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Case Report

Sarcocystis falcatula infection in ringneck parakeets (*Psittacula krameri*) in Minas Gerais, Brazil

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Abstract

Sarcocystis falcatula is an Apicomplexa protozoan parasite recognized for its pathogenicity in birds. This microorganism has a complex life cycle involving definitive hosts, such as marsupials from the Didelphidae family, and intermediate hosts, which include a wide range of bird species. Three male ringnecks (*Psittacula krameri*) from a breeding facility with a history of sudden bird deaths were submitted for post-mortem examination. Gross findings included severe pulmonary congestion and edema, and diffusely congested livers. Histologically there was a severe and diffuse lymphohistiocytic interstitial pneumonia with moderate hyperemia and pulmonary edema. Numerous schizonts morphologically compatible with *S. falcatula* were observed in pulmonary endothelial cells, which were confirmed by immunohistochemistry. There was also moderate random multifocal lymphohistiocytic hepatitis, coalescing multifocal lymphohistiocytic and heterophilic myocarditis, mild coalescing multifocal lymphohistiocytic interstitial nephritis, and severe diffuse splenic congestion. These cases represent the first reported case of *S. falcatula* infection in ringnecks in Minas Gerais, highlighting the need for vigilance and control of *Sarcocystis* infections in captive bird populations.

Keywords: avian pathology*; Psittacula krameri*; ringnecks; sarcocystosis.

Introduction

Sarcocystis falcatula is a protozoan parasite of the phylum Apicomplexa, recognized for its pathogenicity in birds. This microorganism has a complex life cycle involving definitive hosts, such as marsupials of the family Didelphidae, and intermediate hosts, which include a wide range of bird species (1, 2,11,13).

S. falcatula is one of the most common *Sarcocystis* spp. species in the Americas, and its geographic distribution is closely linked to the presence of its definitive hosts, the opossums. Opossums not only harbor *S. falcatula*, but also other species of the genus *Sarcocystis*, such as *S. neurona, S. speeri*, and *S. lindsay*. Genetic lineages of *S. falcatula* show regional variation, with one lineage predominating in North America and another, more common in South America. This genetic diversity leads to the consideration of *S. falcatula* as a species complex, with the term "*Sarcocystis falcatula*-like" used to describe organisms with compatible morphological and molecular characteristics (7,13).

The transmission cycle of *S. falcatula* involves both sexual and asexual reproduction. Birds containing *Sarcocystis* in their musculature are ingested by opossums, and these cysts release bradyzoites through the action of intestinal proteolytic enzymes. The bradyzoites penetrate the lamina propria of the intestinal villi, initiating sexual reproduction

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and the formation of infective sporocysts, which are excreted in the opossum's feces for extended periods. The infective sporocysts are then ingested by birds, either through direct contact with opossum feces or indirectly through contact with paratenic hosts, such as cockroaches (4,5,6,10). Within the intestine of the bird, sporozoites are released and enter the bloodstream, where they undergo asexual reproduction in the vascular endothelium of arterioles, capillaries, and venules in various host organs, such as the lungs, liver, spleen, kidneys, intestines, and skeletal muscles. After the asexual phase, the merozoites formed primarily invade the skeletal muscles and form cysts (4,5).

Mortality due to *S. falcatula* parasitism varies among bird species. New World psittacines are generally resistant to infection and have an asymptomatic clinical course. However, Old World psittacines are highly susceptible, often presenting an acute course with no prior clinical signs, and are considered accidental hosts (10).

Although well-described in various bird families, there are only a few reports of *S. falcatula* infection in psittacines, especially in ringnecks (*Psittacula krameri*). In these animals, severe disease is often observed, including acute pulmonary lesions and even death (7,13). In addition to posing a threat to captive birds, *S. falcatula* infection can also have serious implications for endangered bird species. Given the pathogenic characteristics of the protozoan and the vulnerability of many wild bird populations, the spread of *S. falcatula* in natural environments may represent a significant risk to the conservation of psittacine species and other endangered birds (7,13).

This report aims to describe three cases of *S. falcatula* infection and their gross, microscopic, and immunohistochemical features in three ringnecks (*Psittacula krameri*) in the State of Minas Gerais, Brazil.

