Immune-mediated pure red cell aplasia in a domestic ferret

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Case Description—An 8-month-old spayed female domestic ferret (Mustela putorius furo) was referred for examination to determine the cause of lethargy and severe anemia.

Clinical Findings—Initial examination revealed that the ferret was lethargic but with appropriate mentation. The only other abnormal findings were severe pallor of the mucous membranes, nasal planum, and skin and a PCV of 8%. Pure red cell aplasia (PRCA) was diagnosed on the basis of cytologic evaluation of a bone marrow biopsy specimen.

Treatment and Outcome—Medical treatment included blood transfusions, IM administration of iron dextran, oral administration of antimicrobials and gastrointestinal tract protectants, and SC administration of erythropoietin. Once PRCA was diagnosed, the ferret was orally administered prednisone, cyclosporine, and azathioprine. Nine months after onset of treatment, the PRCA was in remission and the ferret was doing well. Immunosuppressive treatment was discontinued at 14 months after onset of treatment, and 36 months after initial examination, the ferret appeared to be healthy.

Clinical Relevance—It is important that PRCA be considered as a differential diagnosis for a ferret with severe anemia. Prolonged immunosuppressive treatment was successful in the ferret described here. (J Am Vet Med Assoc 2010;237:695–700)

A 0.635-kg (1.4-lb) 8-month-old spayed female domestic ferret (Mustela putorius furo) was referred on an emergency basis to the William R. Pritchard Veterinary Medical Teaching Hospital at the University of California-Davis because of severe anemia. The ferret was purchased at approximately 2 months of age, and it had been vaccinated against distemper and rabies; however, the specific vaccines and the manufacturers of those products were not known. The owners had orally administered milbemycin oxime and fenbendazole to the ferret monthly as heartworm preventatives since the time of purchase.

The owner reported that the ferret had been lethargic and inappetent for the preceding 2 weeks. No other clinical abnormalities had been observed, and there was no history of tick infestation, trauma, or toxin exposure. To the owner’s knowledge, no other medications had been given to the ferret. Results of a CBC performed by the referring veterinarian were consistent with severe anemia (PCV, 3%; reference interval, 43% to 55%; and total WBC count, 3,000 cells/µL) and lymphocytosis (lymphocytes, 70%; reference interval, 43% to 55%; and total WBC count, 3,000 cells/µL [absolute cell count was not reported]).

Physical examination performed at our facility on the day of admission (day 0) revealed that the ferret was quiet but had appropriate mentation. The only abnormality detected was severe pallor of the mucous membranes, nasal planum, and skin. Results of palpation of the abdomen, including the spleen, were unremarkable. The preliminary list of differential diagnoses for anemia in this ferret included gastrointestinal tract bleeding and estrogen toxicosis attributable to remnant ovarian tissue.1,2 Given the signalment, history, and clinical signs in this ferret, other differential diagnoses that were included on the list but considered less likely were infectious diseases, coagulopathies, immune-mediated anemia, hyperestrogenism attributable to hyperadrenocorticism, toxin exposure, renal failure, neoplasia, and anemia of chronic disease.3

The ferret was hospitalized for further diagnostic testing and treatment. The PCV was 8%. The owner provided another ferret to serve as a donor for a blood transfusion. Results of a complete physical examination performed on the donor ferret as well as results of a CBC and biochemical analysis for the donor ferret were all within reference intervals; thus, the donor was used for blood collection. Ferrets are not known to have discernable blood types,4 and transfusion reactions are extremely rare in ferrets; thus, crossmatching was not performed prior to the transfusion. The donor ferret was deeply sedated, and 14 mL of whole blood
was collected from the cranial vena cava. The whole blood was mixed with an anticoagulant (1 part citrate dextrose to 9 parts whole blood). The recipient ferret did not require sedation for the transfusion, and the anticoagulated blood was administered via a cephalic vein and in accordance with standard transfusion protocols for use in small animals. The PCV was 19% after the transfusion.

