Presumed Immune-Mediated Hemolytic Anemia in a Blue-Crowned Conure (Aratinga acuticaudata)

Jeffery S. Jones, DVM, PhD, Jennifer S. Thomas, DVM, PhD, Dipl ACVP, Anne Bahr, DVM, MS, Dipl ACVR, and David N. Phalen, DVM, PhD, Dipl ABVP (Avian)

Abstract: Immune-mediated hemolytic anemia occurs infrequently in poultry and is undocumented in parrots. In this study, we describe a blue-crowned conure (Aratinga acuticaudata) with a strongly regenerative anemia, predominance of round, small erythrocytes (presumed spherocytes), leukopenia followed by leukocytosis, elevated plasma protein, biliverdinuria, and polyuria. Radiography and ultrasonography demonstrated a markedly enlarged spleen. After immunosuppressive treatment with prednisolone, the anemia, abnormal erythrocyte morphology, and biliverdinuria resolved but returned promptly after discontinuation of the therapy. We propose that this conure suffered from an immune-mediated hemolytic anemia.

Key words: hemolytic anemia, immune-mediated, spherocytosis, avian, blue-crowned conure, Aratinga acuticaudata

Introduction

Anemia results from blood loss, hemolysis, or depression of hematopoiesis. In humans, hemolytic anemias are caused by erythrocyte parasites, vascular disease, physical factors, drugs and toxins that damage the erythrocytes, congenital red blood cell (RBC) diseases, and antibodies directed at the RBC (immune-mediated hemolytic anemia [IMHA]). These antibodies may target host proteins that are cross-reactive to epitopes of infectious agents. They may target a drug or a toxin that, in combination with a native epitope, is recognized as foreign (haptinization), or the antibodies may be directed at the host’s own proteins in a true autoimmune disease. Documented causes of hemolytic anemias in birds include infections with Plasmodium species or Salmonella gallinarum, ingestion of crude oil, and heavy metal poisoning. No description of IMHA in a parrot or other companion bird has been reported previously. This blue-crowned conure (Aratinga acuticaudata) is the first documented case of an IMHA in a psittacine.

Case Report

A 17-month-old male blue-crowned conure was presented for anorexia, weight loss, lethargy, and irritability. The bird was housed with a cockatiel (Nymphicus hollandicus) that had been introduced to the household 4 months earlier. The cockatiel had been isolated from the conure until 3 weeks before presentation. A complete blood count obtained the previous week showed hematologic abnormalities that included anemia (packed cell volume [PCV] of 28%; reference range, 40–53%), with a marked increase in the number of polychromatophilic RBCs. The hemoglobin was low (7.4 g/dl; reference range 11–16 g/dl). The animal had a low normal white blood cell (WBC) count (5500 cells/μl; reference range, 5000–9000 cells/μl), was severely heteropenic (660 cells/μl; reference range, 3331–5772 cells/μl), and had a marked left shift (66% of the heterophils were bands; bands are not normally found in the blood of healthy birds). The albumin, aspartate aminotransferase, uric acid, glucose, calcium, and cholesterol concentrations were within normal reference ranges. The total plasma protein concentration (6.6 g/dl; reference range, 2.4–4.9 g/dl) was abnormally high. The albumin to globulin ratio was low (0.40; reference range, 0.55–1.5). A psittacosis elementary body agglutination assay (Texas Veterinary Diagnostic Laboratory, College Station, TX, USA) was negative.

While the history was being taken, the bird slept or sat quietly with ruffled feathers. There was an increased volume of liquid urine in the droppings and the urates were stained yellow (biliverdinuria). The bird weighed 140 g, a loss of 8 g from the
previous week, and had a slight to moderate atrophy of the pectoral muscle mass. The abdomen was doughy and slightly distended on palpation. At this time, the hematologic abnormalities included a PCV of 28%, with a marked increase in the number of polychromatophilic RBCs. The most prominent feature of the hemogram was that most of the RBCs were round (presumed spherocytes) instead of oval and were 47–72% (n = 20) of the average length (widest diameter) (n = 20) of the RBC from a healthy blue-crowned conure (Figs 1 and 2). The conure had a low normal WBC count (4270 cells/μl; reference range, 5000–9000 cells/μl), a severe heteropenia (770 cells/μl; reference range, 5000–9000 cells/μl), and a marked left shift (50% of the heterophils were bands). Rare atypical lymphocytes were observed. The total protein, as estimated on a refractometer, remained elevated (6.0 g/dl; reference range, 2.4–4.9 g/dl). Moderate numbers of gram-positive cocci and rods were observed on a Gram stain of a swab from the oropharynx. A moderate number of gram-positive rods and rare gram-negative rods were found on a cloacal swab.

