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*Case Report*
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# Hemolytic Anemia in Horses Associated with Ingestion of *Pistacia* Leaves

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**Key words:** Equine; Hemolysis; Methemoglobin; Pyrogallol.

## Case 1 and Case 2

A 6-year-old Peruvian Paso-Mustang cross mare was presented to the William R. Pritchard Veterinary Medical Teaching Hospital, University of California, Davis during the fall (October) with a 2-day history of lethargy and icterus. The mare was from a herd of 26 mares and 3 foals from which 5 mares had died (leaving 21) during the preceding 7 days. These mares had varying degrees of colic, ataxia, pigmenturia, pale and icteric mucous membranes, lethargy and inappetance; they died within 48 hours of initial signs. Three of the affected mares had been pyrexic with rectal temperatures ranging from 102 to 102.5 °F. Treatment with nonsteroidal anti-inflammatory medications and oral antimicrobials was initiated on the affected mares, with no improvement in clinical signs.

The herd had been moved to the current property 6 months previously. It consisted of 40 acres of undulating land comprised of native woodland and a planted *Pistacia* orchard (containing *P. atlantica*, *P. terebinthus*, *P. chinensis*). Mares with suckling foals were housed in a separate corral and the remaining mares grazed the entire 40 acres and were supplemented with orchard grass hay. All affected horses were part of the latter group. The horses were provided with county irrigation water, which was piped to troughs, and they had access to the irrigation ditch directly. There were no recent changes in herd management or housing, except for felling of the *Pistacia* orchard shortly before the first horse developed clinical signs; the owner had witnessed the horses eating from trees that had been cut down. The same owner housed 11 stallions on a property 1.5 miles away where they were provided with well water and the same orchard grass hay. None of these horses, or the lactating mares who were housed separately and fed the same hay, exhibited any signs of illness.

**Abbreviations:**

| | |
|---|---|
| CBC | complete blood count |
| CK | creatine kinase |
| HCT | hematocrit |
| HPF | high-power field |
| PCR | polymerase chain reaction |
| PCV | packed cell volume |
| RBC | red blood cell count |
| RDW | red cell distribution width |
| WBC | white blood cell count |

Physical examination revealed tachycardia (56 beats per minute) and icteric oral, ocular, and vulvar mucous membranes. Pertinent laboratory results are listed in Table 1. Many eccentrocytes were noted on the blood smear. The low hematocrit, presence of nucleated erythrocytes, increased RDW, and indirect hyperbilirubinemia are consistent with hemolytic anemia. The eccentrocytes are indicative of oxidative damage to hemoglobin and erythrocyte membrane proteins.

A Coggins test and *Babesia caballi* and *Theileria equi* PCR results were negative. *Leptospira* antibody titers were not indicative of active infection (*L. bratislava* and *L. icterohemorrhagiae* titers were positive at 1 : 100, and *L. Pomona*, *canicola*, *grippothyphosa* and *hardjo* titers were negative). Serum was negative for nitrate, nitrite, monensin and lead, and trace minerals were within acceptable ranges except for an increased iron (3.9 ppm, reference range 0.8–2.5 ppm) consistent with hemolysis, and slightly decreased magnesium (15 ppm, reference range 18–35 ppm) consistent with reduced feed intake. Urinalysis revealed a specific gravity of 1.032, pH = 8, and proteinuria (150 mg/dL, reference range: 0 mg/dL), 6–8 erythrocytes/HPF (reference range 0–2/HPF), and hemoprotein 150 ery/μL (reference range 0 ery/μL) with no other important abnormalities. The urine was negative for the presence of myoglobin, indicating the hemoprotein present was because of

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**Table 1.** Pertinent results for Case 1 and Case 2.

