# Lymphoid Leucosis and Coligranoluma in a Budgerigar *(Melopsittacus undulatus)*

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Budgerigar; coligranoluma; lymphoid leucosis; salpingitis; oophoritis.

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## Abstract

An adult female budgerigar *(Melopsittacus undulatus)* presented with abdominal enlargement. The condition of the bird deteriorated after needle aspiration for cytological examination. The budgerigar was euthanatized and a complete necropsy was performed. Microscopic sections were prepared and stained with hematoxylin and eosin, Gram staining, periodic acid-Schiff (PAS) and acid-fast staining. *Escherichia coli* was isolated in pure culture. Necropsy revealed the presence of granulomatous lesions of varying sizes at different locations and hepatomegaly, oviduct impaction and oophoritis. Histopathologically, typical granuloma with a central area of coagulation necrosis and bacterial colonies surrounded by lymphocytes, macrophages and multinucleated giant cells were found. These granulomas were present in the liver, oviduct and intestinal tract. A sheet of neoplastic cells and disruption of the normal hepatic architecture was seen. The diagnosis was lymphoid leucosis and coligranuloma.

## Introduction

The leukosis/sarcoma group of diseases designates a variety of transmissible benign and malignant neoplasms caused by viruses of the family *Retroviridae*. These avian retroviruses, including avian leukosis virus (ALV)-related viruses, were formerly placed in a subgenus termed avian type C oncornaviruses, but have recently been termed alpharetroviruses (Regenmortel *et al.*, 2000). Avian leukosis virus (ALV) is an avian pathogen that causes neoplastic disease and has also been shown to induce immunosuppression (Lutticken, 1997). In budgerigars, the virus causes neoplasms of the kidneys and gonads. The most common sign seen in association with these tumors is unilateral leg lameness. In a study of this condition, 47% of budgerigars with renal tumors had evidence of avian leukosis/sarcoma viruses (Neumann and Kummerfield, 1983). This virus has also been found in African grey parrots (*Psittacus erithacus*), Amazon parrots (*Amazona spp.*), cockatoos (Cacatuidae) and red shining parrots *(Prosopeia tabuensis)* (Girling, 2003).

Coligranuloma (Hjärre's disease) was first described by Hjärre and Wramby (1945) as a relatively rare disease of the domestic chicken *(Gallus domesticus)*, characterized by typical granulomas of the wall of the intestinal tract and in the liver but not in the spleen (Hofstad *et al*., 1978; Thiede and Krone, 2001). Coligranuloma has been reported in an Amazon parrot, a Hyacinth macaw (*Anodorhynchus hyacinthinus*) and a free-living common buzzard *(Buteo buteo)* (Raphael and Iverson, 1980; Smith, 1987).

To our knowledge, there is no report of the concurrent occurrence of lymphoid leucosis and coligranuloma in cage birds such as budgerigars. The objective of this study was to report on the concurrent occurrence of lymphoid leucosis and coligranuloma in a budgerigar.

## Case Report

In May 2008, an adult female budgerigar with abdominal enlargement was presented to a private veterinary practitioner three weeks after being purchased from a pet shop. On clinical examination, a massively distended firm mass and a smaller soft mass were palpable in the cranial and caudal part of the abdomen, respectively. Feather losses were seen around the masses in the ventral side of abdomen. The bird was alert, had a normal appetite and there were no clinical signs of depression. The color of the urate was light green. The affected bird was referred to the Mehregan clinic for more detailed radiographic studies. Two perpendicular standard avian radiographs were taken from this case. Exposure factors of 85-95 KV and 25 mA in 0.04 - 0.02 seconds were used. Radiographs revealed considerable hepatomegaly; the liver shadow was tremendously enlarged and distinctly outlined. It extended far into the abdominal region while the caudal part of the liver protruded through the skin of the abdomen (Figure 1). A ventrodorsal radiograph of the bird showed an increase in size and

density of the oviduct in the coelomic cavity.

A needle aspirate of the distended masses was obtained and cytological examination revealed a non-diagnostic cellular sample. One week later, the owners reported a decrease in appetite and listlessness. The bird was weak, depressed and had ruffled feathers, and did not resist handling. Attempts to treat the budgerigar with enroflaxacin failed, the budgerigar was euthanatized three days later, and a complete necropsy was performed by the veterinarian.

