

# Immune-mediated hemolytic anemia in an eclectus parrot

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**Case Description**—A 2-year-old female Solomon Island eclectus parrot (*Eclectus roratus*) was evaluated by a veterinarian because of a 4-day history of progressive lethargy, weakness, poor appetite, and inactivity. The bird was referred to a veterinary teaching hospital for further examination.

**Clinical Findings**—Clinicopathologic analyses revealed that the parrot had marked regenerative anemia, autoagglutination, and biliverdinuria. Small, rounded RBCs (thought to be spherocytes) were detected in blood smears. The abnormal findings met the diagnostic criteria for dogs with primary immune-mediated hemolytic anemia. However, analyses of blood samples for lead and zinc concentrations and plasma bile acids concentrations; the use of PCR assays for *Chlamydophila psittaci*, psittacine circovirus 1 (causative agent of beak and feather disease), and polyomavirus; and microbial culture and Gram staining of feces did not reveal a cause for the hemolytic anemia.

**Treatment and Outcome**—Although administration of immunosuppressive doses of cyclosporine was initiated, there was a rapid progression of disease, which led to death of the parrot before this treatment could be continued long-term. Lack of an identifiable underlying disease (confirmed by complete histologic examinations at necropsy) supported the diagnosis of primary immune-mediated hemolytic anemia.

**Clinical Relevance**—Primary immune-mediated hemolytic anemia has not been widely reported in psittacine birds. A comprehensive evaluation and complete histologic examination of tissues to rule out underlying disease processes are required to definitively establish a diagnosis of primary immune-mediated hemolytic anemia in parrots. Primary immune-mediated hemolytic anemia should be considered as a differential diagnosis for regenerative anemia in a parrot. (*J Am Vet Med Assoc* 2007;230:1028–1031)

A 2-year-old female Solomon Island eclectus parrot (*Eclectus roratus*) was examined by a veterinarian because of a 4-day history of progressive lethargy, weakness, poor appetite, and inactivity. The parrot was domestically hatched and had been fed a diet of commercially available formulated pellets supplemented with vegetables and fruits since weaning. The bird was allowed out of its cage but was under direct supervision by the owner during those excursions. The bird was not allowed to interact with the other household parrot, a 4-year-old Congo African grey parrot (*Psittacus erithacus*). The owners reported no recent environmental changes and no prior health problems for either bird. Yearly wellness examinations included a CBC, plasma biochemical analyses, and cytologic examination of feces after Gram staining.

On physical examination by the referring veterinarian, the bird was weak and lethargic. Clinicopathologic analyses revealed that the bird's PCV was 17% (reference interval, 45% to 55%<sup>1</sup>); plasma aspartate aminotransferase activity was slightly high (418 U/L; reference interval, 144 to 339 U/L<sup>1</sup>), but plasma creatine kinase activity was within reference range (147 U/L;

## ABBREVIATION

IMHA Immune-mediated hemolytic anemia

reference interval, 132 to 410 U/L<sup>1</sup>). The referring veterinarian submitted blood samples for PCR analyses to detect polyomavirus and psittacine circovirus 1 (causative agent of beak and feather disease), the results of which were negative. Initial treatment consisted of 1 dose of iron dextran (0.1 mL/kg [0.05 mL/lb], IM) and a B-vitamin complex (0.2 mL/kg [0.1 mL/lb], IM). The parrot was immediately referred to the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania.

On physical examination at the hospital (day 1), the parrot had signs of mild depression and subjectively had weak grasp strength in both feet, delayed wing withdrawal reflex, pale conjunctiva, and a grade 2/6 heart murmur. The parrot voided bright green liquid urates consistent with biliverdinuria.

Blood (2.5 mL) was obtained from the awake bird by venipuncture of the right jugular vein for a CBC, reticulocyte count, plasma protein electrophoresis, and assessments of blood lead and zinc and plasma bile acids concentrations. Swabs of the choanal slit and cloaca were submitted for PCR analysis for *Chlamydophila psittaci*. Feces were obtained for microbial culture and cytologic examination after application of Gram stain.