| | Case 1 | Case 2 | Reference range |
|---|---|---|---|
| RBC (M/μL) | 2.85 | 3.03 | 6.2–10.2 |
| Hemoglobin (g/dL) | 5.1 | 5.7 | 11.2–17.2 |
| HCT (%) | 13.4 | 15.9 | 30–46 |
| RDW (%) | 29.4 | 17.2 | 16–20 |
| NRBC (/100WBC) | 1 | 0 | <1 |
| WBC (/μL) | 12,200 | 5,360 | 5,000–11,600 |
| Bands (/μL) | 366 | 0 | <1 |
| Platelets (/μL) | 308,000 | 190,000 | 100,000–225,000 |
| Fibrinogen (mg/dL) | 500 | 200 | 100–400 |
| Ionized magnesium (mmol/L) | 0.39 | 0.38 | 0.47–0.7 |
| Phosphorus (mg/dL) | 1.6 | 2.5 | 2.1–4.7 |
| CK (IU/L) | 1542 | 594 | 119–287 |
| Triglycerides (mg/dL) | 57 | 43 | 2–41 |
| Total bilirubin (mg/dL) | 8.3 | 7.6 | 0.5–2.3 |
| Indirect bilirubin (mg/dL) | 8.1 | 7.4 | 0.3–1.7 |
| Serum osmolality (mOsm/kg) | 275 | – | 270–300 |
| Lactate (mmol/L) | 2 | – | <1.8 |
| Methemoglobin (%) | 10.5 | 0.6 | 0–3 |

RBC, red blood cell count; HCT, hematocrit; RDW, red cell distribution width; NRBC, nucleated red blood cell count; WBC, white blood cell count; CK, creatine kinase.

hemoglobinuria, also consistent with hemolytic anemia. Polymerase chain reaction test result on urine was negative for *Leptospira* gene sequences. Urine contained 32 mg/mL of pyrogallol (reference range 0 mg/L). The presence of urinary pyrogallol is indicative of ingestion of gallic acid, present in many trees and plants.

The horse was administered ampicillin[a] (20 mg/kg IV once) flunixin meglumine[b] (0.6 mg/kg, IV once) and isotonic, polyionic fluids[c] (25 mL/kg, as a bolus IV). Minocycline[d] (4 mg/kg, PO) was administered until *Leptospira* infection was ruled out 24 hours later. Fluids were administered (isotonic, polyionic, 12.5 mL/kg IV daily) for 3 days. The lethargy, icterus, and pigmenturia gradually resolved over a few days.

A 6-year-old Lusitano mare was presented with Case 1. This mare had no obvious clinical signs of illness but the owner was concerned because of the recent deaths of 5 horses. This mare had similar hematologic and biochemical derangements as did Case 1 (see Table 1). Eccentrocytes were evident on cytologic examination.

Nitrate and nitrite were not detected in serum and serum *Leptospira* titer results were not indicative of active infection. A Coomb's test was negative. Urine was negative for *Leptospira* gene PCR. The urine was positive for pyrogallol at 90 mg/L (reference: 0 mg/L).

The mare was treated with intravenous fluids (isotonic, polyionic 44 mL/kg, followed by 2.2 mL/kg/h), ampicillin (20 mg/kg IV), flunixin meglumine (0.55 mg/kg IV), and minocycline (4 mg/kg PO). Antimicrobial treatment was discontinued the next day after receiving negative *Leptospira r*esults. Activated charcoal[e] (1 mg/kg) was administered to the mare in feed over a 12-hour period in an attempt to adsorb potential toxins from the gastrointestinal tract. The mare remained bright and maintained a good appetite throughout hospitalization. Intravenous fluid therapy was continued for 2 days and oral activated charcoal (0.1 mg/kg q 6 hours) for 3 days. Recheck of creatinine concentration on day 3 was within the reference range (0.9 mg/mL) and there was gradual improvement in PCV up to 21% by day 4 of hospitalization. The mare was discharged after 3 days.

The owner was instructed to move the mares away from access to *Pistacia* trees and to administer activated charcoal to horses observed to ingest *Pistacia* leaves or seeds. Upon moving the mares to a smaller area of the property with no access to *Pistacia* trees, there were no additional illnesses or deaths.

