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Short communication

Pathological and molecular characterization of systemic isosporosis (atoxoplasmosis) in captive green-winged saltator (*Saltator similis*)

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ABSTRACT

Systemic isosporosis, also called atoxoplasmosis or visceral coccidiosis, is a disease that affects birds in general. Pathogenesis of systemic isosporosis and its etiologic agent have not been well characterized, but taxonomically *Atoxoplasma* is currently considered a junior objective synonym of *Isospora*. The present report aimed to describe pathological and molecular findings of systemic isosporosis in captive green-winged saltators (*Saltator similis*) from the State of Espírito Santo, Brazil. In a commercial breeding facility eleven birds with two to nine months of age died from 2015 to 2016. These birds developed nonspecific clinical signs, including bristly feathers, hyporexia, loss of weight, and apathy. Two birds were necropsied, and grossly there were hepatomegaly, splenomegaly, necrosis of lymphoid follicles, hepatic necrosis, and severe enteritis. Merozoites were observed in the heart, small intestine, proventriculus, brain, liver, spleen, and kidneys. 23 S RNA PCR amplicons from DNA extracted from the liver and the intestinal contents had 99% identity with *Atoxoplasma* sp., whereas amplicons of mitochondrial cytochrome c oxidase subunit 1 ha d 97% identity with *Isospora greineri*. In conclusion, this report indicates that systemic isosporosis in green-winged saltator is a disease that affects the spleen, liver, and small intestine, with high mortality for young birds, resulting in significant loses to commercial breeding facilities.

1. Introduction

Systemic isosporosis also named atoxoplasmosis or visceral coccidiosis is an avian disease caused by a coccidian that has an extra intestinal cycle. Transmission occurs by ingestion of sporulated oocysts released from the feces of infected animals (Box, 1970; Levine, 1982; Adkesson et al., 2005). This coccidian proliferates sexually in enterocytes of the duodenum and has the asexual phase inside macrophages, lymphocytes, and natural-killer cells. The pathogen is transported intracellularly through the blood stream to multiple organs, leading to the systemic manifestation of the disease (Sánchez-Cordón et al., 2007; Cushing et al., 2011). The liver is the main organ involved in systemic infection, but lesions are also observed in the spleen, lungs, and the intestine itself (Sánchez-Cordón et al., 2007; Cushing et al., 2011).

Several species of *Isospora* spp. have been identified in feces of free-living and captive green-winged saltators (Coelho et al., 2013). Importantly, in terms of taxonomy, *Atoxoplasma* is considered a junior objective synonym of *Isospora* (Barta et al., 2005). Therefore, atoxoplasmosis will be referred hereto as systemic isosporosis. Nevertheless, there are no previous reports of extra intestinal or systemic isosporosis in green-winged saltators.

Adult birds are generally asymptomatic, whereas systemic isosporosis results in high mortality for young birds (2 to 9-month-old) in captivity (Adkesson et al., 2005; Cushing et al., 2011; Jamriška et al., 2013). Clinical signs described in affected birds are non-specific, including hyporexia, progressive weight loss, reduction of pectoral muscle mass, depression, dehydration, diarrhea, bristly feathering, abdominal distension, and loss of balance (Sánchez-Cordón et al., 2007; Maslin and Latimer, 2009).

Systemic isosporosis has already been described in several species of passerines from different geographic regions, including canaries (Box, 1970; Sánchez-Cordón et al., 2007), tanagers (Adkesson et al., 2005), American goldfinches (Cushing et al., 2011), house sparrows (Cushing et al., 2011), Bali mynahs (Done et al., 2011), Israeli sparrow (Gill and Paperna, 2008), and blue-crowned laughingthrush, a critically

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endangered Chinese passerine (Jamriška et al., 2013). The green-winged saltator (*Saltator similis*) is a passerine of the family Thraupidae, native of Latin America, and popular among breeders, due to its singing and high commercial value. Although it is not at risk of extinction, its population has been declining, mainly due to illegal traffic (Coelho et al., 2013; IUCN, 2016).

The goal of this study was to characterize the pathological and molecular findings in cases of systemic isosporosis in captive green-winged saltators.

2. Material studied

A commercial breeding facility in the State of Espírito Santo (Brazil), began breeding nine years ago, and since then has never succeeded, with empty litters (unfertilized eggs) or death due to undetermined causes. Between 2015 and 2016, six litters with 12 birds in total were obtained, of which only one bird survived. Fecal samples from 11 birds were submitted for parasitological examination, of these two birds were available for necropsy.

After birth, all birds were raised by the mother for 35 days and then separated into individual cages. Clinical signs began approximately one month after being separated from their mother. The eleven birds that died had a history of apathy, hyporexia, and bristly feathering, with average disease progression of one week.

In May 2016, after the death of nine young birds (9/12), a six-month-old female green-winged saltator was submitted for necropsy at the Laboratory of Pathology of Universidade Vila Velha (UVV). Grossly, there were moderate hypotrophy of pectoral muscles. The duodenum had a bright reddish serosa (Fig. 1A) with a thickened wall, and the mucosa had multiple purple spots, and a dark red mucous content. The liver was diffusely brownish with slight evidence of lobular pattern (Fig. 1C). There was moderate hepatomegaly and splenomegaly.

