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HAEMOLYTIC ANAEMIA IN CATS

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Haemolytic anaemia is characterized by reduced erythrocyte survival and is often regenerative and macrocytic-hypochromic. Jaundice may become obvious with serum bilirubin >2mg/dL. If present in cats, hyperbilirubinuria is not a constant finding in contrast to dogs. Intravascular haemolysis is characterized by hemoglobinemia and hemoglobinuria and indicates a more severe disorder. Many causes that have to be considered in the differential diagnosis of haemolytic anaemia in cats.

Immune-mediated haemolytic anaemia (IMHA)
Immune-mediated HA is characterized by antibody production against erythrocytes. In primary (p) IMHA, the stimulus responsible for antibody production is unknown. For a long time pIMHA was considered a rare condition in cats (as opposed to dogs), but recent reports contradict this. There are however some different characteristics in the feline species. There seems to be a slight male predilection. Most cats have severe anaemia upon presentation (PCV<15%). Many cats have splenomegaly (hyperplasia, extramedullary haematopoiesis, haemosiderosis), lymphocytosis, and hyperglobulinaemia. Leukocytosis with a left shift is rare. Liver enzymes can be increased because of hypoxic damage. Hyperbilirubinemia may or may not be present (the latter in case of compensated chronic haemolysis). The diagnosis of pIMHA is based on exclusion of other causes of haemolysis; persistent autoagglutination, increased osmotic fragility or a positive Coombs’ test. Spherocytes are more difficult to distinguish in cats. Mortality rate (+/-25%) is lower than in dogs, and complications such as DIC or thromboembolic events, are less common. Most cats respond favourably to immunosuppressive doses of prednisolone. Relapses are possible.

In secondary IMHA, a specific trigger can be identified (infection (FeLV, FIP, Mycoplasma, Babesia, Cytuzzoonosis), drugs, neoplasia (lymphoma), incompatible blood transfusion). Treatment consists of elimination of the triggering condition, with or without prednisolone to halt immune-mediated destruction of erythrocytes.

Infectious causes

Haemoplasmosis. Feline infectious anaemia is caused by gram-negative epimural parasites. Haemobartonella felis, ‘Ohio strain’ or ‘large form’ was recently renamed as Mycoplasma haemofelis. Haemobartonella felis ‘California strain’ of ‘small form’, which seems to be a low-virulence parasite, was given a candidate species name ‘Candidatus Mycoplasma haemominutum’. Even more recently, a third species was recognized in Switzerland (named Candidatus Mycoplasma turicensis). Dual infections with the former two strains and concurrent diseases (lymphoma, FeLV, FeLV-FIV) may predispose cats to develop more severe signs of anaemia. Ctenocephalides felis may be involved in the transmission. Parasitized erythrocytes are phagocytosed in lymphoid organs. Diagnosis is made by blood smear or PCR. Treatment includes antibiotics such as doxycycline 10 mg/kg/d for 6 weeks, different types of fluoroquinolones, supportive treatment with blood products in severely anaemic animals, and possibly corticosteroids to halt immune-mediated destruction of erythrocytes. Total clearance of organisms, as confirmed by PCR, is rare. A recent study showed better long-term elimination results with pradofloxacin (a new fluoroquinolone with higher in vitro efficacy against gram-negative and gram-positive organisms), at 5 mg/kg/d, for 2 weeks.

Babesiosis. Feline babesiosis is mostly reported in domestic cats in South Africa (Babesia felis, small form) and sporadic cases of unclassified Babesia spp. have been reported in domestic cats from Germany and France. Advances in molecular biology have improved the ability to identify new piroplasma spp in cats. Recently, several reports from Spain and Portugal, and Israel describe infection of domestic cats with subspecies of Babesia canis. The infection is believed to be tick-borne, but the exact vector is still not identified. Babesiosis may result in mild to severe illness depending on the Babesia spp involved, the susceptibility of the host and the presence of co-infections (FeLV, FIV, Mycoplasma). In comparison with canine babesiosis, the disease tends to be more chronic and low-grade. Treatment of choice consists of primaquine phosphate (0.5 mg/kg im or po). Repeated or chronic therapy may be needed and elimination of infection is rare. FeLV. Ten % of FeLV infections result in haemolytic anaemia, either associated with Mycoplasma infection, or by secondary immune mediated mechanisms.