Necropsies were performed on 3 of the dead mares from the herd. These revealed hemoglobin nephropathy as well as hepatic and splenic hemosiderosis. Pyrogallol was present in the kidneys of 2 of the horses. Other findings included slightly low hepatic selenium concentrations (0.27, 0.25 ppm; ref range: 0.3–1.0 ppm) in 2 of the horses, and high liver iron (1700, 880, 520 ppm; ref range: 100–300 ppm) both on wet-weight basis.

A site visit was performed to inspect the property for possible toxin exposure. Sampling of the water, hay, trees, and vegetation was conducted to investigate potential intoxication as the cause of hemolytic anemia. Physical examination and blood sampling of 2 randomly selected herd mates was performed. Physical examination was unremarkable, however both mares were anemic (PCV 22% and 28% respectively, reference 30–46%).

Based on the presence of severe anemia, hemoglobinuria, eccentrocytosis, methemoglobinemia, indirect hyperbilirubinemia, presence of urine pyrogallol, negative Coombs test and negative tests for infectious diseases, a diagnosis of hemolytic anemia, likely associated with an oxidant toxin, was made. Examination of the plants retrieved from the property identified 3 varieties of *Pistacia* tree from the felled orchard, including *Pistacia atlantica*, *Pistacia terebinthus*, and *Pistacia chinensis*. No maple trees, onions, or other plants associated with oxidant damage or hemolysis in horses were found. Two in vitro screening assays using a pyrogallol standard (0.17 mg/mL) as a positive control, confirmed the oxidative properties of *P. atlantica*, *P. terebinthus*, and *P. chinensis* leaf and seed extracts on equine erythrocytes; methemoglobin formation and hemolysis resulted after exposing equine erythrocytes to these plant extracts.[1]

Two weeks after initial evaluation, repeat CBC and serum biochemistry profiles showed improved HCT of 21% and 27% in cases 1 and 2, respectively (reference 30-46%). There was no evidence of eccentrocytes and all other values were within reference ranges. Serum was also submitted for repeat *Leptospira* serology, which revealed no evidence of seroconversion.

## Case 3

A 10-year-old Quarter Horse gelding was presented to Arizona Equine Medical and Surgical Center for

obtundation and pigmenturia. The gelding's pasture mate, a 9-year-old Quarter Horse gelding, had been euthanized earlier the same day for signs consistent with hemolytic anemia and acute renal failure. The horses' pasture consisted of irrigated Bermuda grass and a single tree. The horses were observed to ingest fallen and wilted leaves from this tree. The tree was later identified as *Pistacia atlantica*. On physical examination, the gelding was obtunded, icteric, slightly tachycardic and had an initial PCV of 20% (reference range 30–46%). The gelding was treated with fluids IV (Lactated Ringers Solution (LRS)[f] 2 mL/kg/h for day 1, then 1 mL/kg/h for 6 days), activated charcoal (1 mg/kg q 12h for 2 days, then 30 mL paste PO, q 6 hours), and enrofloxacin[g] (5 mg/kg, IV q 24 h for 7 days). The PCV decreased to 10% within 24 hours of admission (reference 30–46%). The horse remained hospitalized and showed gradual improvement over a 2-week period. Urine *Leptospira* PCR, blood piroplasmosis PCR, and a Coggins test were negative. No other toxic plants were identified in the pasture. The leaves and seeds of the *Pistacia* tree were tested with the same in vitro hemolysis and oxidative assay as for the California cases. On follow-up over 12 months later, a CBC and serum biochemistry panel were unremarkable, and the horse had returned to its previous work level (roping).