Radiographs (Figs 3 and 4) revealed a large mass (2.2 cm in the cranial caudal dimension) in the caudal coelomic cavity displacing the bowel cranially and to the right. The liver was displaced cranially and ventrally, causing the liver to overlap the heart and in the ventrodorsal view. Ultrasonography (Fig 5) demonstrated a single uniformly echogenic mass in the caudal coelomic cavity (2.3 × 2.8 cm) with an echo texture similar to the spleen. During the ultrasound examination, the mass was aspirated, but cytologic examination revealed only blood.

The bird was anesthetized with isoflurane, a bone marrow aspirate was obtained from the tibiotarsal bone, and an exploratory coelomotomy was performed through a ventral midline incision. A grossly normal liver was seen, aspirated, and a biopsy was performed. The large coelomic mass was recognized radiographically and by ultrasound was found to be the spleen. It was also aspirated, and a biopsy was performed.

The bone marrow aspirate was moderately hemodiluted and contained hematopoietic precursors intermixed with mature erythrocytes. The hematopoietic precursor cells consisted of predominantly erythroid cells with very few myeloid cells. Erythrocyte cells were shifted markedly to the left, with increased numbers of rubriblasts and prorubriblasts. The erythroid line did complete maturation, progressing through rubriblasts to mature erythrocytes. Myeloid precursors were sparse. Normal-appearing mitotic figures were increased in numbers. There was no evidence of atypical or immature lymphocytes in the smears. Infectious agents were not observed.

In the liver aspirate, there were numerous rubriblasts, prorubriblasts, and rubriblasts similar to those described in the bone marrow. Although the series appeared to have a left-shift because of an increase in the immature forms, maturation appeared complete. Myeloid cells were rare, with occasional myelocytes, bands, and segmented cells. Plasma cells appeared more numerous. Normally-appearing hepatocytes were rare. Infectious agents were not observed.

The liver biopsy showed generalized mild to moderate hemosiderosis of the hepatocytes. There was a moderate increase in the number of sinusoidal Kupffer cells. Many contained bile pigments, hemosiderin, or both. There was also a mild diffuse
Figure 3. Right lateral radiograph of the blue-crowned conure described in Figure 2. A large mass (arrows), subsequently determined to be the spleen, is visible.

Figure 4. Ventrodorsal radiograph of the blue-crowned conure described in Figure 2. A large mass (arrows), subsequently determined to be the spleen, is visible.
plasmacytosis. The splenic biopsy contained only clotted blood.

Treatment with enrofloxacin (8 mg/kg PO q12h for 16 days; Baytril, Bayer, Shawnee Mission, KS, USA) was initiated because of the heteropenia and left shift. A presumptive diagnosis of IMHA was made on the basis of the hemogram, large spleen, and liver and bone marrow cytology results. Oral prednisolone syrup (3.3 mg/kg q8h; Ethex Corp, St Louis, MO, USA) was administered as the treatment. The prednisolone dose was determined by allometric scaling based on an immunosuppressive dosage of 2.2 mg/kg q24h in the dog.\textsuperscript{15} Because of continued weight loss, the animal was also gavage fed q8h (Exact, Kaytee, Chilton, WI, USA).

After medicating the patient for 5 days, imaging was repeated and the spleen was reduced (1.9 × 2.0 cm). The PCV had recovered to 40%, the WBC count had risen to 7422 cells/μl with 2672 heterophils/μl, and the total protein remained elevated at 5.6 g/dl. A left-shift was no longer present. There was a marked decrease in the number of small, round erythrocytes, and there was a continued prominent regenerative response. The urates were no longer yellow. The conure’s appetite had improved, and it was discharged to the owner’s care.

One week later, the conure presented for anorexia. The owner also reported difficulty in medicating the bird. It now weighed 124 g and had a PCV of 39% and a WBC count of 6000 cells/μl, with 1500 heterophils/μl and a total protein of 5.9 g/dl. Small, round erythrocytes were more common than on the previous blood smear. The bird was hospitalized for tube feeding and continuation of the enrofloxacin and prednisolone. Numerous budding yeasts were observed on a Gram stain of the crop. Nystatin (300 000 IU/kg PO q8h for 4 days; Alpharma, Baltimore, MD, USA) was added to the treatment regimen. After 4 more days of hospitalization, the bird was eating vigorously, was grooming, and had gained 5 g. The PCV was 44%, the WBC count was 18 769 cells/μl, the heterophil count was 4480 cells/μl, and the bird was developing a monocytosis (3566 cells/μl).

The bird was discharged after 5 days of hospitalization on prednisolone alone. After 40 days of prednisolone treatment, the PCV was 43% and the WBC was 31 100 cells/μl. The conure had a heterophilia, without a left shift (18 349 cells/μl), and a monocytosis (3421 cells/μl). Small, round erythrocytes were not observed. Prednisolone therapy was gradually decreased and finally discontinued during the next 2 weeks. The bird was rechecked at this point and was found to have yellow urates and
a PCV of 36%. Approximately 20% of the mature erythrocytes were small and round. The conure was less active. The owner declined further treatment. The bird's condition continued to deteriorate and the owner force-fed it at least once daily to maintain its weight. After 2 weeks of force feeding, the owner called and indicated that the bird may have aspirated food but appeared stable. One month later (5 months after initial presentation), it died.

Pertinent gross necropsy findings included emaciation and splenomegaly, with the spleen's widest dimension being 1.5 cm and its weight being 1.3 g. Multiple 1–3 mm yellow foci were clustered together within the parenchyma of the right lung. Histologically, the bone marrow was hyperplastic, with expansion of both the erythroid and myeloid lineages. There were increased numbers of Kupffer cells within the liver. Many Kupffer cells were laden with pale yellow-green bile pigment. The spleen contained decreased numbers of lymphocytes and a marked increase in histiocytes. Many of the histiocytes also contained pale yellow-green bile pigments. The nodules in the lung were granulomas that contained plant material.

Discussion

Anemia can result from blood loss, failure of erythrocyte production, or hemolysis. Blood loss in this bird was ruled out because there was no evidence of bleeding externally or through the respiratory or gastrointestinal tract while the bird was alive or at necropsy. Additionally, chronic blood loss would have been expected to result in a hypoproteinemia. This bird had a persistent hyperproteinemia. Had the primary source of this bird's problem been failure of erythrocyte production, the erythroid series within the bone marrow should have been hypoplastic. Instead, this bird was appropriately responding to the anemia with erythroid hyperplasia of the bone marrow and with extramedullary hematopoiesis. It was concluded from these observations that the cause of the anemia in this bird was hemolysis.

Only a few causes for hemolytic anemia have been documented in the bird; therefore, it was necessary to review the hemolytic diseases in humans to fully appreciate potential etiologies of hemolytic anemia. In humans, congenital defects may result in erythrocyte membrane defects, enzyme deficiencies, or unstable hemoglobin that make the erythrocyte prone to lysis. Blood parasites may directly invade erythrocytes and result in lysis. Chemicals, including drugs and toxins, can induce a hemolytic anemia by causing Heinz bodies. Clostridium perfringens releases an α-toxin that can cause a sudden, dramatic, and often lethal hemolytic crisis. Vasculitis, microthrombi formed during disseminated intravascular coagulation, and abnormal vasculature within neoplasms can all result in shearing forces that damage erythrocytes and result in subsequent lysis. An important cause of hemolysis in humans is an antibody that targets the RBC membrane.

Although this bird was relatively young (18 months of age), congenital disease resulting in the observed hemolytic anemia in this bird was unlikely. A congenital disease would have manifested earlier in life and would not have responded to immunosuppressive therapy. A toxin-associated anemia in this bird is also unlikely. Heinz body hemolytic anemia secondary to drug or toxin ingestion is well documented in humans. It has been documented to occur in seabirds that have ingested crude oil. But there was no history of toxin exposure or medication of this bird, and Heinz bodies were not observed.

The most common cause of hemolytic anemia in companion birds is heavy metal poisoning. Both lead and zinc can induce a mild to moderate, strongly regenerative anemia. Biliverdinuria or hemoglobinuria, in some species, often accompanies heavy metal poisoning in parrots. The signs and laboratory findings in this conure are not consistent with heavy metal toxicity. Central nervous systems signs are commonly observed in birds with heavy metal intoxication, but they did not occur in this bird. Also, a predominance of small, round erythrocytes has not been described in birds with heavy metal poisoning. Finally, heavy metal toxicity would not have been expected to respond to prednisolone therapy.

