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Case Report—

Hepatic Hemosiderosis in Red-Spectacled Amazons (*Amazona pretrei*) and Correlation with Nutritional Aspects

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SUMMARY. Alimentary habits of free-living Psittaciformes vary significantly among different species. *Amazona pretrei* is under risk of extinction and has very specific free-living dietary habits, which are based on Paraná pine seeds. Hemosiderosis is a pathologic process characterized by intracellular accumulation of iron without other evident lesions. It is associated with increased prevalence of infections, neoplasms, and hepatopathies. The purpose of this study was to quantify hepatic hemosiderin deposits in captive *A. pretrei* and verify their association with nutritional parameters. Liver samples were processed for histopathology and stained with Prussian blue. The sections were analyzed by computerized morphometry to quantify the hepatic hemosiderin deposits. The hepatic hemosiderosis rates showed positive correlation with age and time in captivity. These results suggest that the menus and commercial rations for Psittacidae must be carefully revised.

RESUMEN. *Reporte de Caso*—Hemosiderosis hepática en loros de anteojos rojos (*Amazona pretrei*) y su correlación con aspectos nutricionales.

Los hábitos alimentarios de las aves Psittaciformes de vida libre varían significativamente entre las diferentes especies. La especie *Amazona pretrei* está en peligro de extinción y en vida libre tiene hábitos alimentarios muy específicos, que se basan en las semillas de pino Paraná. La hemosiderosis es un proceso patológico caracterizado por la acumulación intracelular de hierro, sin otras lesiones evidentes. Se asocia con una mayor prevalencia de infecciones, neoplasias y hepatopatías. El propósito de este estudio fue cuantificar los depósitos hepáticos de hemosiderina en aves de la especie *A. pretrei* mantenidas en cautiverio y verificar su asociación con parámetros nutricionales. Se procesaron muestras de hígado para su estudio histopatológico y se tiñeron con azul de Prusia. Los cortes fueron analizados por morfometría computarizada para cuantificar los depósitos de hemosiderina hepática. La cantidad de hemosiderosis hepática mostró una correlación positiva con la edad y con el tiempo en cautiverio. Estos resultados sugieren que los menús y las raciones comerciales para aves Psittacidae deben ser revisados cuidadosamente.

Key words: *Amazona pretrei*, hemosiderosis, iron, liver, morphometry, parrot, Psittacidae

Abbreviations: HE = hematoxylin and eosin; HHR = hepatic hemosiderosis rate; PB = Prussian blue

In physiological conditions, intracellular iron may be stored as ferritin, free in the cytosol and not visualized with routine histological staining methods, or as hemosiderin, which is detected as brown or blue granules, on hematoxylin and eosin (HE) and Prussian blue (PB) techniques. Hemosiderosis is the pathologic process characterized by intracellular accumulation of iron without other evident lesions, whereas hemochromatosis is the excessive accumulation of iron with functional and/or morphological consequences (8,18,25).

The deposits may occur as the result of a primary condition, of hereditary origin, or secondary to intoxications, anemia, excessive iron intake, and other pathological processes (11). Hemosiderosis is also associated with increased prevalence of infections, neoplasms, and hepatopathies (4,5,31).

Pathological hepatic accumulation of iron, disease associated or not, has been described in several families or order of birds, more commonly in Sturnidae, Ramphastidae, Corvidae, Cotingidae,

Ptylonorhynchidae, and Bucerotidae, and rarely in Psittacidae (4,11,12,29,30,32). Hemochromatosis was described in lorries (30), a group of Psittaciformes that consumes pollen, nectar, fruits, and insects (17), in a citron-crested cockatoo, and in other psittacine species (25).

There are about 85 species of Psittacidae in Brazil (2). The red-spectacled Amazon (*Amazona pretrei*) occurs in a restricted area in the south of the country. Its population, currently vulnerable and decreasing, is considered to be at risk of extinction (1). These parrots migrate from the State of Paraná to Rio Grande do Sul, where they nest (15). It is believed that this behavior is associated with the maturation process of Paraná pine seeds (*Araucaria angustifolia*), from February to September, because it represents more than 90% of the diet of the red-spectacled Amazons from April to July in the State of Santa Catarina, Brazil (16).

The alimentary habits of free-living Psittaciformes vary significantly among different species, including fruit, flowers, leguminosae, and coconuts (16). Commercial rations for captive birds are usually formulated for a specific order or family and extrapolate the same nutritional requirements for every species. Birds in captivity may

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Table 1. Distribution of the cases analyzed according to gender, age, time in captivity, presence of parasitic infestation, and HHR.

Case number	Gender	Age (years)	Time of captivity (years)	Presence of parasites ^A	HHR (%)
1	M	8	8	Y	11.25
2	F	8	5	N	1.87
3	F	7	7	N	0.02
4	M	8	8	N	0.64
5	M	2	2	Y	0.31
6	F	0.5	0.5	Y	15.06
7	M	1	1	Y	0.18
8	F	5	5	N	0.20
9	F	1	1	Y	0.75
10	F	0.5	0.5	N	0.20
11	F	10	10	Y	15.72
12	M	10	10	Y	7.65
13	M	10	10	Y	33.21
14	M	10	10	N	13.95
15	F	2	2	Y	0.71
16	F	10	10	Y	3.60
17	M	4	4	Y	0.20
18	M	10	10	Y	21.57
19	M	10	10	Y	11.55
20	M	9	9	Y	8.05

^AGastrointestinal metazoan, genus *Capillaria*: Y = yes, N = no.

receive diets rich in iron and vitamin C and poor in tannins, which favor iron absorption and accumulation (17,27).

In this study, we quantified the hepatic hemosiderin deposits in captive *A. pretrei* and verified its association with age, time in captivity, gender, and presence of parasitic infestation. Possible correlations between natural and commercial diets as sources for intracellular iron accumulation are also discussed.

CASE REPORT

Liver samples from 20 red-spectacled Amazons (*A. pretrei*) were submitted to microscopic examination from January 2006 to December 2009. All birds originated from the same breeder, situated in Santa Maria, in the central region of Rio Grande do Sul, Brazil, and during the whole captivity period were fed commercial ration (Alcon® PsitaBits®, Santa Catarina, Brazil), fresh fruits (banana, orange, kaki, and tangerine), and cereals (corn, oat, wheat, and soybean flour). Identification, age, sex, time in captivity data, and results from parasitological exams (gastrointestinal metazoans) were obtained from the necropsy reports. Eleven of the 20 parrots were male (55%), and nine (45%) female. The age of the birds ranged from 0.5 to 10 years (average 6.3). The average captivity time was 6.2 years, because one of the birds (case 2) was donated at the age of 3. All the birds died spontaneously (Table 1).

Liver fragments were fixed in 10% formalin and routinely processed for histopathology. Five-micrometer sections were stained with HE and PB (23). Morphometric analysis was conducted on histological sections stained with PB. The slides were analyzed with a microscope (Trinocular Olympus® BX 50, Olympus, Melville, NY) equipped with a digital camera (Evolution MP®, 5.1 megapixels, Media Cybernetics, Silver Spring, MD), a computerized image analysis system (Image ProPlus®, version 5.1.2.59, Media Cybernetics), and a 19-inch monitor (model L1950HQ-SN, LG Electronics, São Paulo, Brazil). The center of each fragment, corresponding to the intersection of the two largest diameters, was marked on the cover slip with a pen. Eight images were recorded as TIF files, 50 µm from this point, at 45° intervals, with the same light

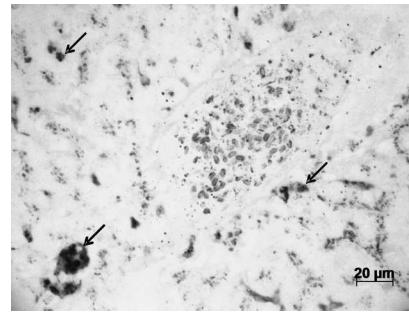


Fig. 1. Liver, case no. 16; mild hemosiderosis (arrows); PB, obj. 40×.

conditions and using the 20× magnification objective. The hemosiderin deposits were semiautomatically delineated, from the lower intensity of blue to the higher intensity, to avoid the measurement of artifacts or background. For practical purposes, a maximum digital zoom of 150× was stipulated. The parameter that was obtained from each field was the percentage of the area that was occupied by hemosiderin. The average of the eight fields was considered the hepatic hemosiderosis rate (HHR).

The average HHR was 7.33% (ranged from 0.02% to 33.21%; Figs. 1 and 2). No statistically significant differences were detected when the cases were grouped by gender or presence of parasitic infestation ($P = 0.3618$ and $P = 0.0908$, respectively, Mann Whitney test). The HHR showed a statistically significant positive correlation with the age (Fig. 3) and time in captivity of the birds ($P = 0.0029$, $r = 0.6295$, and $P = 0.0042$, $r = 0.6113$, respectively, Spearman correlation).

DISCUSSION

Hepatic hemosiderosis has been described in several groups of birds and is usually associated with captive frugivore or insectivore species (14). Reports of hepatic intracellular iron accumulation in Psittacidae are relatively rare and variable. Roskopf *et al.* (25) described a clinical case of hemosiderosis in a citron-crested cockatoo and other psittacine species. A study with 531 birds of 18 orders, conducted in the London Zoological Park, did not find psittaciformes affected by hemosiderosis (28). On the other hand, Cork *et al.* (5) analyzed samples of 180 birds of six different orders, received from rehabilitation centers, breeders, and veterinarians, and detected hepatic hemosiderin deposits in 50 cases, the more severe in psittaciformes and columbiformes. Unfortunately, neither of these two studies regarded the diet and the species of Psittacidae. Costa and Catão-Dias (6) described a case of *Amazona aestiva* with a HHR of 5.7%. Hemochromatosis was identified in captive lorries that were fed diets high in iron (30), and Gancz *et al.* (13) described

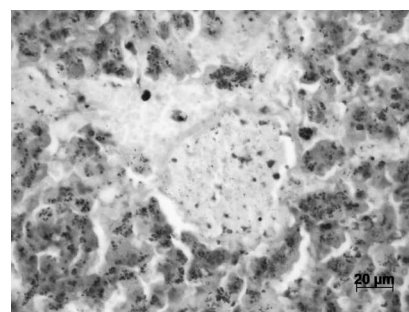


Fig. 2. Liver, case no. 13; severe hemosiderosis; PB, obj. 40×.

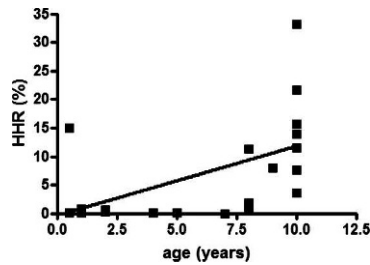


Fig. 3. Graph demonstrating significant positive correlation between age and HHR of the birds ($P = 0.0029$, $r = 0.6295$).

hemosiderin deposits in macaws (*Ara severa* and *Ara militaris*) with diabetes mellitus.

The samples analyzed in the present research represent a population of *A. pretrei* bred in captivity that were fed the same diet, of commercial ration and fresh fruit, during the entire period. For the evaluation of the hepatic iron accumulation, we used computerized morphometry, an accurate technique that correlates biochemical measurement of iron by atomic absorption spectrophotometry (21) or by chemical analysis (24).

The HHRs were similar to the HHRs described by Cubas (9) in Ramphastidae, for which the values varied from zero to 37.2%. Diseases related to excessive iron accumulation are common among these birds (9). Similarly to what was observed with toucans, there was no correlation between the HHR and sex for the red-spectacled Amazons (10).

Because of the diversity of alimentary habits, some species may be more susceptible to iron hepatic accumulation (16,30). It is known that free-living mynahs (*Gracula religiosa*), which eat diets poor in iron and rich in fruits and insects, have greater proportion of and more efficient *Ireg1* transporters in their enterocytes than other birds (19). This can help explain the high susceptibility of mynahs to iron accumulation when fed commercial rations (26). Another constituent of these rations is vitamin C, which decreases intestine pH and facilitates iron absorption (12,17,19,26), favoring hemosiderosis. Moreover, ascorbic acid may promote the reduction of iron, both intra- and extracellularly, predisposing to the formation of hydroxyl radicals by the Fenton reaction (7).

Amazona pretrei kept in captivity and fed with native vegetables ate 10 kg/bird/year of *Araucaria* seed (*A. angustifolia*), while free-living red-spectacled Amazons ate, from April to July, diets composed of more than 90% of *A. angustifolia* seeds (22). The Paraná pine seed *in natura* contains 7.2 mg Fe/kg (3).

The birds analyzed in our study received, during the whole period in captivity, a commercial ration with iron levels of 70 to 120 mg/kg. These levels are markedly high when compared to those of free-living diets, in which Paraná pine seeds are the main item. Another aspect that must be considered is the positive correlation of hepatic hemosiderosis levels to age, as well as to time in captivity. These data suggest that the duration of the ration consumption influenced the magnitude of the hemosiderin hepatic deposits. These findings corroborate the observations of Crissey *et al.* (8) for European starlings.

If we analyze just birds with more than 5 years in captivity, it is interesting to note a wide HHR variation among this subgroup of animals. The mean HHR for males (13.48%, $n = 8$), is more than 3 times higher than the HHR for females (4.28%, $n = 5$). Although no statistical significance was detected ($P = 0.0932$), it is reasonable to suggest an apparent male propensity to develop higher HHRs in captivity when compared to females. The reasons for this trend could

not be precisely identified, but we consider that physiological and/or behavioral aspects might be involved, as well as individual susceptibility. Further investigations are required to clarify this point.

Hemosiderosis is an important pathologic condition for several groups of birds, predisposing them to infections and neoplasms (14). Diets low in iron, between 32 and 48 ppm, are known to reduce the quantities of iron deposits in the liver. When associated with phlebotomy or the use of iron chelants, the decrease is more effective, and it may be applied to the treatment of susceptible birds (20).

The present data allow us to suggest that the menus and commercial rations for Psittacidae must be carefully revised because of their specific alimentary habits, avoiding generalizations for an order or family of birds, since their behavior and nutritional requirements vary notably. Such caution could improve the quality of life in captivity and, therefore, contribute to conservation of the species.

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