## Discussion

The severity and acuteness of clinical signs described in these cases provoked detailed investigation to establish the cause of the acute hemolytic anemia. Although the presence of methemoglobinemia and eccentrocytosis on the blood smear were considered highly suggestive of oxidative damage to the red blood cells,[2,3] other potential infectious causes of hemolysis were ruled out. Testing for nitrate, nitrite, and lead, which have been reported to cause oxidative damage, also yielded negative results. Previously reported oxidizing toxins known to cause intravascular hemolysis in horses include wilted red maple, sugar maple, and silver maple leaves as well as onions.[4–6] None of these were found on the property.

Many of the clinical and clinicopathologic findings of the cases described in this report are similar to those of red maple (*Acer rubrum*) toxicosis.[6] Clinical signs of red maple toxicosis also include weakness, lethargy, icterus, pigmenturia, and even unexpected death.[6] The leaves, especially when wilted in the fall, cause severe oxidative damage to equine red blood cells, resulting in methemoglobinemia and hemolytic anemia.[7–9] A major component of the *Acer* leaves which causes methemoglobin formation has been identified as gallic acid. An amount of gallic acid equivalent to that found in *A rubrum* extract significantly increased methemoglobin concentration, compared to that in extract-free control erythrocytes, but caused less than actual *A rubrum* extract. A potential co-oxidant, 2,3-dihydro-3,5-dihydroxy-6-methoxy-4H-pyran-4-one, was found in the *A rubrum* extract, which may have been responsible for increasing methemoglobin formation above the gallic acid alone.[4]

Pyrogallol has been demonstrated to be a more potent oxidizing agent than either gallic or tannic acid.[10] In a previous study, gallic acid was metabolized to pyrogallol in equine ileum contents to a greater extent than in other gastrointestinal tract tissues.[10] Incubation of tannic acid and *A. rubrum* leaves, individually with ileum contents, produced gallic acid and subsequently pyrogallol. Ileum suspensions formed no pyrogallol when passed through a filter which excluded microbes, suggesting a microbial basis to the pathway.[10] Bacteria isolated from the ileum were found capable of pyrogallol formation. Therefore, gallotannins and gallic acid present in *A. rubrum* leaves can be metabolized by *K. pneumoniae* and *E. cloacae,* found in the equine ileum, to form pyrogallol.[10]

The detailed history provided by the owner of the California cases in our report did not suggest exposure to any known or previously reported hemolytic or oxidative toxins. The presence of pyrogallol in the urine of both affected mares, as well as in the kidneys of 2/3 necropsied horses, suggests ingestion of plants containing pyrogallol, gallic acid, or gallotannins. These findings along with the evidence of red blood cell oxidative damage (eccentrocytes, methemoglobin, hemoglobinuria) suggest a similar pathogenesis to red maple toxicosis. The affected horses had been observed to consume *Pistacia* leaves, which are known to contain gallic acid.[11] In addition, in vitro testing performed using extract from *Pistacia* leaves and seeds, similar to that previously reported to confirm the oxidative and hemolytic properties of *A. rubrum* leaves, was consistent with oxidative and hemolytic effects on equine erythrocytes.[1,4] The high liver iron concentration in all 3 necropsied horses is an expected finding with intravascular hemolysis. It is interesting to speculate whether the slightly low liver selenium concentrations in 2/3 necropsied horses were associated with an increase predisposition to or a result of increase oxidative damage.

*P. atlantica*, *P. terebinthus*, and *P. chinensis* are not native to North America, but are found in California, as well as several other states ranging from the Southwest to the Southeast of the United States. In the Middle East, these trees have been used for nutritional and medicinal purposes.[12] Oxidative and hemolytic effects of *Pistacia* spp. have not been previously reported in horses or other species; the results reported here indicate that these plants are toxic to horses. Interestingly, the California outbreak arose in the fall, the same seasonality as reported with red maple leaf toxicosis, when the leaves are wilted and falling off the trees. The similar incident in Arizona, also associated with access to a *Pistacia atlantica* tree, also occurred at a time when leaves were falling off the tree. While the horses had access to the trees throughout the remainder of the year, we propose that the pathophysiology of *Pistacia* spp. toxicosis is similar to that of red maple in that felled and wilted leaves may be of importance. Furthermore, the felled trees in the California outbreak would have allowed for easy access and ingestion of large

quantities of wilting leaves and seeds by the horses, thus accentuating these effects.

