Severe Heinz body anemia and methemoglobinemia in a kitten with chronic diarrhea

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Summary

A 2-month-old kitten was referred for depression and partial anorexia since 3 days and chronic diarrhea lasting for over 3 weeks. General physical examination showed pale and cyanotic mucous membranes. Blood sample was of brownish appearance. Venous blood gas analysis and complete blood count showed 16% methemoglobin level and severe regenerative anemia with Heinz bodies in about 40% of the erythrocytes, respectively. The kitten was transfused with fresh whole blood and treated with supportive care, antimicrobial and antioxidant agents. The kitten totally recovered. To the authors' knowledge, this represents the first case report of severe Heinz body hemolytic anemia and methemoglobinemia with concurrent chronic diarrhea in a young kitten. Diarrhea resolution coincided with Heinz bodies and methemoglobin disappearance. The possibility that diarrhea might have stimulated an inflammatory state causing release of oxygen radicals and prolonged erythrocytes oxidative damage has been discussed.

Keywords: kitten, anemia, Heinz body, methemoglobinemia

Schwere Heinz-Körper Anämie und Methämoglobinämia bei einem Kätzchen mit chronischem Durchfall

Ein zwei Monate altes Kätzchen wurde wegen Depression und partieller Anorexie seit drei Tagen und chronischem Durchfall während mehr als 3 Wochen überwiesen. Die allgemeine klinische Untersuchung zeigte blasse und zyanotische Schleimhäute. Das Blut war von bräunlicher Farbe. Die venöse Blutgasanalyse und das vollständige Blutbild zeigten 16% Methämoglobin und eine schwere regenerative Anämie mit Heinz-Körpern bei etwa 40% der Erythrozyten. Das Kätzchen wurde mit frischem Vollblut transfundiert, gepflegt und mit Antibiotika und Antioxidanten behandelt. Das Kätzchen erholte sich vollständig. Nach Wissen der Autoren stellt dies den ersten Fallbericht von schwerer Heinz-Körper hämolytischer Anämie und Methämoglobinämie mit gleichzeitig chronischem Durchfall bei einem jungen Kätzchen dar. Die Besserung des Durchfalles fiel mit dem Verschwinden von Heinz-Körpern und Metämoglobin zusammen. Die Möglichkeit, dass Durchfall einen entzündlichen Zustand verursacht haben könnte, der zu Freisetzung von Sauerstoffradikalen und Iänger anhaltenden oxidativen Erythrozyten Schäden führte, wird diskutiert.

Schlüsselwörter: Kätzchen, Anämie, Heinz Körper, Methämoglobinämie

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Introduction

Red blood cells (RBCs) are continuously exposed to oxidant stress under both physiologic and pathologic conditions. The most common consequence of oxidant injury is the formation of Heinz bodies (HBs) and methemoglobin (metHb) (Desnoyers, 2010). Heinz bodies are aggregates of denatured and precipitated hemoglobin within RBCs. Methemoglobin is an inactive form of hemoglobin created when the iron molecule of hemoglobin is oxidized from ferrous state to the ferric form. In normal cats, metHb accounts for less than 3% of total hemoglobin (Rahilly and Mandell, 2015). Numerous substances that cause an increase in HBs induce some degree of metHb but associated clinical signs are typically attributable to the hemolytic anemia secondary to the HBs (Rahilly and Mandell, 2015). In kittens, severe Heinz body anemia was previously reported in a 7-month-old cat with suspected congenital hemoglobin defect (Thompson et al., 1989). In two experimental

studies, HBs formation without anemia was detected after intake of diets with added propylene glycol (Hickman et al., 1990; Bauer et al., 1992).

To the authors' knowledge, this report describes the first case of severe Heinz body hemolytic anemia and methemoglobinemia in a young kitten with concurrent chronic diarrhea. After 4 days of hospitalization, diarrhea resolution coincided with Heinz body hemolytic anemia and methemoglobinemia disappearance.

Case history

A 2-month-old, weighing 600 grams, male Scottish fold was presented for depression and partial anorexia lasting for 3 days. The kitten lived indoors and was fed commercial cat food. The kitten originated from a pet store 3 weeks before and had passed small bowel diarrhea since purchase. Ten days before referring, the kitten was treated empirically with a milbemycin oxime/praziquantel formulation (Milbemax®, Novartis, Varese, Italy). The diarrhea did not resolve. There was no known history of exposure to other drugs or toxic substances.