The CBC revealed marked regenerative anemia (PCV, 17%); the relative reticulocyte percentage was 19.6% (reference interval, 2% to 5%<sup>2</sup>), and absolute

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reticulocyte count was 157,000 reticulocytes/ $\mu\text{L}$  (reference interval unavailable). The WBC count and differential yielded no abnormal results.<sup>1</sup> Evaluation of a blood smear revealed slightly decreased number of thrombocytes, many RBC precursors, and 2+ polychromasia (assessed subjectively by use of a scale of 0 [none] to 3+ [severe]). Marked agglutination of whole blood was evident during preparation of slides for the CBC, and a saline solution agglutination test was performed. This test involved diluting blood with sterile saline (0.9% NaCl) solution (3:1 dilution) in a tube containing EDTA. The tube was then centrifuged, and the remaining supernatant, including the plasma, was removed. The remaining cells were washed several times with saline solution, and the sample was then examined grossly and microscopically. Grossly, autoagglutination was still present; via microscopic examination, this was confirmed to be true autoagglutination and not rouleaux formation. The agglutination was subjectively graded as 3+ on a scale of 0 to 3+ (Figure 1).

Blood lead concentration was reported as  $< 0.05 \mu\text{g/g}$  (0.05 ppm); 0.05  $\mu\text{g/g}$  is the lowest limit of detectable lead concentration in blood for all species at the Pennsylvania State Veterinary Toxicology Laboratory. The blood zinc concentration was reported as 1.03  $\mu\text{g/g}$  (1.03 ppm), which was considered normal on the basis of findings from a study<sup>3</sup> in other psittacine birds; however, no specific reference limits have been determined for eclectus parrots. Results of the PCR assay for *C psittaci* were negative. Microbial culture and cytologic examination (after Gram staining) of feces revealed no abnormal bacterial gastrointestinal flora. The plasma bile acids concentration was mildly high (114  $\mu\text{mol/L}$ ; reference interval, 30 to 110  $\mu\text{mol/L}$ <sup>1</sup>). Plasma protein electrophoresis revealed a large  $\beta$ -globulin fraction (0.65 g/dL; upper reference limit,  $< 0.57 \text{ g/dL}$ <sup>4</sup>), which was consistent with acute inflammation or infection.

Following mask induction with 5% isoflurane in 100% oxygen, the bird was intubated and anesthesia was maintained with isoflurane. Radiographic views of the entire coelom (from the cranial aspect of the neck to tail) were obtained. During this anesthetic episode, bone marrow aspirates were collected from the sternum and the tibiotarsus by use of a 6-mL syringe and a

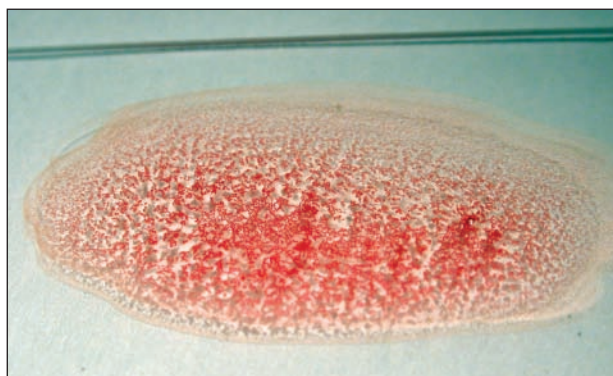


Figure 1—Photograph of the result of a slide autoagglutination test performed with blood of an eclectus parrot examined because of 4-day history of progressive lethargy, weakness, poor appetite, and inactivity. After repeated washing with sterile saline (0.9% NaCl) solution, the observed agglutination of RBCs is still present.