Chemical-induced haemolytic anaemia
Many compounds can form metabolites that interact with oxygen to form free radicals and peroxides that cause oxidative damage to erythrocytes. Three major mechanisms are involved: oxidation of heme iron...
resulting in methaemoglobin (metHb) production (feline erythocytes have less metHb reductase), denaturation of haemoglobin (Hb) (leading to Heinz body (HB) formation) and/or membrane damage resulting in abnormal deformability and iron transport. Erythrocytes with HB have reduced levels of glutathione and ATP, and reduced deformability, which results in reduced survival and possibly haemolysis. Benzocaine, phenazopyridine and acetaminophen can produce severe methaemoglobinemia within minutes to hours. Propofol, onions, acetylsalicylic acid, propylene glycol, benzocaine, methylene blue, d-L methionine, phenazopyridine, vitamin K3, zinc and copper can result in HB formation. Lymphoma, diabetes (ketoacidosis), hepatic lipidosis or hyperthyroidism may increase the amount of HB-containing red blood cells in cats.

**Treatment of drug induced Heinz body anaemia (acetaiminophen toxicity).**
1/ removal of the source of oxidant damage (emesis, gastric lavage, activated charcoal (2g/kg, repeated doses because acetaminophen undergoes enterohpatic recirculation), a cathartic with every third charcoal dose);
2/ supportive therapy (oxygen, fluid therapy, blood transfusion-oxygenyl);
3/ glutathione levels should be restored. Combined use of N-acetylcysteine (NAC), cimetidine and ascorbic acid is more effective then either therapy alone, especially in minimizing hepatotoxicity. Acetaminophen metabolism may be slowed by cimetidine (5-10 mg/kg q6-8h po/iv), which inhibits the hepatic P-450 system. NAC (140-280 mg/kg in 5% dextrose once, then 70 mg/kg q4h po for 3-5 treatments) is a precursor of glutathione and binds toxic acetaminophen metabolites and enhances elimination. Because of the extended half-life of acetaminophen in cats, NAC should be given regardless of time since ingestion. 5-Adenosylmethionine may limit oxidative damage, if administered within 1 hour of acetaminophen ingestion (180 mg q12h for 3 d, 90 mg q12h for 11 days). Vitamin C (20-30 mg/kg q6h po/iv) enhances reduction of metHb to Hb. Corticosteroids and antihistamines are contraindicated.

Zinc. Rarely, cats can suffer from zinc toxicosis, resulting in gastrointestinal irritation, acute renal failure (or multi-organ failure) and intravascular haemolysis with a strongly regenerative response. Increased plasma zinc levels confirm the diagnosis. Treatment consists of foreign object removal and chelation therapy. Calcium EDTA (25 mg/kg q6h po, 2-5 d, dilute in 5% dextrose to decrease local irritation) chelates zinc to form more water-soluble complexes which are more easily excreted in urine. Diuresis minimizes zinc-induced nephrotoxicity. Antacids are recommended, as more soluble zinc salts are formed in the acidic gastric environment.

**Propofol.** Propofol administered to cats on consecutive days can induce oxidative damage to erythrocytes (increased HB formation) and can result in clinical illness after a few days, although haemolysis was not detected after this time.

**Hereditary erythrocyte defects**
Although hereditary causes of haemolysis are generally considered rare they may represent important differential diagnoses if persistent or recurrent, or seen in certain breeds.

Erythrocytic pyruvate kinase (PK) deficiency has been described in Abyssinians and Somalis, and is considered to be a major cause of anaemia in these breeds. Pyruvate kinase is an essential enzyme in the anaerobic glycolysis and insufficient ATP production results in premature erythrocyte membrane failure and haemolysis. Haemolysis is often intermittent and mild and may be well compensated for in cats. A simple mutation specific screening test (University of Pennsylvania, http://www.vet.upenn.edu/penngen) can be used to screen cats from these breeds that are anaemic or will be used for breeding. Interestingly PK deficiency has also been diagnosed in DSH cats (the same PK mutation). Many affected cats have splenomegaly which occasionally becomes massive, as the spleen is considered a major site of red cell sequestration and destruction. Also, the spleen may accumulate iron in the form of haemosiderin and may be infiltrated by lymphocytes. The large spleen may cause inappetence and cachexia. Osteosclerosis and myelofibrosis with subsequent haematopoietic failure (typical features in dogs), have not been demonstrated in cats.

