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CLINICAL PATHOLOGY AND PARASITOLOGIC EVALUATION OF FREE-LIVING NESTLINGS OF THE HYACINTH MACAW (*ANODORHYNCHUS HYACINTHINUS*)

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ABSTRACT: This study evaluated the health status and established hematologic and serum biochemistry parameters for free-living nestlings of the Hyacinth Macaw (*Anodorhynchus hyacinthinus*) from the Brazilian Pantanal (19°51′–19°58′S; 56°17′–56°24′W), for four consecutive years (from December 2003 through December 2006). Physical examinations indicated that all the birds were in good health. Endoparasites and blood parasites were not detected in any of the nestlings, and ectoparasites seemed to be limited to *Philornis* sp. (Diptera: Muscidae). Significantly higher levels of total white blood cells and heterophils, glucose, total protein, triglycerides, and phosphorus were observed in females. In females, higher cholesterol levels and packed cell volumes were observed in older birds, and total white blood cell and heterophil counts were higher in young animals. In males, uric acid levels were higher in older individuals. Wild Pantanal Hyacinth Macaws feed on only two species of palm nuts (*Acrocomia totai* and *Scheelea phalerta*). This limited food habit has a strong impact on population size and may alter the clinical pathology parameters of these birds. Therefore, knowledge of blood levels in normal individuals is essential to assess the physiologic and pathologic condition of wild macaws, to assess the effects of environmental changes on their health, and to contribute to conservation strategies of this endangered species.

Key words: *Anodorhynchus hyacinthinus*, clinical pathology, health status, hematology, nestlings, serum biochemistry.

INTRODUCTION

The Hyacinth Macaw (*Anodorhynchus hyacinthinus*) is the largest psittacine species in the world. It is currently considered endangered because the remaining populations are not only small but also are decreasing rapidly as a result of illegal trapping and deterioration or loss of their habitat (Galleti et al., 2002). The Pantanal wetlands of central Brazil, which are inhabited by the largest wild population of Hyacinth Macaws (Guedes, 2004), have gone through a process of habitat modification or destruction, largely due to human population pressure, cattle ranching, and deforestation (Guedes and Harper, 1995). This situation has increased research efforts related to the conservation and management of Pantanal wildlife species (Seixas and Mourão, 2002); the Projeto Arara Azul (Hyacinth Macaw Project), which is managed by the Instituto Arara Azul (Hyacinth Macaw Institute) and which began in 1990, is one of these projects. Although progress has been made in relation to the conservation and management of this species and to the knowledge of basic aspects of its biology (Guedes et al., 2006; Pizo et al., 2008), little is known about the health status of the Hyacinth Macaw in its natural habitat in Brazil.

Clinical pathology parameters are complementary veterinary tools for disease diagnosis and for the monitoring of the health condition of animals (Karesh et al., 1997; Lanzarot et al., 2001). Hematologic and biochemical reference ranges for wild birds are rare, due to the difficulty in

obtaining samples from wild animals (Masello and Quillfeldt, 2004). Although data for captive, seized, and captive-reared psittacines are available (Rosenthal et al., 2005; Valle et al., 2008), data on wild psittacines are scarce (Masello and Quillfeldt, 2004; Deem et al., 2005), and no information on free-living Hyacinth Macaws is available. Therefore, we established clinical pathology values and assessed the occurrence of parasitism in Hyacinth Macaw nestlings in their natural environment in the Brazilian Pantanal.

MATERIALS AND METHODS

This study was performed in the Miranda Pantanal subregion (19°51′–19°58′S; 56°17′–56°24′W), Mato Grosso do Sul, Brazil. It was approved by the local Ethics Committee of the Lutheran University of Brazil and by the Brazilian Institute of the Environment and Natural Renewable Resources.