Gross necropsy revealed the presence of yellowish-white, granulomatous lesions of varying sizes at different locations. The largest granulomatous lesion (1×2 cm) was a yellow-white, hard, fleshy capsulated mass adjunct to the caudal border of the right lateral lobe of the liver (Figure 2). In the digestive tract, six granulomas were found in the mesentery and wall of the intestine with a diameter of 0.2 to 0.3 cm, and one granuloma was found in the mesentery with a diameter of 0.5×1 cm. The spleen and kidneys were free of any abnormal macroscopic findings. The heart was enlarged and adhered to the cranial border of the right lateral lobe of liver. The oviduct was distended, enlarged and contained caseous material and misshapen, partially formed eggs. The ovaries were wrinkled, black and firm. Collected organs were preserved in 7.2% neutral buffered formalin and were sent to the Baharan Pathology Laboratory in Tehran. Microscopic sections were prepared by routine histological methods and underwent hematoxylin and eosin, Gram, PAS and acid-fast staining. Microscopic sections were referred to the Veterinary Pathology Department of the University of Tehran for further routine pathological studies. The liver was aseptically collected and submitted for bacterial culture. *E. coli* was isolated in pure culture.

In microscopic examination, a typical granuloma with the central area of coagulation necrosis surrounded by lymphocytes, macrophages and multinucleated giant cells was found (Figure 3). These granulomas were present in the liver, oviduct and intestinal tract. In a capsulated mass adjunct to the liver, a granulomatous reaction with typical central necrosis, marginal infiltration and well-formed fibrosis was found. Yellow/gold pigments in hepatic tissue near the lesions and some roughly identical but smaller granulomas were also seen inside the liver. In cardiac tissue, multifocal lymphocytic pericarditis and myocarditis were detected. In lung tissue, mild to moderate hyperemia and slight lymphoplasmacytic infiltration was seen. In the oviduct wall, there was a mass of cells with central necrosis. These cells consisted of macrophages, lymphocytes, heterophils and giant cells which infiltrated around and into the lumen of the glands of isthmus (Figure 3). Many bacterial colonies were particularly seen at the border of the granulomas. An area of necrosis was found in the pseudostratified columnar epithelium of the oviduct (isthmus). A solid sheet with a uniform population of mature and immature lymphocytes with large, round vesicular nuclei and a basophilic cytoplasm was seen, as well as the replacement of the normal hepatic architecture and mitotic figures (Figure 4). Gram staining showed clusters of Gram-negative rods. Ziehl-Nielsen staining and PAS staining were negative for acid-fast organisms and mycotic forms, respectively.

The results of radiography and microbiology, in combination with staining of specimens, provided evidence of a lymphoid leucosis and coligranuloma.

## Discussion

Avian leukosis virus (ALV) is an avian pathogen, which causes neoplastic disease and has also been shown to induce immunosuppression (Lutticken, 1997). Immunosuppressive viral diseases can damage organs directly by viremia and indirectly by the proliferation of neoplastic tissue. Secondary infections can further weaken the immune system. Smith (1987) considered infections with subgroup B viruses to have a more severe immunosuppressive effect compared to subgroup A viruses. An infection with ALV often results in a persistent infection of macrophages, which can interfere with many macrophage functions. In most birds with hepatic disease, clinical findings are often nonspecific in patients with liver or gallbladder alterations. Green or yellow coloring of the urate is a common sign of hepatic disease. The cause is an increase in the excretion of the most important bile pigment in birds, biliverdin (Krautwald-Junghanns, 2001). The most common sign seen in association with neoplasms of the kidneys and gonads is unilateral leg lameness, sometimes with distension of the abdomen in advanced cases (Neumann and Kummerfield, 1983). The clinical signs depend on the origin of the tumor. As lymphoid tissue is usually involved and psittacine birds have no genuine lymph nodes, lymphoid deposits spread throughout organs such as the liver and kidneys and so tumors often appear as infiltrative neoplasms within other organs (Girling, 2003). Enzyme-linked immunosorbent assays (ELISAs) and complement fixation tests have been used in poultry to detect lymphoid leukosis virus in samples of plasma, serum, feces and respiratory and reproductive secretions. However, none of these tests have been shown to work in psittacine birds, making the diagnosis of retroviruses in these species almost impossible (Girling, 2003). In the opinion of Gerlach (1994), differential leucocyte counts are rarely helpful for diagnosis purposes. In polymerase chain reaction (PCR) assays, primer pairs specific for the different ALV subgroups are available. However, the primer pairs may not detect all variants of the viruses (Jordan *et al*., 2008). Coligranuloma appears to be a very rare disease, reported only occasionally in poultry and birds. The pathogenesis is unknown. Coligranuloma

**Figure 1:** Lateral view of the bird showing hepatomegaly. The liver shadow was enlarged and distinctly outlined. It extended far into the abdominal region while the caudal part of liver protruded through the skin of abdomen.