22-gauge needle with a calcium-EDTA-filled hub. The lateral radiographic view revealed a round soft tissue opacity in the midcoelom region, which was interpreted as possible splenomegaly, and a soft tissue opacity covering the ventral aspect of the typical triangular lucency in the region of the caudal air sacs. The ventrodorsal radiographic view revealed a slightly wide and asymmetric cardiohepatic silhouette. Cytologic examination of the bone marrow aspirates was performed by a board-certified clinical pathologist and revealed the presence of many hematopoietic cells. All cell lines were represented, and the myeloid-to-erythroid cell ratio was 1.4:1. The maturation sequences were considered complete, balanced, and morphologically unremarkable, and no causative reason for the marked anemia was determined.

During day 1, while diagnostic results were pending, the parrot received supportive care, including a balanced crystalloid solution<sup>a</sup> administered SC (25 mL/kg [11.4 mL/lb], q 12 h), and was kept in a padded cage that was heated to 29.4°C (85°F). The bird ate a small amount of carrots and bread without assistance, and enteral supplementation was therefore withheld. Additionally, pending the results of the microbial culture and cytologic examination of feces, treatment with enrofloxacin<sup>b</sup> was started (20 mg/kg [9 mg/lb], PO, q 24 h).

On day 2, the signs of depression had worsened although no other notable changes were detected via physical assessment. Another blood sample (0.25 mL) was collected, and a CBC revealed a further decrease in PCV (15%). Mild leukocytosis (WBC count, 16,300 WBCs/ $\mu\text{L}$ ; reference interval, 9 to 15,000 WBCs/ $\mu\text{L}$ <sup>1</sup>) was identified with heterophilia (heterophil count, 14,000 heterophils/ $\mu\text{L}$  [representing 86% of total WBCs; reference interval, 46% to 70%<sup>1</sup>]). Microscopic evaluation of a blood smear revealed a slightly low number of thrombocytes, many RBC precursors, 3+ polychromasia, 3 to 5 anucleated RBCs (erythroplastids)/hpf, and the presence of the occasional RBC with a less pronounced oval shape than a typical avian erythrocyte. A plasma biochemical profile revealed high activities of aspartate aminotransferase (545 U/L) and creatine kinase (2,817 U/L). The bird was anesthetized again. During this anesthetic episode, lateral and ventrodorsal radiography was performed 45 minutes after administration of 20 mL of 100% (wt/vol) barium sulfate/kg (9.1 mL/lb) via crop gavage. Findings indicated that barium had not reached the cloaca after 45 minutes, suggesting delayed gastrointestinal tract transit. The contrast radiographic views also revealed a slightly thick esophageal wall; mild coelomic effusion (as evidenced by separation of the serosal surfaces of the small intestinal loops); and a slightly large proventriculus, which was considered to be within normal limits for this species.

On day 2, treatment with a balanced electrolyte solution and enrofloxacin (as previously prescribed) was continued. Because the parrot was now completely anorexic, crop gavage feedings of a commercially available baby-bird hand-feeding formula<sup>c</sup> (10 mL/kg [4.5 mL/lb], q 8 h) were initiated. Additionally, because of the high suspicion that the bird may have IMHA, administration of cyclosporine<sup>d</sup> (3.5 mg/kg [1.6 mg/lb], PO, q 12 h) was started. By the end of the second day

of hospitalization, the bird's PCV had decreased to 11%, and it had become recumbent. An intraosseous cannula was placed into the left ulna, and an infusion of hemoglobin-based oxygen-carrying fluid<sup>e</sup> (10 mL/kg) was administered via this cannula once during a 2-hour period because a suitable blood donor was unavailable. Following this infusion, the bird was somewhat more responsive and could stand unaided but was still very weak and lethargic. On the morning of day 3, PCV was 9%. The parrot became markedly weaker, and signs of depression worsened drastically; the bird was once again recumbent. Four milliliters of fresh whole blood was collected from a healthy donor eclectus parrot, to which 0.4 mL of citrate-phosphate-dextrose-adenine (anticoagulant) was added. This mixture was administered to the parrot. Approximately 30 minutes following the blood transfusion, the bird collapsed into lateral recumbency and rolled backwards into an opisthotonus position; cardiopulmonary arrest ensued. Resuscitative efforts were unsuccessful.