Case description

This study included three ringnecks (*Psittacula krameri*), two approximately 3-month-old males and one 10-year-old female, from a breeding facility with a history of sudden deaths occurring at approximately every 10 days. The birds were sent to the Universidade Federal de Minas Gerais, Belo Horizonte, Brazil, for post-mortem examination. Grossly, the animals had good body condition. The liver of both male birds had diffuse dark red discoloration associated with diffuse highlighting of the lobular pattern. In contrast, the liver and spleen of the female bird appeared reddish-orange (Figure 1A and B). Lungs were not collapsed and were diffusely and markedly dark red, draining a moderate amount of blood on the cut surface (Figure 1C). Cytologic analyses performed during the necropsy using the squash method on lung samples, rare endothelial cells from dilated vessels containing schizont morphologically compatible with *S. falcatula* were observed (Figure 1D).

Tissue and organ samples were fixed in 10% buffered formalin paraffin embedded in paraffin, sectioned with a microtome (4-µm sections), and stained with hematoxylin and eosin (HE). Histologically, there was a severe diffuse lymphohistiocytic interstitial pneumonia with moderate hyperemia and pulmonary edema. Within endothelial cells of multiple pulmonary vessels, numerous schizonts were seen in longitudinal and transverse sections. Schizonts were elongated, measured 15 to 20 µm in length and approximately 5 µm in cross-sectional diameter, and were morphologically compatible with *S. falcatula* (Figure 2A). In the livers there was moderate multifocal random lymphohistiocytic hepatitis (Figure 2B), and in the female birds, a moderate number of schizonts morphologically compatible with *S. falcatula* were observed within endothelial cells. Additionally, multifocal to coalescent lymphohistiocytic and heterophilic myocarditis (Figure 2C), mild multifocal to coalescent lymphohistiocytic interstitial nephritis (Figure 2D), multifocal subepicardial hemorrhage, and severe diffuse splenic congestion were observed.

Immunohistochemistry was performed for detection of *Sarcocystis* spp. antigens in the lungs. Endogenous peroxidase was blocked with 3.5% hydrogen peroxide at room temperature for 40 min. Nonspecific reactions were blocked using 6% powdered skimmed milk for 1 hour at room temperature. A polyclonal anti-*Sarcocystis* spp. primary antibody was used at a dilution of 1:1000 for 1 hour at room temperature as previously described (7), and the anti-rabbit secondary antibody conjugated with peroxidase (Envisioflex HRP, Dako) was incubated for 30 min at room temperature and in a dark chamber. The chromogen used was the Envision Flex HRP Magenta (Dako) for 5 minutes, followed by counterstaining with hematoxylin and dehydration in increasing alcohol and xylene. A known positive case was used as a positive control (12). Immunohistochemistry showed a large number of *Sarcocystis* schizonts in endothelial cells of dilated blood vessels and sometimes associated with lymphohistiocytic infiltrate (Figure 3A and B).

Discussion

Based on gross, histological and immunohistochemical features, *S. falcatula* was diagnosed in three *P. krameri*.

Infection with *S. falcatula* in birds typically occurs through the ingestion of oocysts or sporocysts present in the feces of definitive hosts, such as opossums of the genus *Didelphis* spp., which are commonly found in environments where captive birds are kept. The oocysts/sporocysts release sporozoites that invade the epithelial cells of the gastrointestinal tract of intermediate host birds (4,11).

Previous studies on birds have reported acute clinical signs, including dyspnea, lethargy, and, in some cases, anorexia. These clinical signs are often nonspecific, which can complicate the initial diagnosis. However, in more severe

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cases, birds may exhibit weakness, partial or total paralysis, and even sudden death (2,12). In the present report, detailed information on the clinical progression and signs was not obtained, likely due to the acute nature of the *Sarcocystis* infection and sudden death of the animals.

During necropsy, the main findings included congestion, edema, and hemorrhage in the lungs, as well as hepatomegaly and splenomegaly. The lungs typically had more pronounced lesions, reflecting the tropism of the parasite for this organ (2,3,6). Microscopic lesions were compatible with sarcocystosis in the lungs, such as the presence of schizonts and cysts in tissues, which was confirmed by immunohistochemistry. Additionally, an inflammatory infiltrate around the *Sarcocystis* was noted, indicating a host immune response, which included pneumonia, myocarditis, nephritis, hepatitis, and myositis, all with lymphohistiocytic inflammatory infiltrate and visualization of intralesional schizonts in the lungs, heart, and liver (2,6,12,13). These findings support the diagnosis of the infection.