Hereditary increased osmotic fragility (OF) is another condition reported in Somali and Abyssinian cats that is characterized by intermittent episodes of (severe) haemolysis resulting in a regenerative and macrocytic anaemia, and thus should be differentiated from PK deficiency. Both conditions have a negative Coombs’ test. Also here, splenomegaly, lymphocytosis and hyperglobulinaemia are common. Erythrocytic OF is severely increased, suggesting an underlying membrane erythrocyte membrane defect. Haemoplasmas and IMHA have to be excluded because both can result in increased erythrocyte OF. Cats with PK deficiency or increased OF are often treated with immunosuppressive (short-term) and anti-inflammatory doses of prednisolone to mainly impair the macrophage system and thereby the premature destruction of erythrocytes. Animals with
severe splenomegaly, inappetence and weight loss, benefit from splenectomy.
A recently described major complication in cats with PK deficiency is the formation of bilirubin choleliths and posthepatic biliary obstruction, secondary cholangiopathy and hepatic failure. Excessive bilirubin production during haemolysis overloads the hepatocyte conjugating capacity, increases the amount of bilirubin monoconjugates and unconjugated bilirubin in bile and results in precipitation as gallstones. In these cases diagnosis can be complicated because jaundice can be caused by both haemolytic and hepatobiliary disease. In human patients with chronic haemolytic disorders and resultant cholelithiasis, cholecystectomy is performed concomitantly with splenectomy. Autosomal dominant congenital erythropoietic porphyria in Siamese cats results in high levels of porphyrins in erythrocytes and viscera with decreased red cell survival and severe haemolytic anaemia, and renal failure as consequences.

**Hypophosphatemia**
Phosphate is an essential component of ATP, the energy source of erythrocytes. Severe hypophosphatemia can result in haemolysis because of reduced red cell membrane integrity.

It has been associated with diabetes mellitus, hepatic lipidosis, refeeding syndrome, oral phosphate-binding antacids and primary hyperparathyroidism. It is most often seen 24-36h after starting insulin therapy in patients with diabetes mellitus due to intracellular phosphate shifts, increased urinary loss and reduced intestinal phosphate absorption.

Phosphate should be monitored at least every 12h in at risk patients and oral or intravenous potassium phosphate can be administered at 0,011-0,017 mmol phosphate/kg/h for 6-12 hours. Development of hypocalcemia is a possible complication.

**References**
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**Blood types and transfusion**
**Medicine in dogs and cats**
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**Canine and feline blood types**
Although over a dozen blood types have been recognized in dogs, only 7 groups are internationally standardized (9 antigens: DEA 1 (1.1, 1.2, 1.3), 3, 4, 5, 6, 7 and 8). Currently typing antisera are available for blood types DEA 1.1, 1.2, 3, 4, 5 and 7. A red blood cell can be either positive of negative for a given blood type. An exception to this is the DEA 1 system, which has subtypes DEA 1.1 (A1), 1.2 (A2) and 1.3 (A3). A dog can be negative to all of the subtypes (DEA 1 negative), or be positive to either one of the 3 subtypes. DEA 7 is a soluble nonerythroid antigen that adsorbs to the red cell surface, possibly resulting in naturally occurring anti-DEA-7 alloantibodies in DEA 7 negative dogs. The importance of these antibodies is debated.

In cats the AB blood group system was identified with three blood types: A, B and AB (incidence +/- 95%, 5%, <1% respectively, and depending on breed and geo-
graphic region). One very important feature in type A and type B cats is the existence of naturally occurring antibodies to the blood type they lack. Especially type B cats have strong naturally occurring natural anti-A antibodies, resulting in acute haemolytic transfusion reactions after a (first) type A blood transfusion, or in neonatal isoerythrolysis in type A or AB kittens born from a type B queen. Maternal antibody is passively transferred to the kitten through the colostrum and absorbed by the gastrointestinal tract within the first 16 hours of life. Haemolysis within hours to days after birth results in pigmentation, anaemia, icterus, anorexia, and sudden death within the first week of life (fading kitten syndrome). Some kittens may have a subclinical course or slough their tail tip at 1-2 weeks of age. Type A queen should not be bred to type B toms. If kittens are born from this combination, the kittens should not be nursed by the type B queen. To improve the kittens immunity, they can receive a plasma transfusion (150 mL/kg iv or ip) with the correct blood type.

