LEEK DIET MAY CAUSE HEMOLYTIC ANEMIA: A CASE REPORT IN A CAT.

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Signalement

A 7 year-old neutered female domestic shorthair cat.

Clinical History

The cat was presented because of the ingestion of a bone. No abnormalities were noted at clinical examination, and an abdominal radiography showed the presence of a bone in the stomach. A medical treatment composed of a leek-based high fiber diet and steroidal anti-inflammatory drugs was decided to try naturally eliminating the bone. At the following presentation, eight days later, no abnormality in the behavior of the cat was reported by the owners.

Clinical findings

At the second clinical presentation, the cat was bright, alert and did not present abdominal pain. The only significant clinical findings were pale mucous membranes and a slight hepatomegaly was suspected.

Diagnostic procedures

Abdominal radiography and ultrasound suggested that the bone had been eliminated. However, a diffuse hepatopathy was suspected. A complete blood cell count, blood film, biochemical and urine analyses were also performed. Results are in tables 1, 2 and 3.

Hematology results obtained with the analyzer Sysmex XT-2000iV® showed a macrocytic normochromic and highly regenerative anemia, and a moderate leukocytosis due to a neutrophilia according to a manual differential count (no differential count was given by the analyzer).A

discrepancy between the platelet count obtained by impedance (PLT-I = 105.10^9/L) and optical technology (PLT-O = 285.10^9/L) was observed. Strongly abnormal dot plots were observed for WBC/BASO, DIFF, RET and PLT-O scattergrams (Figure 1) and two flags were reported by the analyzer: “PLT Abn Distribution” and “WBC Abn Scattergram”. The RET and PLT-O scattergrams were characterized by a new subpopulation localized just between the red blood cell and platelet dot plots. The WBC/BASO scattergram showed a down shift associated with the presence of a “double population”. Furthermore, the main population localized at the bottom of this scattergram seemed to be partially truncated. The DIFF scattergram was characterized by a down shift associated with a large basal dot plot, very few points on the neutrophil dot plot and almost no lymphocyte and monocyte dot plots. The impedance curve of platelets was also truncated on the right side.

On the blood smear, several signs of regenerative anemia have been seen: marked anisocytosis, moderate polychromasia, presence of some basophilic stippling and Howell-Jolly bodies. But the most significant abnormalities on the blood smear were the presence of numerous Heinz bodies on almost all red blood cells, many large free Heinz bodies at the edge of the blood smear, many ghost red blood cells, and rare blister cells (Figures 2 & 3). At microscopic evaluation of the blood smear, the white blood cell population did not seem altered whereas the analyzer had reported an increase (WBC = $22.31.10^9$/L). Some reactive monocytes, rare macrophages, few band cells and toxic neutrophils were observed. Finally, many platelet clumps were present on the blood film.

A new methylene blue staining confirmed the presence of Heinz bodies. The manual reticulocytes count was 2.3% (aggregated reticulocytes), and was lower than the automatic reticulocyte count (6.2%) obtained with the Sysmex XT-2000iV®.

Plasma biochemistry and urine analysis findings included a severe hemoglobinuria and hemoglobinemia, and a moderate increase of total bilirubin and ALAT.

Mycoplasma haemofelis and “*Candidatus* Mycoplasma haemominutum” were excluded as possible infectious causes of regenerative anemia by negative results of PCR.

Based on clinico-pathological findings, a diagnosis of hemolytic anemia was made, and the examination of the blood smear was highly suggestive of an intravascular haemolytic anemia secondary to a severe oxidative injury with formation of Heinz bodies.

Treatment and following:

The cat was hospitalized for treatment and follow-up. A palliative and supportive treatment was performed with antioxydant drugs. The leek ingestion was discontinued.

A monitoring of the hematocrit was performed and remained between 0.15 and 0.18 L/L. The cat was discharged five days after diagnosis with an antioxidant treatment based on the oral administration of S-adenosyl methionine for three weeks. Finally, the cat was clinically normal fifteen days later, and the anemia and hemoglobinuria had disappeared.

Interpretation:

An intravascular hemolytic anemia induced by a severe oxidative injury due to a chronic ingestion of leek was highly suspected.