A necropsy was performed under the guidance of a board-certified veterinary pathologist. On gross examination, moderate multifocal bruising of the integument over the tibiotarsus and keel bone was evident; these were sites used for collection of bone marrow aspirates. Bruising was also detected on the dorsomedial aspects of both thighs, possibly because of the SC administration of fluids. The entire body had a generalized yellow discoloration, similar to icterus in mammals, and moderate splenomegaly was identified. Histologic examination of specimens from all major organs revealed no notable abnormalities. The lack of pathologic findings of underlying disease in the necropsy examination of this parrot further supported the clinical diagnosis of primary IMHA.

## Discussion

The eclectus parrot of this report had primary problems of weakness, lethargy, and anemia. Results of diagnostic testing were compatible with a progressive, hemolytic, regenerative anemia, although no cause of the anemia could be determined. Hence, an initial clinical diagnosis of primary IMHA was made. The rapid progression of the anemia prevented any chance of long-term treatment, and the bird died 3 days after hospitalization (7 days after onset of clinical signs). A complete necropsy examination revealed no abnormalities that could have caused anemia, supporting the clinical diagnosis of primary IMHA.

Potential differential diagnoses for hemolytic anemia in birds include infection (chlamydophilosis, salmonellosis, colibacillosis, clostridiosis, mycoplasmosis, and infection with *Plasmodium* spp or other hemoparasites and viruses), bacterial septicemia, aflatoxicosis or other toxicoses (eg, lead, zinc, or crude oil), neoplasia, drug reaction, vaccine reaction, congenital abnormalities (eg, RBC fragility or enzyme deficiencies), vasculitis, disseminated intravascular coagulation, and primary IMHA.<sup>5-9</sup> Several of these differentials, such as the congenital abnormalities, have not been reported in birds and have been extrapolated from small animal data. Many other differentials, such as infection with *Salmo-*

*nella* spp, were extrapolated from findings of a study<sup>5</sup> in poultry because hemolytic anemia has not been widely reported in parrots. In the parrot of this report, results of the PCR assay for *C psittaci* were negative. The results of the microbial culture and cytologic examination (after Gram staining) of feces were unremarkable, and there was no evidence of enteric pathogenic bacteria. The bird's clinical signs and history were not consistent with either aflatoxicosis or mycoplasmosis. *Plasmodium* spp and other hemoparasites were not detected during microscopic examination of blood smears and were considered to be unlikely infective agents because the parrot was domestically hatched. Results of the PCR analyses for polyomavirus and psittacine circovirus 1 were negative. Blood concentrations of zinc and lead were not high, and there was no known exposure to crude oil. There was no evidence of neoplasia. The parrot had no history of receiving drug treatments or vaccinations. Currently, no tests exist to detect congenital RBC abnormalities in birds, unlike those available for use in small animals. In the parrot of this report, evidence of regenerative anemia with autoagglutination and lack of other detectable diseases led to a clinical diagnosis of primary IMHA.

The results of cytologic examination of bone marrow aspirates collected from the parrot were interesting because they were not representative of findings that are typically associated with a strong regenerative erythroid response in mammals (specifically an increase in erythroid precursors and a low myeloid-to-erythroid cell ratio). However, this can be explained by certain differences between avian and mammalian erythroid cell maturation. In birds, erythropoiesis occurs in the bone marrow primarily, although a large proportion of the maturation of the erythroid cell series may occur intravascularly, especially in birds undergoing an intense regenerative response. Erythroid cell maturation also occurs within bone marrow and other hematopoietic organs such as the liver and spleen.<sup>10</sup> The presence of intravascular erythroid cell maturation is supported by the results of the CBCs performed for the parrot of this report. In blood smears prepared from peripheral blood samples, many erythroid precursors were observed during microscopic examination. Additionally, the presence of many erythroplastids, or anucleate RBCs, detected in the second blood smear evaluation suggested ongoing damage to the erythrocytes,<sup>10</sup> most likely a result of immune-mediated attack. These erythroplastids may be the avian equivalent of mammalian spherocytes. Erythroplastids may be present in scant numbers in hematologic preparations from apparently healthy birds, but the number detected in blood from the parrot of this report was too great to be considered normal.<sup>10</sup>