Once the ferret was stabilized, additional diagnostic testing was performed. Whole-body radiography and abdominal ultrasonography were performed, but no ovarian remnant or other abnormalities were identified. A fecal occult blood test¹ yielded positive results. The fecal occult test also was performed on a sample of the ferret’s diet to rule out a false-positive result; results for the diet were negative. Examination of a fecal sample for parasites yielded negative results. Blood samples were not collected for a CBC, biochemical analysis, or analysis of plasma concentrations of sex hormones at this time because of the severe anemia in the ferret.

Initial supportive treatment included administration of amoxicillin-clavulanate (25 mg/kg [11.36 mg/lb], PO, q 12 h) as a broad-spectrum antimicrobial, administration of famotidine (0.5 mg/kg [0.23 mg/lb], SC, q 12 h) and sucralfate (100 mg/kg [45.45 mg/lb], PO, q 12 h) for possible gastric bleeding, IV administration of fluids, and provision of nutritional support. A single injection of human chorionic gonadotropin (60 U, IM) was given as treatment for potential hyperestrogenism associated with a presumptive ovarian remnant. The following day (day 1), the ferret was more alert and active, and the supportive care was continued. However, on day 4, the ferret’s condition worsened, and the ferret again appeared weak and pale. A blood sample was collected at that time for a CBC and biochemical analysis. All biochemical values were within reference intervals.² Severe nonregenerative anemia (PCV, 10%; reticulocyte count, 0 cells/µL) was identified. Total WBC and platelet counts were within reference intervals.³ A second blood transfusion was performed (18 mL of whole blood obtained from an in-clinic donor ferret). A complete physical examination, CBC, and biochemical analysis also were performed after the transfusion. The PCV was 23% after the second transfusion.

Two days later (day 6), the ferret was anesthetized, and aspirates and biopsy specimens of bone marrow were collected by use of standard small animal approaches from the trochanteric fossa of the femur and the proximal portion of the humerus. Postoperative analgesia was provided via IM administration of buprenorphine (0.03 mg/kg [0.014 mg/lb]). Cytologic evaluation of an impression smear of the bone marrow biopsy specimen revealed a hypercellular sample. An adequate number of both mature and immature megakaryocytes were observed. Cells of the myeloid series were present and appropriately distributed from myeloid stem cells through segmented neutrophils. Cells of the erythroid series were present, but there appeared to be a left shift, with greater numbers of rubriblasts, prorubricytes, and basophilic rubricytes. Only scattered polychromatophilic rubricytes and megaloblasts were detected, with rare polychromatophilic erythrocytes. Increased numbers of small, well-differentiated lymphocytes were found, comprising 44% of the nucleated cells counted (reference interval, < 15% of the total nucleated cell count).⁴ A few plasma cells and macrophages were also observed. These findings indicated an arrest of erythroid maturation, which can be evident with preregenerative anemias or destruction of cells of the later stages. Given that the duration of the clinical signs was greater than the time expected to generate a regenerative response (ie, 3 to 5 days), these findings were more supportive of a selective destruction of erythroid cells in the later stages of maturation and a lack of regeneration. The myeloid-to-erythroid ratio was 4.6:1, as determined by a differential count of 500 cells; reported values for the myeloid-to-erythroid ratio for ferrets range from 1.3 to 3.1.⁵

These findings indicated a severe erythroid hypoplasia, given the associated severe peripheral anemia. Histologic findings for the biopsy specimens were consistent with the cytologic findings. Immunohistochemical analysis by use of a CD3 marker for T cells and a CD20 marker for B cells revealed that most of the lymphoid cells in the bone marrow were mature and of T-cell lineage. Arrest in the maturation of the erythroid cell line was diagnosed, and an immune-mediated reaction was suspected.

Endoscopic examination of the esophagus, stomach, and proximal portion of the duodenum was performed, and no mucosal abnormalities were identified. Gastric biopsy specimens were obtained, and histologic examination revealed no abnormalities. Silver staining and PCR assay of gastric tissues for Helicobacter spp yielded negative results. A blood sample was submitted for evaluation of total iron binding capacity and total iron concentration, and results (total iron-binding capacity, 481 µg/dL; iron concentration, 423 µg/dL) were within canine and feline reference intervals for our hospital (to our knowledge, reference intervals for ferrets have not been published). Despite these results, an injection of iron (10 mg/kg [4.5 mg/lb] IM) and an injection of vitamin B complex (2 mg/kg [0.9 mg/lb] IM) were administered as part of the supportive care.