Discussion & conclusion:

Leek (*Allium porrum*) is a plant of the same family and order as onion (*Allium cepa*) and garlic (*Allium sativum*), *i.e. Alliacaea* family and *Allium* order. Onion and less frequently garlic are known to induce an oxidative injury in domestic animals sometimes causing a hemolytic anemia [1][2][3][4][5][6][7]. In dog and cat, no case of hemolytic anemia secondary to leek ingestion has yet been published to our knowledge. It would be logical to think that all these plants (onion, garlic and leek) should have some similar compounds which may be responsible for oxidative injury and hemolytic anemia. These toxic compounds have been particularly studied in onion because of the frequency of such poisoning in domestic animals, especially in dogs. In fact, the cat is not known to like eating food with onions or garlic, except for baby-food which can contain onion powder and is sometimes proposed to ill cats by their owners [4][5]. The toxic compounds of onions are not completely elucidated. However, it has been demonstrated that several organosulfide compounds responsible for an oxidative injury are present, such as n-propyldisulfide, sodium n-propylthiosulfate and probably others [8][9][10].

In our case, we cannot know the exact dose of leek consumed by the cat. We just know that there has been a chronic ingestion for eight days. The formation of Heinz bodies in cats has been described in several diseases (diabetes mellitus, hyperthyroidism and lymphoma…) or in case of administration of drugs and exogenous molecules (acetaminophen, propofol, propylene glycol, methylene blue, benzocaine or onion). We have highly suspected that it was a case of leek poisoning which had induced the severe noticed oxidative injury by exclusion of previously mentioned causes and because hemolysis has stopped after withdraw a leek administration [11].

The Heinz body is the result of clumping of denatured hemoglobin on the inner surface of the red blood cells [11]. The patho-physiological mechanism of the common formation of Heinz bodies in cat

is based on the high sensitivity of feline hemoglobin to oxidant compounds because of the presence of eight cysteine sulfhydryl groups (only four in the dog) and the capacity of feline hemoglobin to dissociate from tetramer to dimers more readily than in other species. The oxidation of hemoglobin can result in Heinz body and methemoglobin formation [10]. However, hemolytic anemia is not always observed in such case because the spleen of cats (with its non-sinusoidal structure) is inefficient to eliminate the damaged red blood cells containing Heinz bodies [10]. An intravascular hemolysis occurs in case of highly fragile RBC population secondary to dramatic damages on RBC membranes induced by the presence of numerous or large Heinz bodies [10].

In this case, the clinical signs of the cat were similar to those described in previously published cases of onion poisoning in cats: no severe clinical signs since the cat was bright; only pale mucous membrane and hemoglobinuria were noticed at the clinical examination. The clinical signs previously described with onion poisoning were less intense in cats than in dogs, except in case of a high dose or chronic ingestion with lethargia and weakness, pale mucous membrane, icterus, tachycardia, heart murmur, polypnea, and sometimes digestive signs [1][4][6].

The clinicopathologic findings observed in our case are partially in agreement with those described in the case of onion poisoning; the hemolysis seemed to be more severe than in classical onion poisoning, since marked hemoglobinuria and hemoglobinemia were observed. The formation of many Heinz bodies was the main abnormality, associated with signs of a regenerative anemia compatible with a hemolytic anemia. In an experimental study of onion poisoning by ingestion of baby food containing onion powder, Heinz body formation was shown to be dose-dependent [5]. This formation started within the first hours after the ingestion of onion with an immediate decrease of the hematocrit followed by an increase in the reticulocyte count [2][4][5][6]. The anemia is not always observed in onion poisoning, except when the ingestion is chronic or massive [6]. Unfortunately, the kinetics of the anemia and of reticulocyte count could not be investigated in our case. Finally, eccentrocytes have been described in garlic and less frequently in onion poisoning in dogs, but these abnormal erythrocytes were not observed in our case [3][7].

One of the key modifications noticed in the hematological results in our case were abnormal dot plots given by the Sysmex XT-2000iV® analyzer.

On the RET and PLT-O scattergrams, a new subpopulation was observed, localized just below the red blood cells dot plot and above the platelet dot plot. A discrepancy in platelet count obtained with the optical technology of the Sysmex XT-2000iV® has been previously linked to the presence of many ghost cells in the case of an immune mediated hemolytic anemia; the ghost cells seemed to be at the top of the platelet but included in the dot plot [12]. The atypical population observed on our scattergrams was different from the previously described cases and could represent the free Heinz bodies according to their intermediate size or the many ghost cells observed on the blood smear

(Figure 3). In human medicine, some fragmented RBC, such as micro-spherocytosis in the case of acute burn, Heinz bodies and ghost cells were also described to disturb the platelet count measured by impedance counting, but less intensively by cytometry. For all these reasons, we can be confident that the platelet count has also been distorted, but unfortunately we could not estimate the error due to the presence of Heinz bodies and ghost cells because many large platelet clumps were detected on the blood smear.