The definitive diagnosis of sarcocystosis in ringnecks can be confirmed based on clinical signs, necropsy findings, and histopathological analysis. Immunohistochemistry can be employed to identify the presence of *Sarcocystis* antigens in the affected lungs, liver, heart, and other organs, and it should be used particularly in cases where the agent is not visualized, but lesions are compatible with those observed in this disease. This technique provides a definitive confirmation of the parasite infection, complementing the microscopic findings observed during necropsy (2,6,12,13). Advanced diagnostic methods, such as molecular techniques, have proven effective in the precise identification of the parasite. PCR (polymerase chain reaction) can be employed to detect *Sarcocystis* DNA in affected tissues, offering a definitive confirmation of the infection. Additionally, serological techniques, such as ELISA

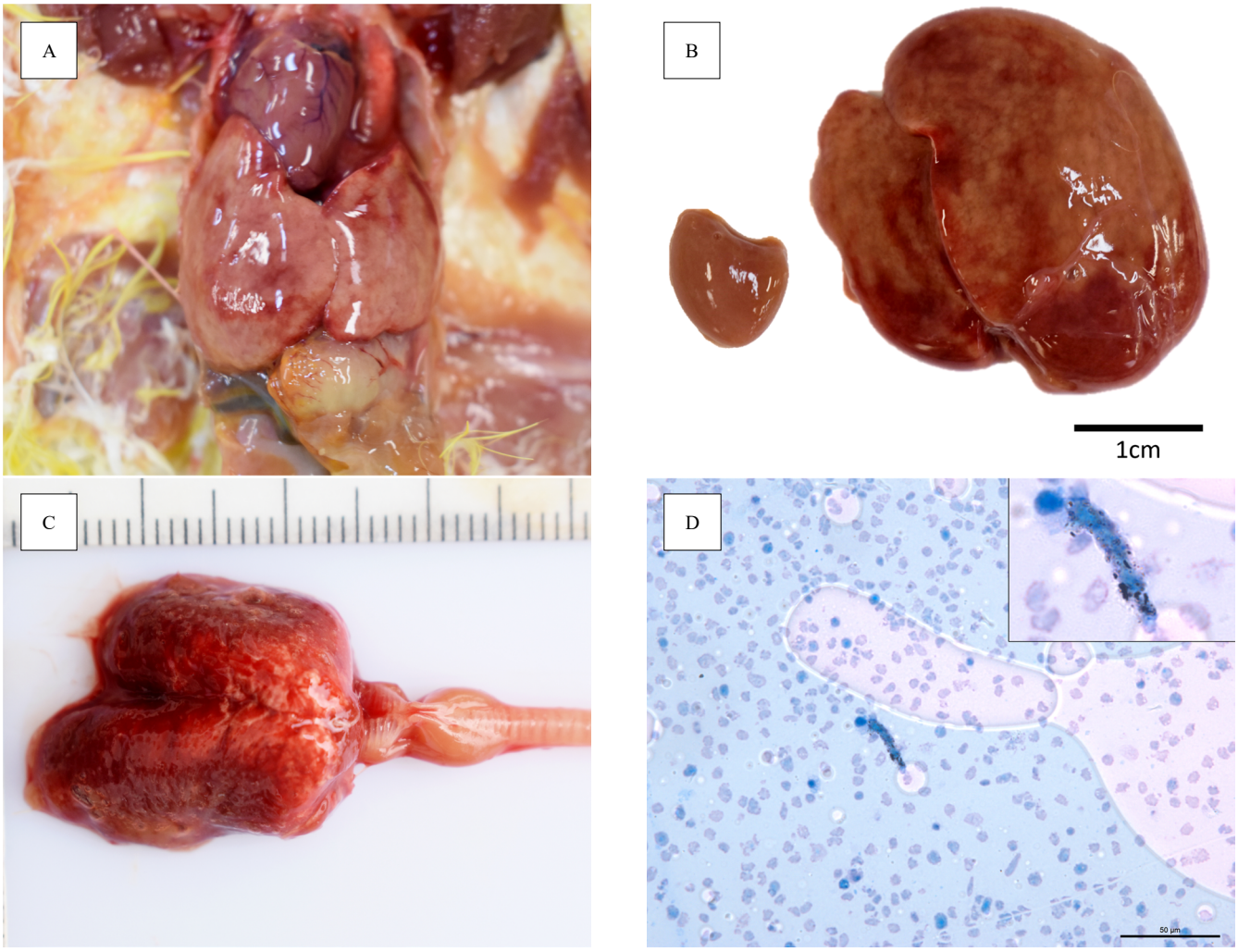

Figure 1. *Sarcocystis falcatula* infection in ringnecks (*Psittacula krameri*). A- Celomic cavity showing diffusely orange-red liver, enlarged with multifocal areas of lobular pattern accentuation. B- Liver and spleen diffusely orange-red, enlarged, and exuding a small amount of blood on sectioning. C- Lung non-collapsed, diffusely and intensely dark red, exuding a moderate amount of blood on sectioning. D- Lung cytology showing endothelial cells with schizonts compatible with *S. falcatula* (inset).

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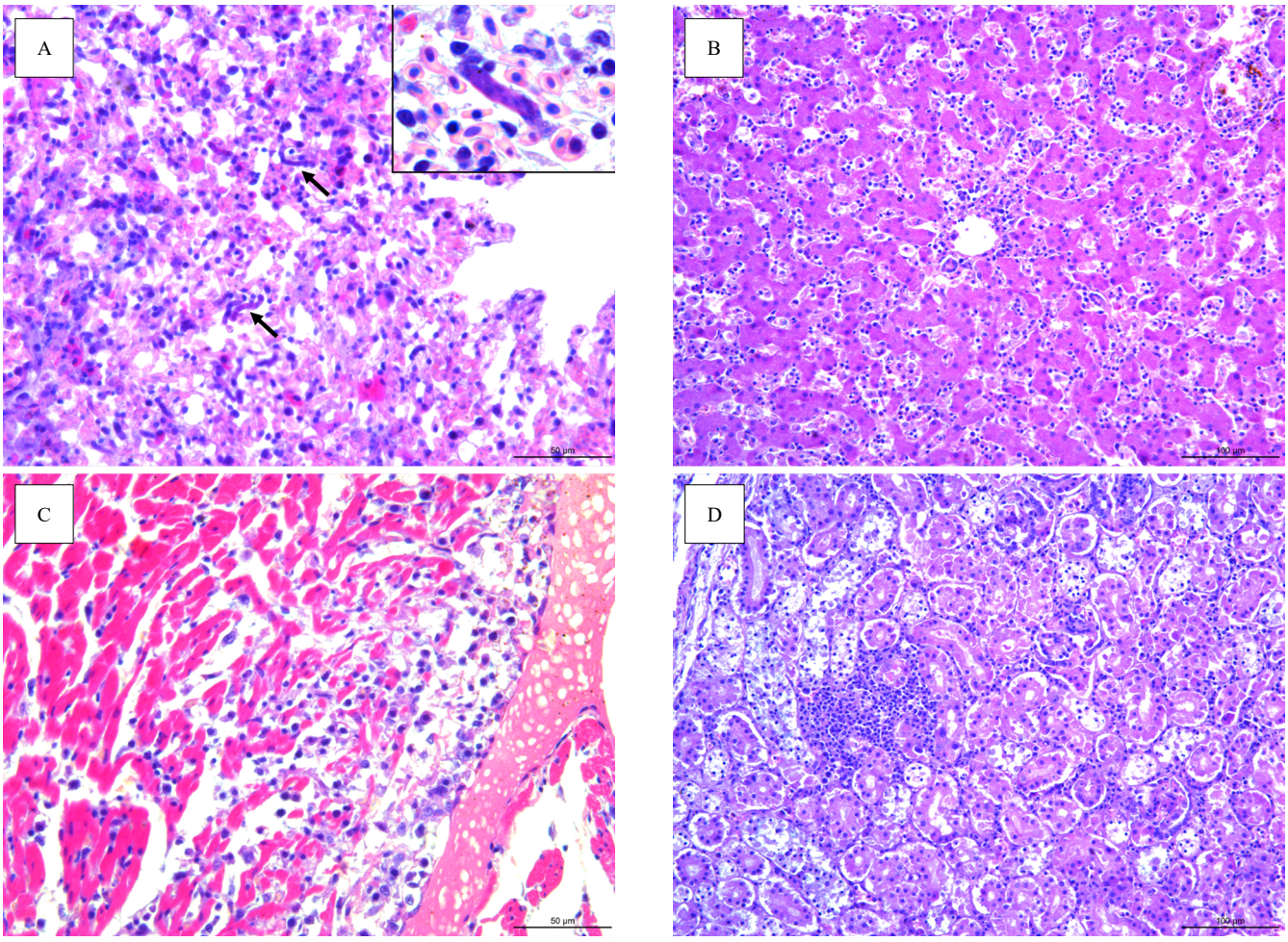