On the basis of the results of diagnostic tests obtained at this time, a tentative diagnosis of PRCA was made. Estrogen toxicosis appeared unlikely because of a lack of pancytopenia, a lack of outward clinical signs (such as vulvar enlargement and alopecia), and a lack of remnant ovarian tissue identified during ultrasonography.⁶ A slide agglutination test was performed, and red cell agglutination was not detected. A RBC antiglobulin test (ie, Coombs’ test) was not performed because it was not available for ferrets. A plasma antinuclear antibody test yielded negative results.

Pure red cell aplasia can be congenital or acquired.⁷ Acquired autoimmune-mediated mechanisms are thought to be the main process for PRCA in cats, dogs, and humans.⁸ Parvoviruses have been postulated as a cause for this disease in dogs and humans, and FeLV subgroup C can cause PRCA in cats.⁹ Acute infectious parvovirus can affect domestic ferrets; thus, an in situ PCR assay was performed on the bone marrow biopsy specimen in an effort to detect evidence of this virus in this ferret. The PCR assay yielded negative results. Cells were cultivated from the bone marrow biopsy specimen and cultured. Cells were evaluated by use of electron mi-
crosomy for evidence of retroviruses. However, evaluation by use of TEM yielded negative results, and use of DEM yielded inconclusive results. During DEM of the bone marrow cell culture, a few particles with osmotic tails were identified that could have been associated with selected retroviruses, bunyaviruses, or togaviruses. However, the pleomorphic and infrequent nature of these particles did not allow for definitive diagnosis of viral infection in the bone marrow cells. A tick-borne disease antibody panel was performed in an effort to rule out exposure to rickettsial diseases that might have been a cause of the anemia in this ferret. Evaluation of plasma samples for antibodies against *Ehrlichia canis, Ehrlichia equi, Babesia canis,* and *Rickettsia rickettsii* revealed that the ferret was seronegative for each of these rickettsial diseases.

On day 8, prednisone (2 mg/kg, PO, q 12 h) was added to the treatment protocol because of its immunosuppressive effects; amoxicillin-clavulanate, famotidine, and sucralfate were discontinued; and administration of omeprazole (0.7 mg/kg [0.32 mg/lb], PO, q 24 h for 10 days) was initiated. On day 10, the ferret had an increase in activity and alertness. The PCV was 28%, but there was still no evidence of regeneration (reticulocyte count, 0 cells/µL). The ferret was discharged to the owner with instructions for the owner to continue administering the medications until a follow-up examination was performed in approximately 2 weeks.

On day 23, the ferret was readmitted to our hospital because of lethargy; severe nonregenerative anemia was again identified (PCV, 11.4%; reticulocyte count, 0 cells/µL). Cyclosporine* (4 mg/kg [1.8 mg/lb], PO, q 12 h) was administered as a second immunosuppressive drug. Two weeks later, the PCV was 23%, and a regenerative response was identified for the first time (reticulocyte count, 315,000 cells/µL). Additional follow-up examinations were recommended at weekly intervals to perform CBCs and reticulocyte counts and to measure trough plasma concentrations of cyclosporine (Figures 1 and 2).

The ferret was reported to be doing well at home until an examination on day 86 again revealed severe nonregenerative anemia (PCV, 8%) with marginal reticulocytosis (reticulocyte count, 6,960 cells/µL). A third blood transfusion was performed (30 mL; 10 mL of blood from each of 3 ferrets identified by the owner of the ill ferret). Each of the donor ferrets was evaluated and sedated before blood collection as previously described; blood also was collected as previously described. The PCV was 28% after the third transfusion.