A discrepancy between manual and automatic reticulocyte counts with respectively 2.3 % and 6.2 % of reticulocytes were observed, and could be attributed to: the poor separation between RBC and reticulocyte dot plots, the modified damaged RBC, a possible discrepancy in aggregated and punctuated reticulocytes or less probably to the presence of Heinz bodies or ghost cells [13]. Spurious counts of reticulocytes have been described in presence of Heinz bodies in human medicine [14]. Heinz bodies have also been reported to cause abnormal RBC dot plot with the Technicon H-1® (Bayer), in a case of onion poisoning with baby food in a cat [4]. A hyperchromatic subpopulation of red blood cells associated with an abnormally high MCHC has been reported. This abnormality was explained by the increase of the refractive index of the red blood cells due to the presence of Heinz bodies in these cells and free in suspension. This abnormality was not observed in our clinical case.

White blood cell count cannot be validated in our case because of the greatly abnormal scattergrams. In fact, on the WBC/BASO scattergram, the main dot plot localized at the bottom of the graphic was truncated by the horizontal axis. This unexpected dot plot seems to be composed of smaller particles than leukocytes associated with a low fluorescence, and could potentially be composed of many Heinz bodies or cell fragments such as ghost cells. Similar abnormalities have been previously described with the basophil cytogram obtained with Technicon H-1®, which also displayed a population of particles which seemed to be smaller than leukocytes and were present in a large number: these particles had been highly suspected to be Heinz bodies [4]. In our case, the DIFF scattergram was also abnormal; a large population of cells was observed at the bottom of the graphic, which could be ghost cells or Heinz bodies, but we have no explanation for the very few points on the neutrophil dot plot and the absence of other ones (monocytes, lymphocytes and eosinophils).

As a conclusion, two main observations were made with this case. Firstly, on a technological point of view, dramatic spurious cells counts can be measured in case of hemolytic anemia with Heinz bodies with the Sysmex XT-2000iV® thus scattergrams should be cautiously observed before validation of results. Secondly, on a clinical point of view, practitioners should be aware of the possible toxicity of leek before prescription of a high fiber diet based on this plant to facilitate the elimination of foreign bodies. Finally, leek ingestion should not be recommended especially in ill cats, at least if their disease is classically related with an oxidative injury.

Table 1: Hematology results obtained by Sysmex XT-2000iV®and the manual differential and reticulocyte counts

Analytes	Data	Reference Interval
HGB (g/dL)	**5.7**	9.8-16.9
RBC (.10^{12}/L)	**3.4**	6.7-11.4
HCT (L/L)	**0.18**	0.29-48
MCV (fL)	**52.1**	33.6-48.3
MCH (pg)	**16.8**	12.1-16.6
MCHC (g/dL)	**32.2**	32.9-39.1
PLT-I (.10^{9}/L)	105	39-417
PLT-O (.10^{9}/L)	285	72-457
WBC (.10^{9}/L)	**22.31**	3.70-18.66
Neutrophils (.10^{9}/L)	**19.63**	1.45-9.62
Lymphocytes (.10^{9}/L)	1.56	1.18-10.36
Monocytes (.10^{9}/L)	0.67	0.09-0.82
Eosinophils (.10^{9}/L)	0.22	0.16-1.81
Reticulocytes (.10^{9}/L)	**209.1**	14.1-104.5
Reticulocytes (%)	6.15	-
Manual Aggregated Reticulocytes (%)	2.3	-
PCR analysis (*Mycoplasma haemofelis* and "*Candidatus* Mycoplasma haemominutum")	Negative	Negative