Plasmodium species are blood parasites with the potential to cause hemolytic anemia in birds. These infections, however, are rare to nonexistent in captive-raised parrots in North America, and these organisms were never seen in repeated blood smears from this bird or in its tissues. There was no evidence of a clostridial infection or osmotic stress in this bird clinically or on the postmortem examination.

In humans, immune-mediated, extravascular hemolytic anemia is characterized by an acute or chronic, mild to severe anemia, a prominent spherocytosis, hyperbilirubinemia, splenomegaly, and erythroid hyperplasia of the bone marrow. A polyclonal or monoclonal increase in the gamma globulins is also common. The definitive diagnosis of IMHA in humans requires the detection of antibodies or complement on the surface of erythrocytes.
mediated hemolytic anemia may also be a sequella to virus infections, including those resulting from the Epstein-Barr virus. Neoplasia, in particular lymphoproliferative disorders, may also induce IMHA.9

In birds, the only disease resembling IMHA was described in chickens infected with Salmonella gallinarum, and it was speculated that IMHA occurred in chickens infected with other members of the Enterobacteriaceae. These birds were prone to severe extravascular hemolytic anemia that was characterized by biliverdinuria, marked reticulocytosis, extramedullary erythropoiesis in spleen and liver, erythroid hyperplasia of the bone marrow, splenomegaly, and erythropagocytosis and siderosis within the spleen. Immune-mediated hemolytic anemia was suspected in these birds, because at the onset of anemia they were found to be positive to both the indirect and direct Coombs test.11 In this case, enteritis was never observed and only low numbers of gram-negative bacteria were present in the cloacal Gram stain. But we cannot rule out possible intestinal colonization with Salmonella species or other Enterobacteriaceae because a fecal culture was not performed.

The cause of this suspected IMHA may relate to its transient heteropenia and myelosuppression of the bone marrow. In humans, neutropenia is most often secondary to viral infections, administration of drugs that are toxic to neutrophils, an overwhelming bacterial infection with sepsis, and rarely immune-mediated disease.17 Immune-mediated neutropenia has occurred concurrently with IMHA, but only rarely. In this condition, the bone marrow, instead of being leukopenic, exhibits a marked myeloid proliferation.17 An overwhelming bacterial infection would have been expected to kill this bird in a few hours to days. The patient could not have survived a week without antibiotic therapy. Given that there was no history of drug administration, a drug-induced suppression of heterophil production was also unlikely.

The recent introduction of a cockatiel into the conure’s environment suggests possible exposure to a viral pathogen. Experimental infections with a herpesvirus causing Pacheco disease has been shown to cause a severe neutropenia.18 Unfortunately, at the time of presentation, an assay capable of consistently detecting this virus in the live bird was not available, and serology for this infection was not performed.

The drug of choice for treatment of IMHA is prednisolone at immunosuppressive dosages.15,19 Certain etiologies of IMHA are highly responsive to prednisolone, whereas others are not. In refractory cases of IMHA, other immunosuppressive
drugs including azothiaprim, cyclophosphamide, danazol, or cyclosporine A can be substituted for, or used in combination with, prednisolone.15,19 Even when multiple immunosuppressive drugs are used in combination, some cases of IMHA do not respond to treatment. These options would have been explored if the owner had consented to additional treatment. The dose of prednisolone was determined by metabolic scaling. Treatment regimens, although necessary, are not without possible undesirable side effects. The risks of treatment with these drugs must be weighed against their benefits.

In conclusion, we described a possible extravascular hemolytic anemia in a blue-crowned conure. The clinical pathologic, imaging, and necropsy findings strongly suggest that this conure represents the first documented case description of IMHA in a psittacine bird. Clinicians should consider IMHA as a differential for birds with anemia, especially if erythrocytes resembling spherocytes and an otherwise unexplained splenomegaly are present. Several diagnostic tests (eg, direct and indirect Coombs tests, plasma electrophoresis, osmotic fragility test, and specific assays for viruses) were not performed with this conure. However, they should be considered when psittacine birds present with anemia and splenomegaly.

References