Individual evaluations and blood samples were obtained for 91 free-living Hyacinth Macaw nestlings during four breeding seasons (December) from 2003 through 2006. Nests in trees were reached using climbing equipment, and the birds were carefully taken from the nest. Each bird underwent a physical examination as described by Samour (2000), and birds were weighed and blood samples (5 ml) were collected from the basilica (wing) vein. These procedures were performed by the same veterinarian (M. C. Allgayer), for quality assurance control (Bowerman et al., 2000). About 2 ml of blood was placed in anticoagulant (BD Vacutainer® K3 EDTA tubes, 7.5% solution, 0.068 ml, 5.1 mg), and 3 ml was transferred to tubes without anticoagulant. Immediately after collection, two blood smears from each nestling were made and air-dried. Feces were collected opportunistically from any bird that defecated during handling or before release ($n=51$) and were preserved in 10% buffered formalin for later analysis. After sample collection, all nestlings were tagged with open stainless-steel leg bands, and a microchip was implanted in the pectoral muscle. The birds were then carefully returned to the nest and subsequently monitored until they eventually left it.

Sex was determined through chromohelicase-DNA-binding gene amplification, by using blood samples (Miyaki et al., 1998). Positive male and female samples as well as negative controls (water) were used in each amplification reaction.

Fecal samples were examined 60 days after collection, at 100× total magnification, by using sodium chloride flotation and detergent sedimentation methods. Fecal flotation was performed by mixing 1 g of feces in a vial with sodium chloride (311 g of NaCl/1,000 ml of water); the mixture was sieved through a tea strainer to eliminate large debris. A coverslip was placed on top, and after 15 min the coverslip was transferred to a slide and examined. The sedimentation method was performed by mixing feces with a 0.5% soapy water solution. This mixture was placed in a 50-ml centrifuge tube, left to rest for 5 min, and then the solution was decanted. This process was repeated until the solution became clear and the entire sediment sample had been examined (Patton, 2000).

Hematologic analyses were done within 6 hr of sampling by the same veterinarian (M. C. Allgayer) according to standard hematologic methods. Packed cell volume (PCV) was determined by centrifugation in a microhematocrit centrifuge for 5 min at 10,000 × G. Total white blood cells (WBCs) were counted in blood in dilution fluid (1:100) in a Neubauer hemocytometer (Natt and Herrick, 1952).

Blood smears were fixed in 99% methanol, stained with Giemsa solution, and sent to the clinical pathology and parasitology laboratories of the Lutheran University of Brazil. Blood smears were examined under 1,000× magnification for the WBC differential count (in 200 cells; Fudge, 2000a). The entire blood smear was examined for blood parasites.

For serum biochemistry, blood samples were allowed to clot at room temperature for 1–2 hr and then centrifuged for 10 min at 3,000 × G. Serum aliquots were stored for 30 days at −20 C until analysis. Samples were assayed for: creatine kinase (CK), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), amylase, lipase, alkaline phosphatase (ALP), lactate dehydrogenase (LDH), cholesterol, triglycerides, total protein, glucose, uric acid, urea, magnesium, and phosphorus. All analyses were processed in a Bio TP-Analyser Basic semiautomated analyzer (Equipar, Curitiba, Paraná, Brazil). Although other parameters could be included in the biochemical panels, the amount of blood serum obtained from each nestling was insufficient to allow the estimation of all of these parameters. Therefore, the parameters analyzed were those recommended by Clubb et al. (1991) for health evaluation of birds.

Statistical analyses were performed with SPSS 10.0.5 (SPSS Inc., Chicago, Illinois,

USA). Comparisons between males and females and between age groups (25- to 80- and 81- to 107-day-old birds) were performed using the unpaired Student's *t* test. The age intervals were based on the information that the maximum weight is reached by nestlings at approximately 80 days of age. After that, the nestlings lose approximately 20% of their weight until they fledge, which is a critical physiologic event that may influence hematologic and biochemical parameters (Lanzarot et al., 2005). Bird weights at different ages were compared with literature information through one-way analysis of variance. Before the statistical tests, the samples were checked for normal distribution. Differences were regarded as significant at $P<0.05$.

RESULTS

Physical examination indicated that all the birds were healthy, in good condition, and no abnormalities were detected when the birds were in the nests (approximately 110 days, from hatching to fledging; Guedes and Harper, 1995). All nestlings fledged successfully; nestlings included 37 males and 54 females. Ages of nestlings on the day when blood samples were collected ranged from 25 days to 107 days of age and were estimated based on a reported fledging period of 110 days (Guedes and Harper, 1995). Weight measurements obtained from these nestlings varied from 734 g to 1,561 g, with a mean of 1,275.59±135.27 g. No difference was observed between males and females, and the data are in accordance with the growth rates reported by Abramson (1995).

