDS patients, the clinical pattern of infection is similar to those reported in disseminated MAC infections, including fever, weight loss, abdominal pain, hepatosplenomegaly, anaemia and possibly early death (2, 6, 17). In avian patients, necropsy findings were pectoral muscle atrophy, hepatomegaly with lack of visible granulomatous lesions, and small intestinal wall thickening (7, 10, 13). Severe lesions caused by this pathogen, observed in the central nervous system, lungs, cervical air sacs, and adrenal glands without involvement of the gastrointestinal tract, was described in European goldfinch (Carduelis carduelis) with polyoma co-infection (13). Difficulties or inability of M. genavense to grow on standard solid media delayed its identification (2, 5, 6, 14, 16). In many cases, the amplification by PCR or similar techniques represents the only possibility of detecting and identifying M. genavense in tissue samples (4). BACTEC 12B broth and BACTEC 13A (blood, bone marrow) are recommended media for M. genavense culture. For M. genavense, the median detection time was 20 d (range, 7 to 54 d) for 13A, 8 d (range, 6 to 40 d) for 12B, and 41 d (range, 35 to 47 d) for solid media (Löwenstein-Jensen medium with sodium pyruvate or Middlebrook 7H10 medium) (15).

Material and Methods

Case history. A 7-year-old lineolated parakeet (Bolborhynchus lineola) was submitted for necropsy. The owner reported that a pair of parakeets had been bought six years earlier. They probably came from imports. Both birds were in a bad condition with signs of
diarrhoea and dyspnoea. Enrofloxacin had been used on setback and then itraconazole was used with recovery over the remaining five years. About one year before its death, the bird became less active, with intermittent diarrhoea and indigested seeds in faeces. Presumptive diagnosis was proventricular dilatation disease (PDD). The parakeet showed a decreasing appetite; the owner fed it Prosohe (Bristol Myers Squibb, USA).

**Histopathology.** Tissue samples were fixed in 10% buffered formalin and paraffin sections were prepared. The sections were stained with haematoxylin and eosin (H&E) or according to the Ziehl-Neelsen (Z-N) method.

**Culture.** Liver, kidney, and intestinal samples were kept at -20°C for one month before delivering them to mycobacterial culture. After decontamination, the samples were cultured for six weeks on BD BACTEC™ 460TB 12B Middlebrook 7H12 medium in BACTEC 460 TB system under aerobic conditions (11).

**DNA Extraction.** DNA was extracted from the frozen liver, spleen, and lungs (25 mg each) with 5% Chelex (Bio-Rad Laboratories, Canada) method (18). As a positive control we used suspensions of *M. genavense* strains kindly provided by Prof. Françoise Portaels (Institute of Tropical Medicine, Antwerp, Belgium).

**PCR.** *Chlamydia psittaci*, psittacine beak and feather disease (PBFD), budgerigar fledgling disease (BFD), psittacine herpesvirus (PsHV-1), and psittacine adenovirus PCR tests were done with standard procedures.

**Specific Mycobacterium genavense SYBR® Green real-time PCR.** A selection of specific *M. genavense* primers was supported by Bacon Designer software 7 (PREMIER Biosoft International, Canada). The chosen forward primer MG 25-s (GAATCCGCTGCTGCTCTG) was located at nucleotides 378 to 395 *Mycobacterium genavense* hypothetical 21 kDa protein gene (4) and MG25-as (TCAATGTAGTCCTGTCCGAAC) corresponded to nucleotides 313 to 291. Real-time PCR amplification was carried out in a total-volume 50 µl using Brilliant® SYBR® Green QPCR Master Mix (Stratagene, Canada) containing 1 µl of each primer and 2 µl of DNA template. The PCR was performed in the Stratagene® Mx3005P™ cycler (Stratagene, Canada), with the following protocol: initial denaturation: 10 min at 95°C followed by 40 cycles consisting of denaturation at 95°C for 30 s, annealing at 55°C for 60 s, and elongation 72°C for 60 s. The fluorescence data were collected during the elongation step. After termination of the reaction by a final extension step at 72°C for 5 min, a DNA-melting curve was generated to verify the correct product by its specific melting temperature. Melting-curve analysis was performed by heating at 95°C for 1 min, followed by cooling to 55°C for 30 s and subsequent heating to 95°C for 30 s. For each real-time PCR reaction, the software associated with Stratagene® Mx3005P™ system determined a threshold of cycle number (Ct). The specific melting temperature value of the real-time product was about 83°C.

**Results and Discussion**

The bird was in poor body condition, with feather depletion around the beak and on its cheeks. The upper-jaw corneal structure was also damaged. In the lower 1/2 of the neck symmetrical lobular “thymus like” structures bent together in neck lordose area were observed. The liver was slightly enlarged and tender, with multifocal grey discoloration. The spleen was significantly enlarged (12 x 18 mm) and yellowish (Fig. 1). The proventriculus was enlarged (Fig. 1) with an excess of mucus with little blood stains. In the proventriculus thymus region irregular, a focally-ulcerated outgrowth (10x7x4 mm) was found (Fig. 2). In the small intestine, a towel-like hypertrophy of mucus membrane was present.

*Chlamydia psittaci*, psittacine beak and feather disease (PBFD), budgerigar fledgling disease (BFD), psittacine herpesvirus (PsHV-1), and psittacine adenovirus PCR tests were negative.

No aerobic bacteria were cultured on standard media.

A histopathological examination revealed brain congestion, diffuse inflammation cell (macrophages, giant cells) infiltration of the intestine mucosa, and numerous acid-fast bacteria (AFB) in infiltrating cells (Fig. 3). Infiltration of macrophages and giant cells with numerous AFB were found also in the spleen, liver (Fig. 4), proventriculus, lungs, and thymus-like structure. Spleen and thymus-like structure constitution was blurred. In the proventriculus glandular dysplasia and mucous membrane hypertrophy were present.

The lack of typical caseous changes in the organs aroused suspicion of Mycobacterium genavense infection; therefore, mycobacterium identification tests were performed.

Samples of the liver, spleen, lungs, and kidneys were examined by real-time PCR (Fig. 5). *M. genavense* DNA was amplified in all the tested samples with the largest quantity in the spleen.

Positive mycobacterium growth on BD BACTEC™ 460TB 12B Middlebrook 7H12 was after 10 d for the intestines and kidneys.

Despite many world reports (7, 8, 10, 13, 14) describing *Mycobacterium genavense* as an important mycobacterial pathogen for birds, this case is the first documented in Poland. Earlier investigation confirmed the presence of this bacterium in healthy parrot faecal samples (11). Probably most cases are unrecognisable, due to atypical changes, different from most mycobacterial infections (8, 13, 14). In this case histopathologic examination was crucial to the diagnosis of mycobacteriosis, because of the presence of typical microscopic changes and numerous acid fast bacteria. The very difficult culture of this microorganism is also responsible for the extremely rare diagnosis of *Mycobacterium genavense* infections before the PCR test era. The source of these infections remains unknown - few birds from the same indoor aviary were positive in *M. genavense* PCR tests (cloacal swabs) and all 10 parrots were treated for mycobacteria for four months, and they are under regular veterinary control.
Fig. 1. Significantly enlarged spleen next to moderately distended proventriculus.

Fig. 2. Irregular, outgrowth in lower part of proventriculus and isthmus with ulcerated bleeding foci.

Fig. 3. Hepatic mycobacteriosis. Multifocal infiltration with macrophages containing mycobacteria visualised by acid fast-positive staining (Ziehl-Neelsen, 100x).

Fig. 4. Intestinal mycobacteriosis with mycobacterial organisms in macrophages demonstrated by acid-fast staining (Ziehl-Neelsen, 200x).

Fig. 5. A) Thermal profile, B) Amplification plots with standards and examined samples, C) Dissociation curve, D) Standard curve.
References