Figure 2. *Sarcocystis falcatula* infection in three ringnecks (*Psittacula krameri*). A- Lung. Intense diffuse histiocytic and lymphoplasmacytic interstitial pneumonia with schizonts (arrows) morphologically compatible with *Sarcocystis falcatula* in the vascular endothelium (inset). HE, Bar: 50 µm. B- Liver. Moderate multifocal random lymphoplasmacytic and histiocytic hepatitis. HE, Bar: 100 µm. C- Heart. Moderate multifocal to coalescent lymphoplasmacytic and histiocytic myocarditis. HE, Bar: 50 µm. D- Kidney. Mild multifocal to coalescent lymphoplasmacytic and histiocytic interstitial nephritis. HE, Bar: 100 µm.

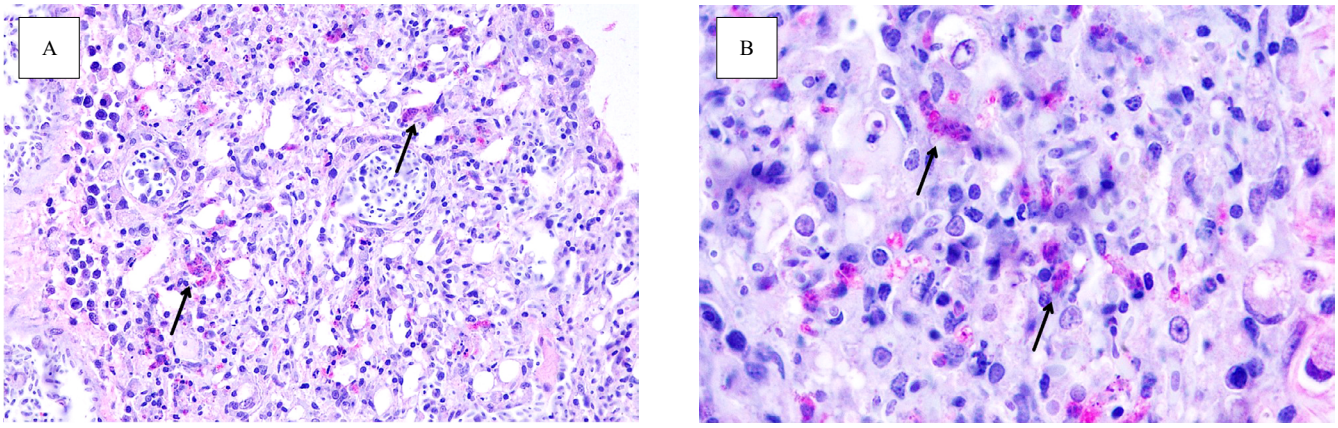

Figure 3. Immunohistochemistry for detection of *Sarcocystis* spp. antigens in ringnecks (*Psittacula krameri*). A- Lung, immunostaining of schizonts in magenta (arrows), 400x. B- Lung, immunostaining of schizonts in magenta (arrows) associated with histiocytic and lymphoplasmacytic infiltrate, 1000x.

(enzyme-linked immunosorbent assay), may be useful for detecting specific anti-*Sarcocystis* antibodies (2,5). Treatment of sarcocystosis in ringnecks generally involves the use of antiparasitic medications, such as sulfadiazine (9,12).

Early recognition of clinical signs, along with advanced diagnostic techniques, is essential for effective management of the disease. Furthermore, appropriate preventive measures are crucial to reduce the risk of infection and ensure the continued health and well-being of both captive and wild birds. The spread of the parasite in threatened bird populations could represent a significant risk to the survival of these species in natural environments and to biodiversity (6,9).

Our finding highlights the importance of surveillance and control of *Sarcocystis* infections in captive birds. Preventive measures, such as proper sanitary management and vector control, are essential to reduce the risk of infection by *S. falcatula* and other *Sarcocystis* species for exotic bird populations.

Conflict of Interest

The authors declare no competing interests.

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