No blood or fecal parasites were observed, but fat droplets were found in all fecal samples. Ectoparasites (larvae) were detected in three (3%) animals during 2003 and 2006, and scars suggesting the occurrence of parasitism were observed on the head and wings of six (6%) nestlings. The larvae were not collected for identification, but they were identified in the field as *Philornis* sp. (Diptera: Muscidae).

The hematologic parameters (mean± SD) for the nestlings are listed in Table 1. Significantly higher values for total WBCs ($P=0.01$) and heterophils ($P=0.01$) were observed in females. Total WBCs ($P=0.04$) and heterophils ($P=0.02$) were also significantly higher in the young females, and higher PCV ($P=0.04$) levels were observed in the older females. No difference was detected in any hematologic parameter in males of any age. No basophils were identified in blood smears, and polychromasia and anisocytosis were observed in the morphology of erythrocytes.

Biochemical values are presented in Table 2. Glucose ($P=0.04$), triglycerides ($P=0.02$), total protein ($P=0.02$), and phosphorus ($P=0.01$) levels were significantly higher in females. In females, higher amylase levels ($P=0.02$) were observed in young animals, and higher cholesterol levels ($P=0.02$) were observed in older nestlings. In males, uric acid levels were significantly higher ($P=0.03$) in older birds.

DISCUSSION

This is the first study of clinical pathology parameters for Hyacinth Macaw nestlings living in their natural environment. Although knowledge of the health status of endangered species is important for the development of conservation programs, no information for this species in the wild has been available. It is important to use healthy animals to establish reference values. Although Weber et al. (2002) stated that it is difficult to determine the health condition of free-living animals, all the birds studied here fledged successfully in the expected period, approximately 105–110 days, suggesting that they were healthy. This period was estimated through observation of nests with birds at different ages, from egg through fledging, every year (Guedes and Harper, 1995) for the past 19 yr.

The absence of blood and intestinal parasites in samples collected for four consecutive years leads to the conclusion

TABLE 1. Hematologic parameters (means±SD) in free-living Hyacinth Macaw nestlings according to sex and age.

Parameter[a]	Males			Females		
	25–80 day olds	81–107 day olds	Total[b]	25–80 day olds[c]	81–107 day olds[c]	Total[b]
PCV (%)	35.2±3.9 (5)[d]	36.2±7.2 (4)	35.6±5.2 (9)	26.7±5.8 (4)[c]	36.6±2.8 (5)[c]	33.2±6.6 (9)
WBCs ($\times 10^9$/l)	10.8±4.5 (11)	8.6±4.7 (12)	9.5±4.6 (23)[b]	20.0±8.8 (10)[c]	10.3±2.9 (10)[c]	15.2±8.1 (20)[b]
Heterophils ($\times 10^9$/l)	7.8±3.4 (11)	6.1±4.1 (12)	6.8±3.7 (23)[b]	15.6±6.5 (10)[c]	7.4±2.7 (10)[c]	11.7±6.5 (20)[b]
Heterophils (%)	70.8±8.1 (15)	69.6±9.9 (14)	70.1±8.7 (29)	74.7±9.0 (19)	65.4±13.7 (12)	71.5±11.7 (31)
Lymphocytes ($\times 10^9$/l)	2.8±1.4 (11)	2.7±1.4 (12)	2.7±1.3 (23)	4.2±2.4 (10)	3.1±0.8 (10)	3.7±1.9 (20)
Lymphocytes (%)	28.0±7.5 (15)	29.9±10.0 (14)	29.1±8.5 (29)	24.6±9.4 (19)	34.3±13.9 (12)	28.0±11.9 (31)
Monocytes ($\times 10^9$/l)	0.1±0.02 (11)	2.0±1.4 (12)	0.1±0.03 (23)	0.5±0.02 (10)	0.2±0.04 (10)	0.3±0.2 (20)
Monocytes (%)	1.4±0.5 (15)	2.0±1.4 (14)	1.6±0.8 (29)	1.4±0.5 (19)	1.3±0.5 (12)	1.3±0.5 (31)
Eosinophils ($\times 10^9$/l)	0.1±0.03 (11)	0.4±0.1 (12)	0.2±0.2 (23)	0.2±0.0 (10)	0.1±0.0 (10)	0.16±0.09 (20)
Eosinophils (%)	1.0±0.0 (15)	1.3±0.5 (14)	1.3±0.6 (29)	1.0±0.0 (19)	1.0±0.0 (12)	1.0±0.0 (31)

[a] PCV = packed cell volume; WBC = white blood cells.