**Does the ideal canine donor exist?**

Recommendations have been made that canine donors should at least be DEA 1.1 and 1.2 (and some suggest also DEA-7 negative). As most dogs are DEA 4 positive (>98%), this type was suggested as the universal donor type, although recently a severe haemolytic transfusion reaction was seen following a DEA 4 positive transfusion to a negative dog. The significance of DEA 3 and 5 remains to be determined. The donor also should have normal von Willebrand factor (vWF) concentration. As dogs do not have clinically significant naturally occurring antibodies against red cell antigens implicated in immediate haemolytic transfusion reactions, first transfusions in dogs rarely result in incompatibilities. Therefore, in emergency situations, pre-transfusion testing can be omitted. However, there is a high risk of sensitization and if possible, DEA 1.1-negative blood should be given.

**New blood types**

Recently, an acquired, transfusion-induced alloantibody was discovered in a Dalmatian dog. The newly discovered red cell antigen was called ‘Dal’. In the overall (non-Dalmatian) dog population, this is a ‘high frequency antigen’ (incidence of >92-99% in the general population), but some Dalmatians seem to lack this antigen. The clinical significance of this Dal-antigen is yet to be determined but possibly these dogs are at risk for haemolytic transfusion reactions, once sensitized by a blood transfusion.

In cats, because of the occurrence of naturally occurring antibodies against red cell antigens, blood typing is always necessary. Moreover, incompatible cross-match results or haemolytic transfusion reactions occur in (not previously transfused) AB compatible cats, suggesting that other naturally occurring alloantibodies may be present in some individuals. Recently, a new alloantibody formed against a common antigen (‘Mik’) was identified in 4 cats with no transfusion history. Cats lacking the ‘Mik’ antigen can have naturally occurring anti-Mik antibodies, and are at risk for haemolytic transfusion reactions, even after an AB-compatible transfusion. This underscores the importance of the crossmatch test before every transfusion.

**Donor screening for infectious diseases**

If dogs with a travel history in endemic areas are to be used as a donor, they should be negative for babesiosis, leishmaniasis and ehrlichiosis (by serology or PCR). Recently, in two regions in the Netherlands (south of Arnhem and Den Haag), several babesiosis cases were diagnosed in dogs with no travel history. Also, *Babesia canis* infected *Dermacentor reticulatus* ticks were found on several dogs in these specific regions. Therefore, it seems prudent to test potential donor dogs from these regions for *Babesia canis*. The ACVIM consensus statement on blood donor selection (2005) also recommends testing for brucellosis. Testing for Lyme disease is not recommended, disease transmission by blood transfusion has not been documented.

Cats should be excluded if free roaming, and FeLV and/or FIV positive. The ACVIM consensus statement also recommends testing for Mycoplasma spp. (blood smear or PCR) and possibly Bartonella spp. (blood culture, PCR or serology), although clinical bartonellosis has not been identified after transfusion.

**Indications for transfusion with specific blood products (component therapy)**

There are many advantages in the use of blood components over fresh whole blood. With component therapy, the patient can be specifically treated with the component needed (eg plasma in patient with rodenticide intoxication, packed red cells (PRC) in normovolemic anaemic patient with chronic renal failure), therefore 1 donor can provide transfusion support for 2 different patients. Also the risk of adverse transfusion reactions is decreased.

Whole blood can be collected in blood bags containing ACD (acid citrate dextrose) or CPDA-1 (citrate phosphate dextrose adenine) as preservatives. Viability of erythrocytes is increased by addition of dextrose, adenine and phosphate, therefore storage time is increased up to 3 weeks at 4°C. In contrast, heparin and citrate alone only permit storage up to 24 hours.
Whole blood transfusion expands plasma volume by increasing osmotic pressure and restores oxygen carrying capacity (tissue reoxygenation). Therefore, indications for whole blood use in veterinary patients include acute loss of over 50% of total blood volume or the need for several blood components. Dogs with severe bleeding caused by immune mediated thrombocytopenia (IMPT) may need a fresh whole blood transfusion to restore red blood cells and provide some platelets. However, the platelets will be utilized immediately and will not cause a significant rise in the platelet count.

Packed red cells are produced by centrifugation of fresh whole blood and removal of +/- 200 mL of plasma. This results in a viscous preparation with a PCV of approximately 75%, flow can be improved by adding normal saline. Storage time can be increased for up to 37 days by adding certain additive solutions which contain factors needed for energy metabolism of erythrocytes (adenine, dextrose, mannitol, NaCl). This results in a blood product with a PCV of 55-65 %.