Physical examination

General physical examination showed mild hypothermia (37.8 °C), 6% dehydration, pale and slightly cyanotic mucous membranes. Blood samples were taken for routine laboratory evaluations. The collected blood showed a brownish appearance. Venous blood gas analysis revealed 16% metHb level [reference interval (RI): 0.5% to 3%] and 9.3 mmol/L lactates value (RI: 0.5 to 2 mmol/L). Complete blood count (CBC) showed regenerative anemia, moderate leucocytosis with neutrophilia, marked thrombocytosis (Tab. 1). Blood smear microscopy (May Grunwald Giemsa) revealed anisocytosis, polychromasia, hypochromasia and poikilocytosis (acanthocytes, schistocytes, elliptocytes and keratocytes). Numerous RBCs showed single large round projections of membrane or clear round inclusion identified as HBs (Fig. 1A). In blood smears prepared with new methylene blue stain (NMB), HBs were visualized up to 43% of RBCs (Fig. 1B). Left shift and marked toxic changes were also observed (Tab. 1). Biochemical profile was normal except for hypokalemia (2.9 mmol/L, RI: 3.9 to 5.5 mmol/L). No alterations were revealed by abdominal ultrasonography and echocardiography. A diagnosis of Heinz body anemia of unknown origin was posed. At this time, no obvious cause for chronic diarrhea was found.

Treatment

The kitten was admitted to the Intensive Care Unit and was treated intravenously with 40 mEq/L potassium chloride added to Ringer lactate solution started on dose of 7.5 ml/h for restoring hydration and normal potassium level; amoxicillin/clavulanic acid at 20 mg/kg twice daily (Amoxicillin/clavulanic acid, Teva, Milano, Italy) and omeprazole at 1 mg/kg twice daily (Omeprazole, Mylan, Milano, Italy) for prevent gastrointestinal mucosal barrier disruption. S-adenosylmethionine at 90 mg/kg once a day (Denosyl®, Candioli. Beinasco, Italy) was given as antioxidant agent. Approximately four hours after admission, the kitten was normothermic, active and started eating itself. It showed small bowel diarrhea characterized by feces as puddles, watery, flat without texture (Fecal scoring Nestlè Purina: 7). A

Table 1: Haematology results during hospitalization and at discharge from the hospital. Abnormal values are shown in bold.

Parameter	Day 1	Day 2	Day 6	Reference interval
RBC ($\times 10^{12}$/L)	**4.0**	**2.42**	**4.2**	6.0-10.1
Hb (g/L)	**57**	**55**	**70**	81-142
Hct (%)	**16%**	**10%**	**25%**	28-47
MCV (fL)	42	42	**59**	41.3-52.6
MCH (pg)	14.2	**22.9**	**16.6**	12-16
Reticolocytes ($\times 10^9$/L)	**123.5**	**377.9**	**203.9**	15-81
WBC ($\times 10^9$/L)	**37.46**	**57.84**	12.83	6.3-19.6
Mature neutrophils ($\times 10^9$/L)	**22.85**	**32.97**	6.22	3.0-13.4
Band neutrophils ($\times 10^9$/L)	**5.99**	**5.20**	**0.51**	0-0.3
Metamyelocytes ($\times 10^9$/L)	**2.99**	**1.16**	**0.13**	0.0
Lymphocytes ($\times 10^9$/L)	5.43	**8.39**	3.34	2.0-7.2
Monocytes ($\times 10^9$/L)	0.19	**1.45**	**1.67**	0-1.0
Eosinophils ($\times 10^9$/L)	0.3	**12.15**	1.41	0.3-1.7
PLT ($\times 10^9$/L)	**1372**	**1999**	219	156-626

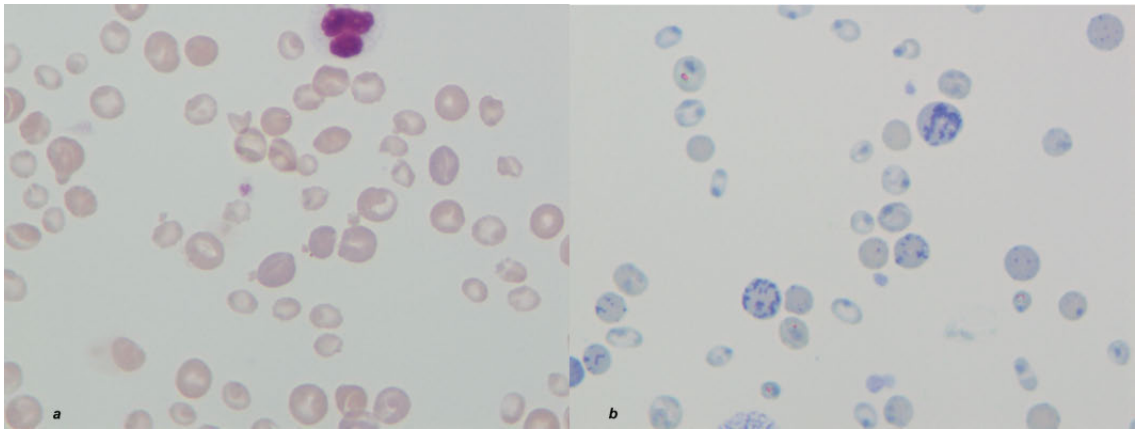

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Figure 1: a - Anisocytosis, hypochromasia, polychromasia and numerous Heinz bodies projecting from erythrocites membrane (May Grunwald Giemsa, ×1000). b - Reticolocytes and Heinz bodies appearing as erythrocites round blue inclusions (New methylene blue, ×1000)

faecal sample for parasitological analysis was submitted. On the 2nd day, the kitten was severely depressed and anorexic. A new CBC revealed RBCs decrease with increased reticulocytes. Blood smear evaluation showed marked anisocytosis, polychromasia and hypochromasia, numerous HBs, schistocytes, acanthocytes, eccentrocytes and erythrocytic ghosts. In blood smears stained with NMB, HBs were identified in 40% of RBCs. Leucocytosis with neutrophilia, lymphocytosis, monocytosis and eosinophylia increased while bands neutrophils and metamielocytes decreased as well as toxic changes (Tab. 1). After blood group typing, the kitten was transfused with group A fresh whole blood. The post-transfusional hematocrit (HCT) reached 21% (RI: 28% to 47%). During the following 3 days of hospitalization, HCT improved and hypokaliemia recovered. Venous blood gas analysis showed 2.5% metHb level and 1.5 mmol/L lactates value. The kitten was active, normothermic and eating with appetite. On the 4th day of hospitalization, feces were very moist but had distinct shape (Fecal scoring Neslté Purina: 5). On the 5th day diarrhea disappeared, however faecal analysis by zinc sulfate flotation returned positive for *Isospora felis*. On the 6th day, CBC still showed a mild regenerative anemia. Platelets and leukocytes were in the normal range with slight monocytosis. Blood smear microscopy did not reveal any HBs. Bands neutrophils and metamielocytes decreased and only occasional toxic changes were noted in neutrophils (Tab. 1). Venous blood gas analysis was normal. On the same day, the cat was discharged from the hospital with oral amoxicillin/clavulanic acid at 20 mg/kg twice daily (Clavaseptin®, Vetoquinol, Bertinoro, Italy) for one week. At the end of antimicrobial therapy, the kitten was administered with chlortetracycline hydrochloride at 25 mg/kg once a day for 15 days (Isospen®, Teknofarma, Torino, Italy) for coccidiosis therapy. Two months after the initial presentation, general physical examination, CBC and venous blood gas analysis did not show any abnormalities. No HBs were observed and faecal parasitological exam did not show any parasite.

Discussion

In this kitten, RBCs oxidative damage was supported by the presence of HBs in about 40% of the RBCs and methemoglobinemia that conferred the typical brown-chocolate colour to blood sample and mild cyanosis to mucous membrane. Severe hyperlactatemia was also indicative of decreased tissue oxygen delivery (Rosenstein and Hughes, 2015). Cyanosis appears at metHB levels of 12% to 14% or more, while clinical signs of hypoxia begin at a metHB of 20% with coma and death if metHB reaches 80% (Rahilly and Mandell, 2015). Feline hemoglobin is more susceptible to oxidative damage than that of other mammalian species due to the easy dissociation from a tetramer to a dimer form and the presence of eight reactive sulfhydryl groups on the molecule with resultant acceleration of hemoglobin auto-oxidation. In most species, hemolytic anemia results because of sequestration, pitting and lysis of HBs containing-RBCs by the mononuclear phagocyte system, particularly in the spleen (Harvey and Kaneko 1977; Christopher et al., 1990). The feline spleen is non-sinusoidal and lacks efficient pitting function. It is inefficient at removing HBs from RBCs which enter through the large pores in splenic pulp venules without deformation and circulate relatively unimpeded in the spleen (Chistopher et al., 1995). In this species the mechanisms by which HBs result in RBCs destruction and hemolytic anemia remain unknown, although multiple cellular abnormalities that may shorten RBCs survival as reduced glutathione and adenosine triphosphate, increased rigidity of the membrane and abnormal membrane surface charge distribution have been identified in HBs

containing-RBC (Christopher et al. 1990; Desnoyers, 2010). In cats, the combination of increased susceptibility of the hemoglobin to oxidative damage and the splenic ultrastructural variation makes that healthy individuals may have HBs in 96% of circulating erythrocytes. The nature of the damage, the amount of affected hemoglobin within a cell and individual variations seem to determine whether a given cat develops clinically significant hemolysis (Rahilly and Mandell, 2015). Patients with Heinz body anemia can have normal serum bilirubin level because HBs are not necessarily metabolized to bilirubin. Jaundice was observed in cats intoxicated with acetaminophen or phenazopyridine because toxicity causes liver damage in addition to hemolytic anemia (Harvey, 1995). This kitten experienced Heinz body anemia without jaundice.