The trough plasma concentration of cyclosporine had decreased during the 2 weeks preceding the third blood transfusion on day 86, and there was concern as to whether the cyclosporine had been correctly stored or administered. Nonetheless, azathioprine* (1 mg/kg [0.45 mg/lb], PO, q 24 h for 7 days) and erythropoietin* (40 U, SC, q 48 h for 3 injections) were administered. The cyclosporine dosage was increased (6 mg/kg [2.73 mg/lb], PO, q 12 h). Five days later (day 91), the PCV had increased, with major evidence of regeneration (PCV, 37%; reticulocyte count, 1,522,500 cells/µL). In addition, the trough plasma concentration of cyclosporine had increased substantially (1,200 ng/mL).

Analysis of plasma concentrations of sex hormones that included androstenedione, estradiol, and 17-hydroxyprogesterone was first performed on day 43, and only a mild increase in the plasma concentration of estradiol was identified (182 pmol/L; reference interval, 30 to 180 pmol/L). Concentrations of sex hormones were reevaluated on days 98, 163, 259, and 328, and the plasma estradiol concentration on those days was 186, 152, 122, and 137 pmol/L, respectively. Concentrations of androstenedione remained within the reference interval on all 5 of those days (3.49, 3.80, 0.35, 5.20, and 3.10 nmol/L, respectively; reference interval, 0 to 15 nmol/L). The concentration of 17-hydroxyprogesterone was within the reference interval on days 43, 98, 163, and 328 (0.12, 0, 0, and 0.15 nmol/L, respectively; reference interval, 0 to 0.8 nmol/L) but was substantially increased (12.6 nmol/L) on day 239.

Abdominal ultrasonography was performed on days 43, 98, 163, 239, and 328, and no evidence of an
ovarian remnant or enlarged adrenal glands was identified. It was recommended that the referring veterinarian perform follow-up evaluation of sex hormone concentrations and ultrasonography to determine whether the ferret had hyperadrenocorticism.

The PCV of the ferret ranged from 35% to 43.5% for the 12 months after its last anemic crisis, and administration of cyclosporine and prednisone was continued for immunosuppression throughout that time. Results of CBCs and trough plasma concentrations of cyclosporine were also evaluated during that time (Figures 1 and 2). Trough plasma concentrations of cyclosporine fluctuated (range, 96 to 1,200 ng/mL). Values for plasma biochemical analysis remained within reference intervals, except for an intermittent mild increase in alanine transaminase activity. Repeated abdominal ultrasonographic examinations did not reveal evidence of abnormal liver architecture. The increase in alanine transaminase activity was assumed to be associated with the transient effect of corticosteroid and cyclosporine use and was not further evaluated. Amoxicillin-clavulanate was prescribed at the time of each hemolytic crisis as a preventative measure for possible infection attributable to overt immunosuppression. Sucralfate administration as a gastric protectant was continued for several months and was discontinued as the prednisone dose was tapered gradually to 0.5 mg/kg, PO, every 12 hours. Administration of prednisone and cyclosporine was discontinued at 14 months after initial admission of the ferret, at which time the ferret appeared to be in complete remission. At 15 months after initial admission (ie, 30 days after the cessation of all drugs), the PCV was 37.1%.

At 36 months after initial admission (ie, 21 months after the cessation of all drugs), the ferret was reported to be doing well at home, and there were no clinical signs of recurrent anemia or hyperadrenocorticism. The nonregenerative anemia appeared to have been managed by use of immunosuppressive treatment, which supported the diagnosis of immune-mediated PRCA in this ferret.

**Discussion**

The juvenile spayed female ferret described here had presumed PRCA that responded to immunosuppressive treatment. Pure red cell aplasia is a hematologic disorder characterized by a marked decrease in concentration of erythroid precursors in the bone marrow in the face of normal granulopoiesis and thrombopoiesis, which typically results in severe nonregenerative anemia. The disorder has been described in dogs, cats, and humans, but to our knowledge, this is the first report of PRCA in a domestic ferret. Examination of bone marrow specimens usually reveals arrest in maturation at some stage of the erythroid precursors; if left untreated, erythroid precursors may be completely absent.

Pure red cell aplasia can be congenital or acquired. A congenital form of the disease has been described in humans and in a dog. In addition, PRCA can be caused by other conditions, such as viral and retroviral infections and drug toxicity. Parvoviruses have also been postulated as a cause for this disease in dogs and humans, and FeLV subgroup C can cause PRCA in cats.