**Figure 2:** A yellow-white, hard, fleshy granulomatous lesion (1×2 cm; black arrow) adjunct to the caudal border of the right lateral lobe of the liver.

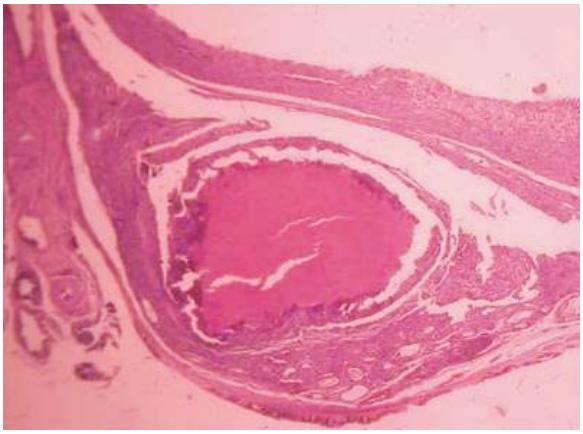

**Figure 3:** Histological section of one of the granulomas of the oviduct showing the collagen capsule and an inflammatory cell layer consisting of leucocytes and central cell debris. H&E stains, ×10.

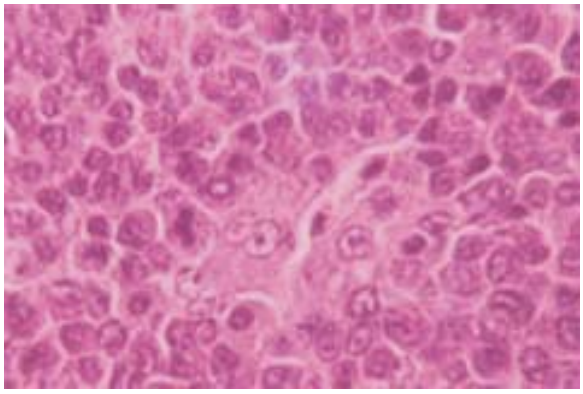

**Figure 4:** Liver showing sheets of neoplastic cells and disruption of the normal hepatic architecture. H&E stains, ×40.

commonly presents in the intestinal tract and liver of poultry but not in the spleen. In case reports of a common buzzard and an Amazon parrot, the wide dissemination of granulomas was shown in the spleen and pancreas (Thiede and Krone, 2001; Raphael and Iverson, 1980). This budgerigar presented with lesions in the liver, oviduct and intestinal tract. Factors such as a limited feed intake, vitamin A deficiency, hot weather, parasitic infestation and genetic susceptibility have been suggested as predisposing risk factors for coligranuloma in poultry (Biely and March, 1962; Jordan *et al.*, 2008; Narula and Kuppuswamy, 1969; Riddell, 1972; Woloszyn *et al.*, 1962). This budgerigar might have been stressed by similar causes. All of these reports suggest that the presence of coligranulomas cannot be explained by infection with *E. coli* alone. However, *E. coli* must have acted as a primary pathogen when the immune resistance of this bird was lowered by an immunosuppressive viral disease, such as avian leukosis.

Causes of oviduct impactions include excess mucin or albumin secretion secondary to cystic hyperplasia or inspissated egg material in the magnum (Joyner, 1994). *E. coli* is the most common isolated coliform bacterium from various species (Joyner, 1994; Gross, 1991). The salpingitis may be descending, secondary to air sacculitis or pneumonia, or ascending, from the distal uterus, vagina, or cloaca (Romagnano, 1996). In this case, salpingitis has been associated with oviductal impaction. Oophoritis, or an infection of the ovary, is frequently associated with systemic bacterial disease (Peckham, 1965).

Coligranuloma, the chronic form of *E. coli* infection, is characterized by the presence in many tissues of granulomas surrounded by giant cells (Raphael and Iverson, 1980). Coligranuloma must be differentiated from tuberculosis and mycotic granuloma. Primarily, avian tuberculosis involves the intestinal tract and the liver (Cooper, 1985). This case was differentiated from tuberculosis by Ziehl-Nielsen

staining, from mycotic granuloma by PAS staining. Lesions of colibacillosis, a subacute form of *E. coli* infection, as a fibrinopurulent pericarditis, air sacculitis and peritonitis were not found in this budgerigar.

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