In cats, paracetamol intoxication, hypophosphatemia and consumption of baby food containing onion powder may result in severe anemia by oxidative injuria (Adams et al., 1993; Aronson and Drobatz, 1996; Robertson et al., 1998). Phenazopyridine, methylene blue, topical benzocaine, propylene glycol, skunk musk, zinc, naphthalene, methionine, crude oils and repeated use of propofol are recognised causes of feline RBCs oxidative damage and potential causes of anemia (Rahilly and Mandell, 2015). Increased levels of HBs and mild anemia have been associated with hyperthyroidism, lymphoma and diabetes mellitus, particularly with ketoacidosis. High number of HBs has been observed in cats with abscesses, urologic disease, chronic renal failure, intestinal obstruction, peritonitis, stomatitis and upper respiratory tract diseases (Christopher, 1989). In this kitten, none of the aforementioned diseases was detected. The history ruled out drugs, toxic substances or ingestion of foreign zinc bodies as possible cause of Heinz body anemia in the last 3 weeks before referring. Foreign bodies were also not detected by abdominal ultrasound examination. Although Heinz body anemia has not been previously reported in association with gastrointestinal diseases, the authors hypothesized that the chronic diarrhea in this kitten may have stimulated an inflammatory state responsible for the release of oxygen radicals and subsequent prolonged RBCs oxidative damage. Inflammation is considered a key component of gastrointestinal disease (Balmus et al., 2016). The leukograms of this cat showing neutrophilia with left shift, lymphocytosis, monocytosis and eosinophilia along with thrombocytosis were indicative of inflammatory/infectious state. In humans and animals, acute or chronic intestinal inflammation has been associated with oxidative stress due to an imbalance between the oxidant substances and antioxidant cellular system as the result of either a reactive oxygen and nitrogen species overproduction or a decreased antioxidant activity (Balmus et al., 2016; Guerrero and Acosta, 2016; Tian et al., 2017). Increased oxidative stress indices in erythrocytes from dogs with viral gastroenteritis and from calves with undifferentiated diarrhea were demonstrated (Ranjan et al., 2006; Panda et al., 2008). It was proposed that oxidative events occurring peripherally in somatic tissue may result in HBs formation as a result of scavenging of reactive oxygen species by erythrocytes (Christopher, 1989). In diabetic cats, the release of oxygen radicals by activate phagocytes in inflammatory lesions may contribute to oxidative damage to RBCs in the circulation and high number of monocytes have been associated with inflammation and HBs formation in ketoacidotic individuals (Christopher et al., 1995).

In this kitten, the primary cause of chronic diarrhea has not been certainly established. Diarrhea due to infectious, viral, parasitic or bacterial, causes is a common problem in young cats. Particularly, if the patient is systemically ill, viral and bacterial causes should be considered (Cook, 2008). To the authors's knowledge, food hypersensibility and steroid-responsive diarrhea have not been described in so young kittens. This kitten fed an appropriate commercial food before hospitalisation and continued to eat the same food after resolution of gastrointestinal clinical signs making unlikely food responsive diarrhea. According to the authors, in this kitten an infectious diarrhea is likely. Coccidia probably were not the primary cause of diarrhea since it resolved before the administration of antiparasitic therapy. A gastrointestinal viral enteritis cannot be ruled out. Enteric viruses are common in feline community and breeding (Herrewegh et al., 1997). Several bacterial species have been associated with diarrhea in cats such as *Campilobacter* spp, *Clostridium perfringens*, *Enterococcus* spp and Coliform bacteria (Cook, 2008; Little, 2012). Intestinal bacteria can also act as opportunistic agents (Magne, 2006). Although the presence of antibiotic-responsive diarrhea is unclear in cats, some cats with chronic undefined diarrhea show clinical improvement with antibiotic therapy (Cook, 2008; Suchodolski, 2016). This may be due to the eradication of occult pathogens or due to an alteration in the host response to endogenous flora (Cook, 2008).

In conclusion, this report describes the first case of severe Heinz body hemolytic anemia and methemoglobinemia with concurrent chronic diarrhea in a young kitten. The authors proposed that diarrhea may have stimulated an inflammatory state causing release of oxygen radicals and prolonged erythrocytes oxidative damage. However, a causal relationship between anemia and diarrhea cannot conclusively be established although diarrhea resolution coincided with Heinz bodies and methemoglobin disappearance.

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