In situ hybridization PCR assay of the bone marrow biopsy specimen obtained from this ferret yielded negative results for Aleutian disease virus, a parvovirus that affects mink and ferrets. Retroviruses have not been specifically described in ferrets, but it has been suggested that certain diseases in ferrets may be associated with retroviruses. The osmotic tail particles that were detected in the bone marrow cell culture for this ferret can be associated with some viruses, but they were too pleomorphic and infrequent to confirm a viral cause in this case. Treatment with recombinant human erythropoietin or other specific drugs and exposure to some toxins are all potential causes for PRCA.

In humans, pregnancy has also been associated with immune-mediated PRCA. Acquired autoimmune-mediated mechanisms are thought to be the main process for PRCA in cats, dogs, and humans because antibodies destroy erythroid precursors in the bone marrow.

Antinuclear antibody titers were evaluated in this ferret, and results were negative; it was the only screening test available (in addition to the slide agglutination test) that could be used to assess autoimmune hemolytic diseases in ferrets. In addition, PRCA has also been associated with neoplasia, such as thymoma or leukemia. However, no clinical evidence for autoimmune disease or neoplasia was found in the ferret described here. Therefore, the diagnosis of PRCA was inferred on the basis of exclusion of other diseases; however, it is possible that some other disease process not detected during the diagnostic evaluation could have been the inciting cause for the severe nonregenerative anemia in this ferret.

Anemia is a common clinical finding in ferrets and is a manifestation of many disease processes. Therefore, PRCA should be differentiated from aplastic anemia and immune-mediated hemolytic anemia. Clinical signs of disease in the juvenile spayed female ferret reported here differed from those described in ferrets with estrogen-induced aplastic anemia. Aplastic anemia in ferrets is usually a result of hyperestrogenism in sexually intact females that fail to ovulate, but this is no longer common in the United States because almost all female ferrets are spayed when they are juveniles. However, aplastic anemia can develop in spayed female ferrets that have an active ovarian remnant. Aplastic anemia has been described in adult female ferrets and is most commonly associated with severe pancytopenia. Clinical findings include petechia and ecchymosis in subcutaneous tissues, alopecia, a large vulva, and an increase in sexual behavior. Interestingly, hyperestrogenism caused by hyperadrenocorticism in ferrets does not commonly result in clinical signs such as pancytopenia, petechia, and ecchymosis, which are commonly identified with estrogen toxicity. Immune-mediated hemolytic anemia is typically characterized by a regenerative anemia represented by appropriate reticulocytosis or nonregenerative anemia with a hypererythroid bone marrow. To our knowledge, immune-mediated hemolytic anemia has not been described in ferrets.

Mild increases in the estrogen concentration were identified on the plasma sex hormone panel, and it is
intriguing that the estradiol concentration was at the high end of the reference interval in a young spayed ferret. Repeated measurement of hormone concentrations was performed because there was reason to suspect increased hormonal activity that could have been the result of an ovarian remnant or hyperestrogenism associated with hyperadrenocorticism. The mild increase in estradiol concentration was not identified until the anemia was already in remission, so the importance of the potential hormonal disturbances in relation to the initial onset of PRCA in this ferret is unknown and likely not clinically relevant. However, the PCV of this ferret did not completely return to the reference interval. It is possible that this ferret had other underlying disease conditions, including hyperadrenocorticism, that could have caused anemia, but those conditions were not clinically apparent and were not identified during multiple examinations. Remnant ovarian tissue was not identified during repeated ultrasonographic examinations, but failure to observe remnant ovarian tissue during ultrasonography is not sufficient to rule out this possibility. No evidence of abnormal morphology of the adrenal glands was identified during multiple ultrasonographic examinations performed at the times at which concentrations of sex hormones were evaluated. Hyperadrenocorticism was strongly suspected by day 259 (a time at which the 17-hydroxyprogesterone concentration increased substantially); however, the importance of this with regard to the original onset of PRCA in this ferret could not be ascertained because the ferret was already in remission by the time the increase in 17-hydroxyprogesterone concentration was detected. No other clinical signs such as vulvar enlargement or alopecia commonly associated with estrogen toxicity or hyperadrenocorticism in ferrets were identified at any time in the ferret described here. Interestingly, 1 form of immune-mediated PRCA in humans is associated with pregnancy.21 The pathogenesis of PRCA during pregnancy in humans is largely unknown. It has been suggested that hormonal disturbances can cause anemia through direct effects on red cell precursors or by induction of autoimmunity.