Theoretical treatment options for parrots with primary IMHA include administration of corticosteroids, cyclosporine, and cyclophosphamide. Immunosuppressive agents have not been widely used in birds, and treatment options for primary IMHA must be extrapolated from small mammals. Of the various drugs used in small mammals for treatment of primary IMHA, corticosteroids, cyclosporine, and cyclophosphamide are the only drugs that have been used in birds, and even so, these drugs have not been widely used in the



pet-bird population.<sup>11-13</sup> Although corticosteroids have anti-inflammatory, antineoplastic, and immunosuppressive effects in birds as in mammals, the use of those drugs in birds has been controversial. Birds are highly susceptible to the adverse effects of corticosteroids. Reported common adverse effects of corticosteroids in parrots include gastrointestinal tract disturbance and ulceration, polyuria and polydipsia, hyperphagia, poor growth, hypercholesterolemia, lipemia, excess hepatic glucose deposition with increased risk of hepatic lipidosis, steroid-induced diabetes mellitus, iatrogenic hyperadrenocorticism, iatrogenic hypoadrenocorticism, immunosuppression, high plasma liver enzyme activities, and glucosuria.<sup>14-17</sup> The use of cyclosporine (including appropriate dosing regimens) has been studied primarily in research poultry.<sup>11-13,18</sup> Cyclosporine is a potent T-cell suppressor that interferes with antibody actions and macrophage function and may elicit more immediate effects than other immunosuppressive drugs. It is also not myelosuppressive, as are cyclophosphamide and azathioprine. Additional research would be needed to ascertain whether the potential benefit of corticosteroid administration in parrots with IMHA outweighs the risk of serious adverse effects. The decision not to use corticosteroids in the parrot of this report was based primarily on the known potential adverse effects and the authors' negative clinical experience with these drugs in parrots.

A previous case of presumed primary IMHA in a parrot was described by Jones et al<sup>19</sup>; a 17-month-old blue-crowned conure parrot (*Aratinga acuticaudata*) had a strongly regenerative anemia with predominance of round, small erythrocytes (which were suspected to be spherocytes), leukopenia followed by leukocytosis, high plasma protein concentration, biliverdinuria, and a markedly large spleen. Resolution of the anemia and biliverdinuria and restoration of erythrocyte morphology occurred after prednisolone treatment was administered and redeveloped after discontinuation of the prednisolone.<sup>19</sup> Although, to our knowledge, that appears to be the first reported case of possible primary IMHA in a psittacine, several diagnostic tests to completely support the diagnosis of primary IMHA were omitted. Tests that were not performed included a Coombs' test, assessment of blood lead and zinc concentrations, plasma protein electrophoresis, microbial culture of feces, and viral assays. Because infectious diseases are known to induce IMHA in mammals,<sup>20</sup> and because heavy metal toxicosis is known to cause hemolysis in birds,<sup>8,9</sup> omission of these tests from the diagnostic workup of that conure parrot leaves room for the possibility that an underlying condition triggered the hemolytic anemia. Presence or absence of autoagglutination was also not reported for that bird. Findings of such tests would definitively have supported the diagnosis of primary IMHA in that parrot.

On the basis of our experience with the bird of this report, we conclude that primary IMHA may develop in parrots and should be considered as a differential diagnosis in an anemic psittacine. Further studies are needed to evaluate the prevalence, clinical course, and

prognosis of primary IMHA in psittacines, as well as investigate additional treatment options.

- a. Normosol-R, Abbott Laboratories, Abbott Park, Ill.
- b. Baytril, Bayer Healthcare LLC, Shawnee Mission, Kan.
- c. Exact, Kaytee, Chilton, Wis.
- d. Neoral, Novartis Pharmaceuticals, East Hanover, NJ.
- e. Oxyglobin, Biopure Corp, Cambridge, Mass.

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