Samples of the liver, spleen, pancreas, proventriculus, ventriculus, small intestine, large intestine, kidneys, lungs, pectoral muscles, heart, bone marrow, and brain were fixed in 10% buffered formalin, and submitted for routine histological processing. In addition, an imprint of the duodenal mucosa was made on a glass slide, stained with Panoptic fast staining, and analyzed under light microscopy.

Table 1
Total of breeding birds, grouped by age, submitted to parasitological examination – Faust method – from fecal samples.

Age (years)	Gender	Number of birds
> 04	Male	04
02	Male	03
> 04	Female	04

Imprints from the duodenal mucosa demonstrated a mixed population of cells constituted by moderate amounts of macrophages, lymphocytes, erythrocytes, and enterocytes. In the cytoplasm of macrophages and lymphocytes there were variable numbers of small unicellular organisms, which were round to oval, with clear cytoplasm and a dark, eccentric nucleus, and sometimes forming a half moon at the edge of the cell (merozoites) (Fig.1B).

Microscopically there was moderate multifocal necrosis of the peribronchial and peribronchiolar lymphoid follicles; severe lympholysis in the spleen; severe diffuse lympho-histiocytic necrotizing hepatitis (Fig. 1D); mild focal endocarditis; moderate diffuse lympho-histiocytic nephritis; moderate diffuse lympho-histiocytic and plasmocytic proventriculitis; and severe diffuse necro-hemorrhagic lympho-histiocytic enteritis. In all these organs there were large numbers of intracytoplasmic merozoites in macrophages, lymphocytes, and epithelial cells (Fig. 1E). Small numbers of merozoites was also rarely observed in endothelial cells of the choroid plexus in the brain and within mononuclear cells in the bone marrow.

After the necropsy, a parasitological examination of the feces pool of all breeding birds (Table 1) was carried out using the Faust method, a centrifugal-flotation technique in zinc sulfate. A small amount of oocysts morphologically compatible *Isospora* spp. were observed.

In the next litter (September 2016), another two-month-old female green-winged saltator died, and was submitted to necropsy, with findings similar to the previous bird, but less severe, and also compatible with systemic isosporosis. At necropsy, imprints of the spleen, liver, and duodenum were performed. In all these organs, a large number of merozoite-filled macrophages and lymphocytes were observed.

At necropsy, samples of the liver and intestinal contents were

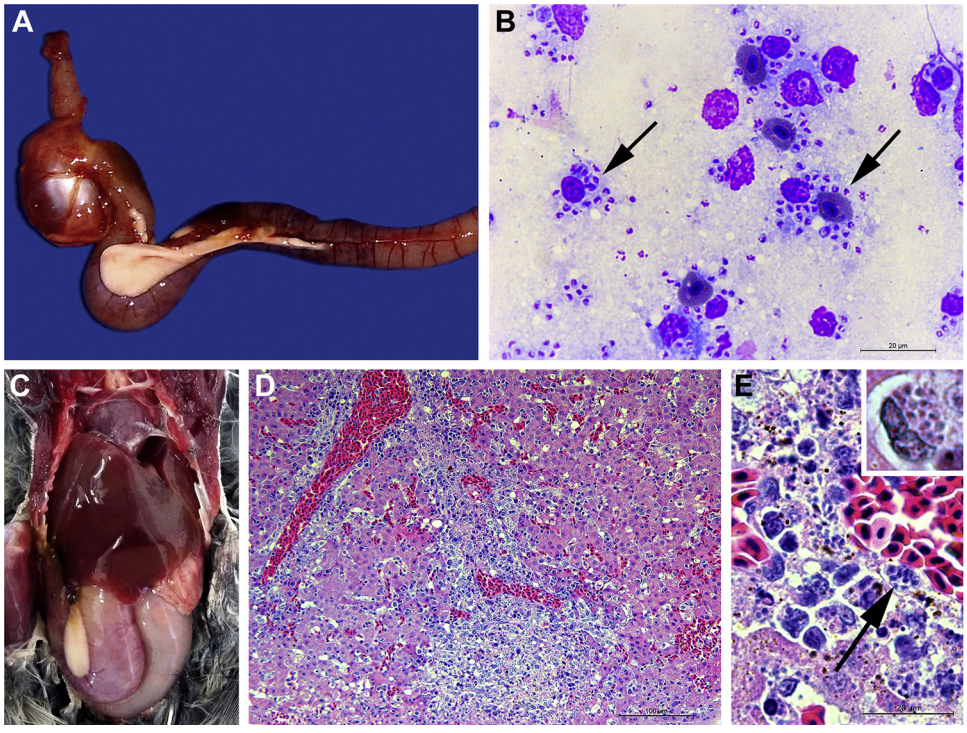

Fig. 1. A. Duodenum. Marked segmental congestion of intestinal serosa restricted to the duodenum. B. Cytologic preparation from the duodenum (imprint) with several merozoites of*Isospora* sp. within the cytoplasm of macrophages (black arrow) and lymphocytes, Diff Quick. Bar = 20 µm. C. Liver. Marked hepatomegaly with diffusely brownish discoloration. D. Liver. Multifocal to coalescing severe lympho-histiocytic and necrotizing hepatitis, HE, Bar = 100 µm. E. Higher magnification of (D) with numerous merozoites (black arrow) in Kupffer cells, lymphocytes, and hepatocytes. Inset: higher magnification of a macrophage (Kupffer cell) containing merozoites, HE, Bar = 20 µm (E).