In the ferret described here, PRCA responded to combined and prolonged immunosuppressive treatment with prednisone and cyclosporine.26,27 Other options for the treatment of PRCA include cyclophosphamide and antilymphocyte globulin. Cyclophosphamide has been used for treatment of PRCA in human and veterinary medicine,10,13,28 and antilymphocyte globulin has been used in human patients.6,26 Cyclosporine was chosen for the ferret of the present report because of its immunosuppressive properties and the relatively rapid response to this drug in other species, which can be crucial for a successful outcome in patients with severe anemia.20,30 Azathioprine also was administered but for <1 week, and it probably did not play a role in the response to treatment because it requires several weeks for the immunosuppressive effects of azathioprine to become evident in other species; thus, it is not commonly prescribed for treatment of PRCA in other species.8,31 When the ferret relapsed at day 86 (PCV, 8%), erythropoietin was administered (3 doses). Administration of erythropoietin may have been associated with the marked release of reticulocytes into the circulation that was evident 5 days after initiation of this treatment (Figure 2). However, the regenerative response could also have been associated with the increased trough plasma concentration of cyclosporine that was detected on day 86, which may have been involved in suppressing immune-mediated destruction of erythroid precursors. In other species, erythropoietin treatment generally is effective only after administration for several weeks, and its usefulness in patients with physiologically normal renal function is questionable. Furthermore, administration of recombinant erythropoietin is listed as a possible cause for PRCA in other species,6,32,33 thus, the use of erythropoietin to treat the anemic crisis in the ferret described here was of dubious benefit.

On the basis of the results of diagnostic testing and the response to immunosuppressive treatment, we believe that the PRCA identified in the ferret described here was induced by immune-mediated mechanisms. To our knowledge, this is the first report of PRCA in a domestic ferret. Further studies are needed to understand the pharmacological effects of immunosuppressive drugs (such as cyclosporine) in ferrets.

References
From this month’s AJVR

Physiologic effects of nasopharyngeal administration of supplemental oxygen at various flow rates in healthy neonatal foals

David M. Wong et al

Objective—To evaluate the effects of various flow rates of oxygen administered via 1 or 2 nasal cannulae on the fraction of inspired oxygen concentration (Fi{sub}O{sub}2) and other arterial blood gas variables in healthy neonatal foals.

Animals—9 healthy neonatal (3- to 4-day-old) foals.

Procedures—in each foal, a nasal cannula was introduced into each naris and passed into the nasopharynx to the level of the medial canthus of each eye; oxygen was administered at 4 flow rates through either 1 or both cannulae (8 treatments/foal). Intratracheal Fi{sub}O{sub}2, intratracheal end-tidal partial pressure of carbon dioxide, and arterial blood gas variables were measured before (baseline) and during unilateral and bilateral nasopharyngeal delivery of 50, 100, 150, and 200 mL of oxygen/kg/min.

Results—No adverse reactions were associated with administration of supplemental oxygen except at the highest flow rate, at which the foals became agitated. At individual flow rates, significant and dose-dependent increases in Fi{sub}O{sub}2, Pa{sub}CO{sub}2, and oxygen saturation of hemoglobin (Sa{sub}O{sub}2) were detected, compared with baseline values. Comparison of unilateral and bilateral delivery of oxygen at similar cumulative flow rates revealed no differences in evaluated variables.

Conclusions and Clinical Relevance—Results indicated that administration of supplemental oxygen via nasal cannulae appeared to be a highly effective means of increasing Fi{sub}O{sub}2, Pa{sub}CO{sub}2, and Sa{sub}O{sub}2 in neonatal foals. These findings may provide guidance for implementation of oxygen treatment in hypoxicemic neonatal foals. (Am J Vet Res 2010;71:1081–1088)