Figure 1: Sysmex XT 2000iV® scattergrams from the blood specimen of the cat

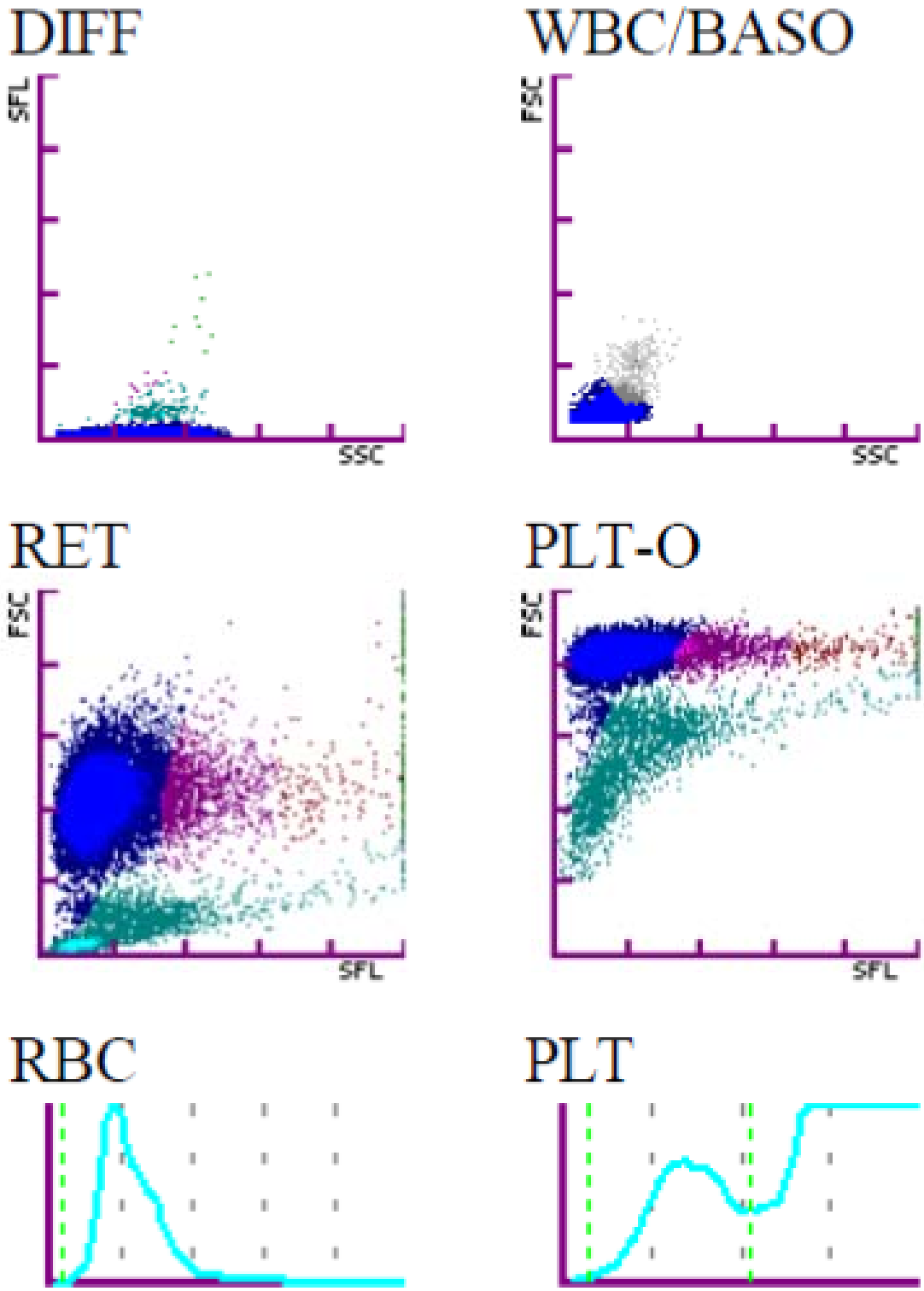

Figure 2: Picture of the blood smear edge (1000x, modified May-Grünwald Giemsa staining). Black arrow: Heinz Bodies; white arrow: free Heinz bodies

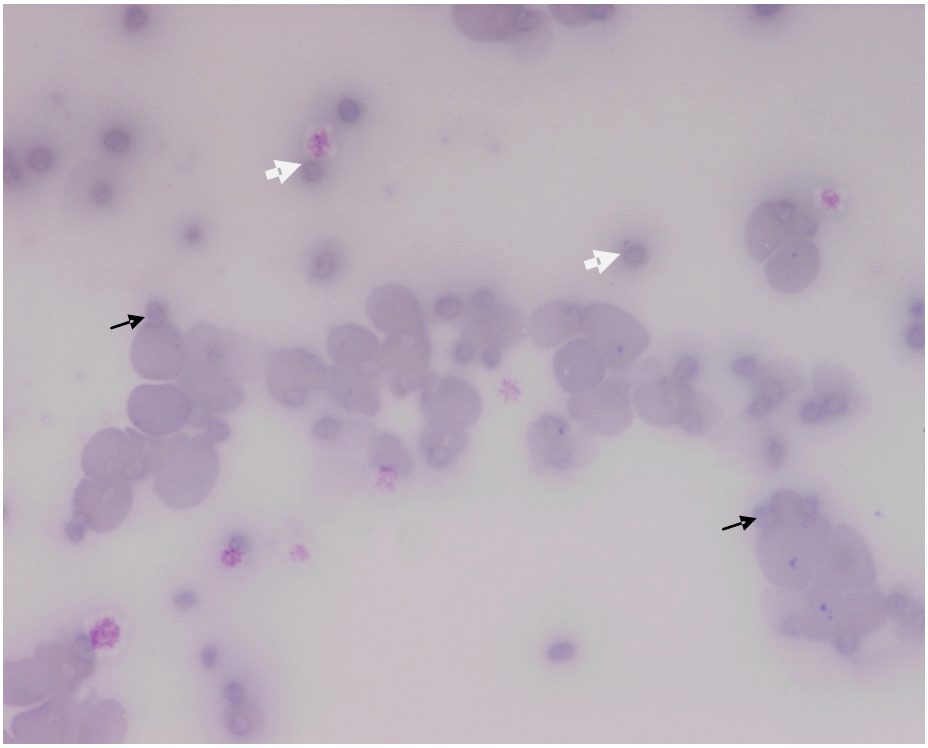

Figure 3: Picture of the blood smear (1000x, modified May-Grünwald Giemsa staining). Red arrow: blister cell; black arrow: Heinz Bodies

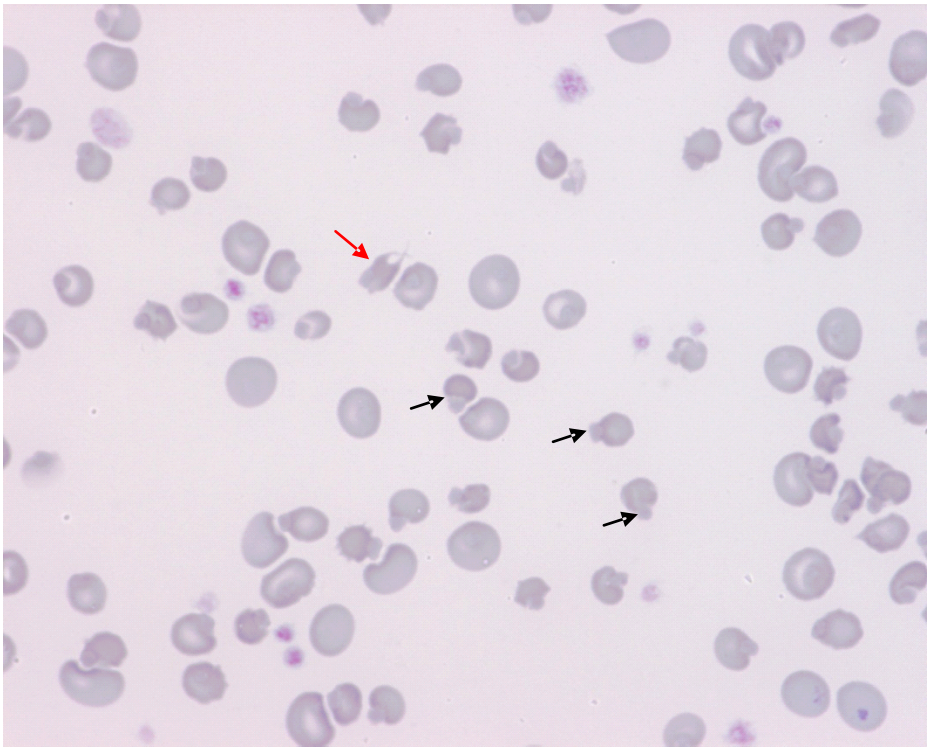

Table 2: Urinalysis results performed on centrifuged urine

Analytes	Data	Reference Interval
Source	Cystocentesis	-
Color	**Dark red**	Light yellow
USG	1.025	1.030-1.060
Sediment	Negative	< 5 cells / 40 PF
Dipstick	Unreadable	-

Table 3: Biochemistry results on plasma obtained by VetTest® (Idexx Laboratories)

Analytes	Data	Reference Interval
Color of the plasma	**Red**	Clear
Creatinine (µmol/L)	56	27-186
ALT (U/L)	**146**	20-100
PAL (U/L)	21	10-90
Total Bilirubin (µmol/L)	**13.0**	1.7-9.9
Total Protein (g/L)	75	50-82
Albumin (g/L)	30	22-44

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