[b] Significant differences between sexes.

[c] Significant differences between ages.

[d] Numbers in parentheses correspond to sample size.

TABLE 2. Serum biochemical parameters (means±SD) in free-living Hyacinth Macaw nestlings according to sex and age.

Parameter[a]	Males			Females		
	25–80 day olds	81–107 day olds	Total[b]	25–80 day olds[c]	81–107 day olds[c]	Total[b]
CK (U/l)	217.5±201.7 (11)[d]	214.8±173.2 (14)	221.9±183.6 (25)	147.6±172.0 (21)	229.9±160.6 (11)	175.9±170.2 (32)
AST (U/l)	34.5±20.5 (11)	39.7±24.2 (13)	37.4±22.7 (24)	32.0±15.3 (19)	34.1±21.6 (12)	32.8±17.7 (31)
ALP (U/l)	554.1±164.5 (12)	525.8±157.5 (13)	525.3±144.8 (25)	416.7±127.9 (21)	499.7±267.2 (15)	451.3±199.1 (36)
GGT (U/l)	8.2±3.3 (07)	5.6±3.4 (10)	6.7±3.6 (17)	7.8±4.8 (15)	6.4±4.4 (08)	7.2±4.6 (23)
Cholesterol (mmol/l)	3.1±0.9 (15)	3.0±1.2 (09)	3.0±1.0 (24)	2.8±0.5 (19)[c]	3.6±1.0 (13)[c]	3.1±0.9 (32)
Magnesium (mmol/l)	1.0±0.3 (06)	1.3±0.5 (06)	1.1±0.4 (12)	1.0±0.1 (03)	1.0± 0.3 (09)	1.0±0.3 (12)
Uric acid (mmol/l)	0.1±0.1 (12)[c]	0.3±1.1 (12)[d]	0.2±0.1 (24)	0.2±0.1 (21)	0.2±0.1 (15)	0.2±0.1 (36)
Urea (mmol/l)	3.9±1.2 (17)	3.7±0.9 (14)	3.9±1.0 (31)	4.3±1.3 (28)	4.0±1.2 (17)	4.2±1.2 (45)
Glucose (mmol/l)	9.9±1.6 (12)	9.4±1.3 (10)	9.7±1.2 (22)[b]	11.4±2.6 (15)	10.3±1.6 (11)	10.9±2.3 (26)[b]
Amylase (U/l)	364.9±70.3 (13)	368.1±87.3 (11)	363.7±78.2 (24)	429.7±139.4 (18)[c]	334.8±61.6 (13)[c]	389.9±121.6 (31)
Total protein (g/l)	21.4±2.5 (08)	18.6±5.0 (12)	19.8±4.4 (20)[b]	22.5±5.3 (13)	24.9±6.6 (12)	23.6±6.0 (25)[b]
Triglycerides (µmol/l)	1.1±0.2 (13)	0.9±0.3 (08)	1.0±0.3 (21)[b]	1.3±0.3 (19)	1.1±0.4 (13)	1.2±0.4 (32)[b]
Lipase (U/l)	35.3±31.6 (05)	46.7±45.4 (02)	35.6±31.0 (07)	27.8±12.9 (10)	36.4±24.5 (08)	31.6±18.8 (18)
LDH (U/l)	209.7±79.6 (05)	183.5±57.5 (06)	195.4±66.1 (11)	139.9±56.1 (09)	225.7±123.9 (04)	166.3±87.4 (13)
Phosphorus (mmol/l)	1.4±0.5 (07)	1.8±0.9 (09)	1.7±0.8 (16)[b]	2.9±1.2 (08)	2.6±1.5 (10)	2.7±1.4 (18)[b]

[a] CK = creatine kinase; AST = aspartate aminotransferase; ALP = alkaline phosphatase; GGT = γ-glutamyltransferase.

[b] Significant differences between sexes.

[c] Significant differences between ages.

[d] Numbers in parentheses correspond to sample size.

that there is a low prevalence of parasitism in macaw nestlings, in this ecosystem. Similar low levels of parasitism have been reported in other free-living South American psittacines (Bennett et al., 1991; Gilardi et al., 1995; Karesh et al., 1997; Rooney et al., 2001; Deem et al., 2005).

The fat drops observed in all the fecal samples analyzed are probably due to the nestlings' diet, which is based exclusively on the nuts of two species of palm, *Acrocomia totai* and *Scheelea phalerta*. These nuts contain more than 60% of ether extract level, and a digestibility level of approximately 68% (Guedes and Harper, 1995). In addition, according to Carciofi et al. (2002), Hyacinth Macaw nestlings have a digestive strategy characterized by low digestibility of ether extracts and energy and high digestibility of protein and minerals.

The larvae and scars detected in these birds did not seem to impair their growth, probably because of low infestation levels. Although the larvae were not morphologically or molecularly identified in this study, the field identification of *Philornis* sp. (Diptera: Muscidae) is consistent with previous reports of this parasite in this macaw population (Guedes and Harper, 1995; Guedes et al., 1999).

According to Bounous et al. (2000), hematologic determinations in free-living species can be influenced by physiologic effects of handling stress. In the present study, the nestlings were carefully managed to reduce handling time; however, although maximal care was taken in handling birds and collecting samples, we cannot exclude the possibility of stress influence on the present results.

The mean observed PCV value was within the range reported for captive juvenile macaws (*Ara* sp.; Clubb et al., 1991) and free-living juvenile Scarlet Macaws (*Ara macao*; Karesh et al., 1997). According to Lanzarot et al. (2005), PCV values are lower in young birds, which may be due to the adaptation to flight, when the need for oxygen is greatly increased. In this study, PCV values were lower in young females than in older females, but no differences were observed in males in terms of age. We cannot suggest a reasonable explanation for this result.

Variability in size and color of the cells is normal in peripheral avian erythrocytes. A slight alteration was observed in the Hyacinth Macaw nestlings studied here. According to Fudge (2000a), slight polychromasia and anisocytosis are considered normal in birds.

The mean total WBC count was higher in females than in males and higher in young females than in older females. Similar findings have not been reported previously in other birds and may be a characteristic of this species. According to Padilla et al. (2003), total and differential WBC counts vary widely in avian species, probably reflecting interspecies variability and differences in capture, restraint, and blood collection methodologies. An elevated total WBC count may be attributed to higher stress levels, social interactions, capture, and growth rate in younger animals (Marco et al., 1997).

Heterophils were the most numerous WBC in these macaws, as reported previously in free-living *Amazona aestiva* (Deem et al., 2005), captive *Amazona guildingii* (Deem et al., 2008), and captive *Ara* sp. (Harr et al., 2005). The levels of heterophils and eosinophils were within the range reported previously for juvenile and older macaws (Clubb et al., 1991; Harr et al., 2005). The lymphocyte levels obtained in our study were within the range reported for *Ara* sp. by Harr et al. (2005) but lower than those described for *A. macao* (Karesh et al., 1997). These differences could be due to age, stress, immune stimulation, or species particularities (Fudge, 2000b).

Basophils were not identified in the present study, suggesting the absence of inflammatory disease in the nestlings and confirming the good health of the animals analyzed. According to Montali (1988), the

exact function of basophils in birds is unknown, but they seem to participate in the initial phase of the acute inflammatory response. The monocyte levels found were within the range previously described in psittacines (Harr et al., 2005; Foldenauer et al., 2007) but lower than the values reported by Karesh et al. (1997) for juvenile macaws.

Serum biochemical values have been used in birds to investigate changes in nutritional state (Alonso-Alvarez and Ferrer, 2001), reproductive status (Merino and Barbosa, 1997), body condition (Masello and Quillfeldt, 2004), physical condition of nestlings (Villegas et al., 2002), and health status (Karesh et al., 1997).

Total proteins play an important role in transport of vitamins, hormones, enzymes, and electrolytes. Their concentrations in birds have been reported as being approximately half those observed in mammalian species (Fudge, 2000b). In the present study, the total protein contents were lower than those described for older psittacines (Fudge, 2000b). Karesch et al. (1997) analyzed Scarlet Macaws and also found lower levels of total protein in younger birds than in subadults. This decrease may occur due to the rapid growth and increase in muscle mass or due to protein deficiency in the diet during the nestling phase. Apart from this, according to Joyner et al. (1992), total protein levels increase with age in wild psittacines; this increase seems to be due to an increase in antibody concentration (Lanzarot et al., 2005).

In this study, glucose levels were lower than those reported in older and younger psittacines (Karesh et al., 1997; Fudge, 2000b). These low levels are not linked to glycolysis by erythrocytes, because, as opposed to mammals, glycolysis in blood samples stored for up to 2 hr is minimal for avian erythrocytes. The reason is that birds depend primarily on fatty acids as an energy source, rather than glucose (Lumeij, 1997). Therefore, the low glucose levels measured are either characteristic of this species or attributable to methodologic differences.

The cholesterol level found is within the range reported for a variety of psittacine species (Fudge, 2000b). Triglyceride concentrations were higher in females than in males, similar to those reported for free-living black stork (*Ciconia nigra*) nestlings (Lanzarot et al., 2005). Triglyceride levels have been reported to vary with climate, hormone influence, gender, and diet (Harr, 2002).

In this study, uric acid levels were within the range reported in other psittacine species, but urea concentrations were higher than the values reported for a variety of other psittacines (Altman et al., 1997; Valle et al., 2008). Uric acid levels in our study were higher in older males. These values may be associated with protein content in food, quantity of ingested food, amino acid requirements for protein synthesis, water consumption, and urine elimination (Costa et al., 1993). Urea levels are normally low in birds, increasing in dehydrated animals. The joint evaluation of urea and uric acid is useful to differentiate between dehydration and renal pathology (Harr, 2002). Although the present result could suggest some degree of dehydration of the animals, all nestlings were clinically examined before blood collection and no indication of low hydration was observed; therefore, it is possible that these differences may be due to different methods of urea analyses.

Inorganic phosphorus levels were within the interval reported in Scarlet Macaw nestlings, but magnesium concentrations were lower than the values observed in this species (Karesh et al., 1997). These differences could result from sample conservation or variations in laboratory methodologies used for the assays (Bounous et al., 2000).

Creatine kinase and LDH activities in Hyacinth Macaw nestlings were within the interval reported by Polo et al. (1998) and Fudge (2000b). However, AST levels were consistently lower and GGT levels were

higher in the present study than those described by Clubb et al. (1991) in juvenile macaws. γ-Glutamyltransferase is probably specific to biliary and renal epithelium in birds, but the clinical utility of GGT for the diagnosis of biliary diseases in birds has not been appropriately evaluated (Harr, 2002). Aspartate aminotransferase activity currently is considered to be a very sensitive but nonspecific indicator of hepatocellular disease in birds, and it also is frequently used with the muscle-specific enzyme CK to differentiate between liver and muscle damage. Because the animals were clinically healthy, the low levels of AST are probably due to laboratory differences or species specificity.

Alkaline phosphatase levels in our study were higher than those reported in adults of other avian species (Valle et al., 2008). Alkaline phosphatase is present in nearly all tissues and organs, in particular in liver and bone, and is associated with osteoblastic processes (Lumeij, 1997). The increase in serum ALP activity observed here is associated with nestling growth and may be due to increased osteoblastic activity in younger birds.

The amylase and lipase values are within the ranges reported previously in psittacines (Altman et al., 1997; Fudge, 2000b). The reason for the higher amylase levels observed in young females is unknown, because no indication of disease was observed. In mammalian species, amylase is associated with pancreatic disease, but data on this enzyme are limited for avian species (Deem et al., 2005).

This study on Hyacinth Macaw nestlings in their natural environment provides information that may be useful for the interpretation of laboratory findings in future conservation studies of this endangered species especially related to evaluating its health status under changing environmental conditions.

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