Indications include increasing oxygen carrying capacity in patients with loss of less than 50% of their blood volume and normovolemic anaemic patients (nonregenerative anaemia, haemolytic anaemia, chronic external blood loss). Packed red cell preparations are especially useful when volume overload is a concern (eg patients with cardiac disease).

When (compatible) whole blood or packed red cells are not available in an emergency situation, a hemoglobin-based oxygen-carrier or blood substitute can be used. Oxycel®, or polymerized bovine hemoglobin is approved for use in dogs, and seems to be safe in cats. Advantages include longer shelf life, direct availability, no need for crossmatching or bloodtyping and absence of infectious disease transmission.

In veterinary medicine, specific coagulation factor concentrates are not available. Therefore, fresh frozen plasma (FFP) and cryoprecipitate are the main treatment options for coagulation disorders. Fresh FP is plasma that has been separated from erythrocytes and frozen at ≤-18°C within 6 hours of collection. After that period of time, procoagulant activity of factor VIII will be lost within 24 hours (labile coagulation factor).

Fresh FP is used to treat acquired coagulation disorders e.g. vitamin K deficiency (as is rodenticide intoxication, bile duct obstruction, liver insufficiency, malassimilation or chronic oral antibiotic use), DIC or hepatic insufficiency. Congenital causes include hemophilia A and von Willebrand’s disease.

Cryoprecipitate is obtained by slowly thawing at 4°C FFP, centrifugation and removal of the supernatant. Freezing at -18°C allows storage for 1 year. Cryoprecipitate is rich in von Willebrand factor (vWF), factor VIII and XIII and fibrinogen, and therefore ideal for patients with von Willebrand disease, hemophilia A and hypo/ dysfibrinogenemia. The cryosupernatant contains adequate amounts of factor IX, which makes it useful for treatment of hemophilia B.

Fresh FP also contains anti-coagulation factors such as antithrombin III. This protein can be lost in large amounts in certain disease processes (protein losing enteropathy, protein losing nephropathy, Cushing’s syndrome…), favoring thrombo-embolic events. Another component of FFP is α-macroglobulin, a natural anti-protease, which is believed to replace consumed α-macroglobulins during acute pancreatitis. In absence of these anti-proteases, proteolytic enzymes released will activate kinin, coagulation, fibrinolytic and complement cascade systems, resulting in diffuse intravascular coagulation and possibly death. A recent study, however, failed to demonstrate a beneficial effect of FFP in patients with acute pancreatitis.

Frozen plasma (FP) is plasma that has been frozen more than 6 hours after centrifugation, or, when FFP is frozen for longer than 1 year. The albumin in FP can be preserved for 5 years. This plasma is not indicated for use in patients in need of labile coagulation factors (factor V and VIII) or vWF, such as in hemophilia A, DIC or von Willebrand disease. Plasma transfusions are not indicated for protein replacement in patients with protein loss or for nutritional support. A dose of 6-10 mL/kg every 8 hours is required to increase albumin concentration with 0.5 g/dL.

Platelet-rich plasma can be prepared from fresh whole blood by centrifugation at lower speed. However, many difficulties exist because the amount of platelets from one donor is low, platelet lifespan is short and adverse transfusion reactions. In IMTP transfused platelets are rapidly destroyed.

**Management of transfusion reactions**

Treatment of acute immunologic (haemolytic) transfusion reactions (due to the presence of antibodies against erythrocytes) is often difficult. Transfusion should be stopped and supportive therapy instituted (fluid therapy). Treatment with glucocorticoids is controversial.

Nonhaemolytic fever (usually because of the presence of antibodies to donor white blood cells) may be treated with antipyretics if needed. If fever is caused by bacterial contamination of blood products, supportive therapy (fluids) and antibiotics are given.
Urticaria (due to antibodies to donor plasma proteins) are treated by short-acting corticosteroids (methylprednisolone succinate 30 mg/kg iv once or dexamethasone 4-6 mg/kg iv once) or antihistamines (diphenhydramine 2 mg/kg iv). Transfusion can usually be restarted at lower rate. If volume overload occurs, diuretics (furosemide 2-4 mg/kg iv) and if needed vasodilators are given. Hyperkalemia can be caused by in vitro leakage of potassium from erythrocytes. Standard treatment for hyperkalemia is instituted (0.9% NaCl, insulin + dextrose...). Hypocalcemia is treated with calcium gluconate (10%), 50-100 mg/kg iv over 30 min, with ecg monitoring.

References: