Opis przypadku *Case report*

Trichomoniasis, psittacine circovirosis and clostridial infection in a budgerigar

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Summary

An adult female budgerigar (Melopsittacus undulatus) was presented with severe dyspnea and microscopically diagnosed with advanced Trichomonas infection. The bird was bought two weeks before presentation and kept with two males in the same room. Because of the negative prognosis the budgerigar was euthanized. Further investigation revealed massive yellow caseous masses in the oropharynx which caused upper larynx blockage, liver massive necrosis and small intestine haemorrhagic inflammation. Psittacine circovirus infection was confirmed with PCR method, while Clostridium perfringens, Enterococcus sp., Enterobacter sp. were cultured from intestine samples. These mixed protozoal and bacterial infections were most probably the effect of immunosuppression caused by Psittacine Beak and Feather Disease.

Keywords: budgerigar, trichomonas, circovirus, clostridium

Trichomoniasis is a common disease in domestic and feral pigeons but also described in other species, including budgerigars (*Melopsittacus undulatus*) (3, 9, 12, 15, 16). Prevalence of *T. gallinae* infection in captive budgerigars in Australia ranged from 0% to 11.4% compared with 46% in wild senegal doves (*Streptopelia senegalensis*) (15). Prevalence of trichomonad infection at United States was 1.7% in house finches, 0–6.3% in corvids, and 0.9% in mockingbirds (2).

Budgerigars usually show no oral lesions. Affected birds often exhibit regurgitation, dysphagia, weight loss, listlessness, palpable mucous in the oropharynx and crop and, in severe cases, vomiting blood and death (10).

Protozoal and bacterial diseases in birds often depend on immunosuppression. Circoviruses are recognized as frequent and serious viral immunosuppressive factors in parrots and pigeons. The psittacine circoviral infection (PBFD) was first described in Australian cockatoos in early 1970s (17). To this day it has a worldwide distribution and many species of parrots have been affected (14). Major clinical signs of PBFD are often severe immunosuppression, feather loss and dystrophy (14).

A case report

Case history. An adult female budgerigar with severe dyspnea, greenish ureates and poor condition of its pectoral muscles was presented in the Ambulatory for pet and wild birds of the Faculty of Veterinary Medicine in Warsaw. The bird had been bought 2 weeks earlier and was kept with two males. The owner had noticed breathing problems in this bird about a week before. Clinical examination revealed a firm mass localised in the pharyngeal region (fig .1). Wet swabs from the oropharynx revealed multiple active *Trichomonas gallinae* protozoa. Because of severe dyspnoea and bad prognoses the budgerigar was euthanized.

The necropsy examination showed massive yellow caseous masses in the oropharynx which caused upper larynx blockage (fig. 2). The liver was marble and enlarged, the spleen was moderately enlarged. In the small intestine a brown-red semifluid content was present. Parathyroid glands were moderately enlarged.

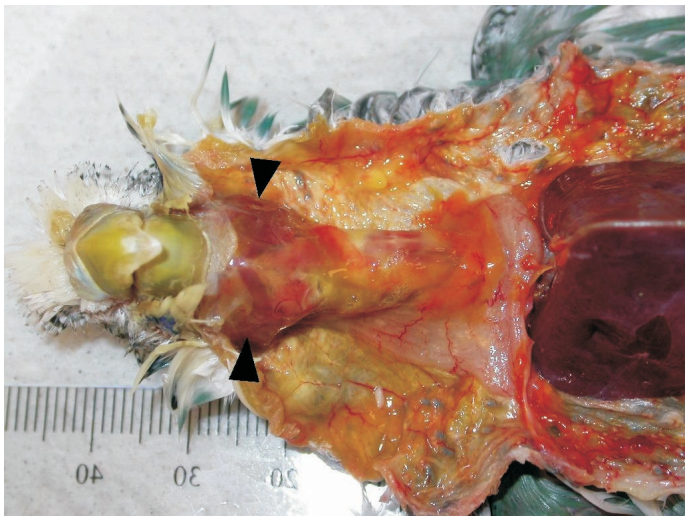

Fig. 1. Distension of pharyngeal region caused by necrotic masses in the lumen of oropharynx

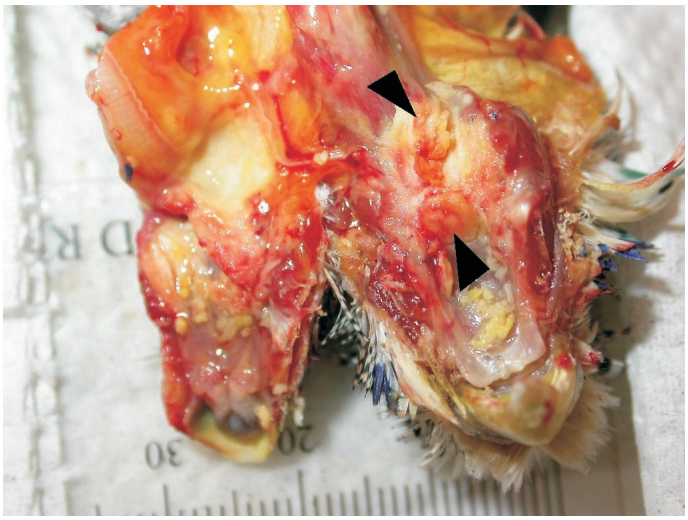

Fig. 2. Necrotic masses typical for *Trichomonas sp.* infestation, caused mainly respiratory distress, but also they are good medium for pathogenic bacteria including clostridia

Cytology. Slides from the liver, lungs and intestinal content were stained with Hemacolor® and examined under a magnification of 1000 ×. Stained print slides from the liver and lungs revealed numerous inflammation cells, rod shaped bacteria, also in macrophages. Numerous bacteria with different morphology including large, clostridia like bacilli were present in the intestinal content.

Histopathology. Hyperaemia, vacuolar hepatocytes degeneration, and disseminated coagulative hepatocytes necrosis with infiltration of the mixed inflammatory cells were present in the liver (fig. 3). Additionally, features of activation and regeneration of hepatocytes was observed multifocally. Massive infiltration of juvenile lymphoid cells in the spleen was shown. The proventriculus and intestine microscopic examination revealed that mononuclear cells' infiltrations scattered multifocally, and gastric pits fibrosis and atrophy were present.

The brain was hyperaemic with neurones degeneration, glial proliferation with advanced neuronophagy. Kidney hyperaemia, parenchymal degeneration and multifocal necrosis of tubular epithelium with bilirubine accumulation were present. Pharyngeal submucosa was infiltrated by mononuclear cells with pyogranulomatous formation and the presence of bacteria clusters.

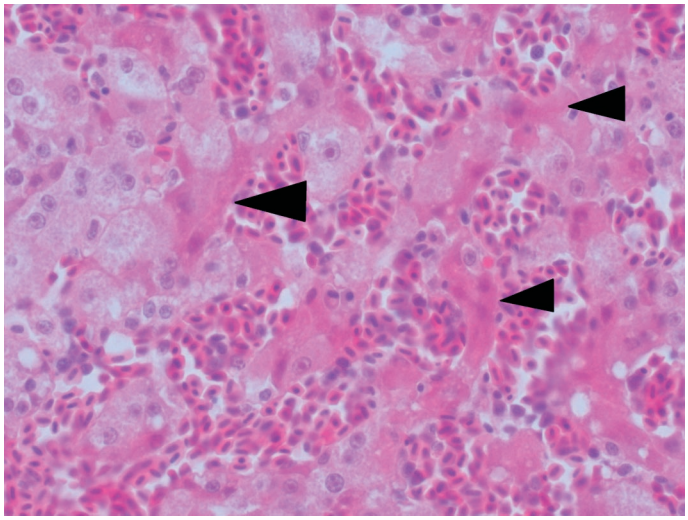

Fig. 3. Disseminated coagulative hepatocyte necrosis (arrow) with severe hyperaemia. Haematoxylin-eosin stain, magnification 400 ×

Bacteriology. Liver and intestine samples were placed on the blood Columbia agar, McConkey and SF media and cultured under aerobic condition at 37°C. Moreover, anaerobic culture from the obtained samples was performed on Schaedler broth medium (under paraffin). *Enterococcus* sp., *Enterobacter* sp. and *Clostridium perfringens* were isolated from the intestinal sample.

PCR tests were performed according to Ypelaar (22) – Psittacine circovirus and Johne (13) – for avian polyoma virus. *Trichomonas* sp. ITS1/5.8S/ITS2 region (7) was amplified according to the methodology described by Gaspar da Silva et al. (6). DNA was isolated with AX Tissue Mini Kit (A&A Biotechnology, Gdynia, Poland).

The oral mucosa, liver and intestinal samples were received and investigated by PCR for *Trichomonas* lTS-1/5.8S1TS-2 region, and the liver and intestine additionally for psittacine circovirus and avian polyoma.

Results and discussion

Liver and intestinal samples researched for psittacine circovirus were positive, PCR for polyoma virus was negative. Analysing the tissue samples with the trichomonad-specific primers, DNA of *T. gallinarum* was found only in the oral sample. Product length 369 bp confirmed the species of the protozoa.

Trichomoniasis is the disease common in young and/or immunocompromised birds. In the current case it seems that immunosuppression was a major cause of the problem. Two others budgerigars which were in close contact with affected female were checked with fresh wet mount of a crop swab a few times with a parasite negative result. They were also psittacine circovirus negative in PCR.

Trichomonas life cycle is a concern by direct oral contact between birds, and its spread through drinking water is also important (10). All parakeets in the

owner's home use the same water source, but the water intake in budgerigars is much less then in pigeons.

The histopathology and PCR examination did not reveal the presence of *Trichomonas gallinae* in the liver and intestinal tissue. This signifies that trichomoniasis was limited only to the upper alimentary and respiratory tract.

Enterococcus sp. often exist in parrots as normal gut flora, but enteritis and septicemia caused by *Enterococcus hirae* have been reported in 10 psittacine species (4, 20). In canaries, *Enterococcus faecalis* can cause chronic tracheobronchitis (18). Diseases caused by *Enterobacter* sp. are uncommon in pet birds, but immunocompromised hosts may be susceptible to an opportunistic infection with this bacteria (8).

The most pathogenic bacteria cultured in the studied case was *Clostridium parfringens*. Clostridial enteritis, usually caused by *Clostridium perfringens*, is becoming more commonly recognized in psittacines, especially nectar eaters such as lories and lorikeets. Clostridial organisms in large numbers can cause acute necrohemorrhagic enteritis (5, 18). In captive birds, an abrupt change of diet is a common history preceding an outbreak of the disease (8). In the presented case *C. perfringens* was probably the main cause of hemorhagic intestine inflammation; the presence of numerous large bacilli in intestinal content uphold this suspicion.

Psittacine beak and feather disease virus (PBFDV) is one of several avian circoviruses. This virus is enzootic in many species of free-ranging Australian parrots and has also been found in free-ranging African parrots. Currently, in the United States PBFDV is most commonly seen in lovebirds (*Agapornis* spp.), budgerigars, lories, lorikeets, *Eclectus* spp. and African grey parrots (*Psittacus erythacus*) (21).

Circoviruses frequently cause degeneration or necrosis in lymphatic organs, causing immunesuppression (11). These viruses are thought to target precursor T cells depleting populations of both helper ($CD4^+$) and cytotoxic ($CD8^+$) T cells (1), and mortality is usually the result of a secondary bacterial or fungal infection (19). In the presented case, mainly a circovirus infection but also stress connected with owner changing could be the cause of the activation of multiple pathogens.

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