Further research is required to identify the exact pathophysiology of *Pistacia* tree toxicosis, the toxic principles involved and the quantities required to cause clinical disease in horses. Until that time, it is clear that horses must be isolated from these trees to prevent acute hemolytic anemia and death.

## Footnotes

[a] Hanford Pharmaceuticals, Syracuse, NY
[b] Banamine, Schering Plough Animal Health, Summit, NJ
[c] Baxter Healthcare Corporation, Deerfield, IL
[d] Watson Pharmaceuticals, Parsippany, NJ
[e] Toxiban, Lloyd Inc, Shenandoah, IA
[f] Abbott Animal Health, Abbot Park, IL
[g] Baytril, Bayer HealthCare Animal Health, Shawnee Mission, KS

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*Off-label Antimicrobial Declaration:* Authors declare no off-label use of antimicrobials.

## References

1. Walter KM, Moore CE, Bozorgmanesh R, et al. Oxidant-induced damage to equine erythrocytes from exposure to Pistacia atlantica, Pistacia terebinthus, and Pistacia chinensis. J Vet Diag Invest 2014;26:821–826.

2. Reagan WJ, Carter C, Turek J. Eccentrocytosis in Equine Red Maple Leaf Toxicosis. Vet Clin Path 1994;23:123–127.

3. Harvey JW, Stockham SL, Scott MA, et al. Methemoglobinemia and eccentrocytosis in equine erythrocyte flavin adenine dinucleotide deficiency. Vet Pathol 2003;40:632–642.

4. Boyer JD, Breeden DS, Brown DL. Isolation, identification, and characterization of compounds from *Acer rubrum* capable of oxidizing equine erythrocytes. Am J Vet Res 2002;63:604–610.

5. Stair EL, Edwards WC, Burrows GE, Torbeck K. Suspected red maple (Acer rubrum) toxicosis with abortion in two Percheron mares. Vet Hum Toxicol 1993;35:229–230.

6. Alward A, Corriher CA, Barton MH, et al. Red Maple (Acer rubrum) Leaf Toxicosis in Horses: A Retrospective Study of 32 Cases. J Vet Intern Med 2006;20:1197–1201.

7. George LW, Divers TJ, Mahaffey EA, Suarez MJH. Heinz Body Anemia and Methemoglobinemia in Ponies Given Red Maple (*Acer Rubrum L.*) Leaves. Vet Pathol 1982;19:521–522.

8. Divers TJ, George LW, George JW. Hemolytic anemia in horses after the ingestion of red maple leaves. J Am Vet Med Assoc 1982;180:300–302.

9. Tennant B, Dill SG, Glickman LT, et al. Acute hemolytic anemia, methemoglobinemia, and Heinz body formation associated with ingestion of red maples leaves by horses. J Am Vet Med Assoc 1981;179:143–150.

10. Agrawal K, Ebel JG, Altier C, Bischoff K. Identification of protoxins and a microbial basis for red maple (*Acer rubrum*) toxicosis in equines. J Vet Diagn Invest 2013;25:112–119.

11. Jouky M, Khazaei N. Compare of extraction of phenolic compounds from *Pistacia atlantica* in different solvents. In: Anninos P, Rossi M, Pham TD, Falugi C, Bussing A, Koukkou M, eds. Advances in Biomedical Research, Proceedings. World Scientific and Engineering Acad and Soc: Athens, Greece; 2010:361–365.

12. Bozorgi M, Memariani Z, Mobli M, et al. Five Pistacia species (*P. vera*, *P. atlantica*, *P. terebinthus*, *P. khinjuk*, and *P. lentiscus*): A review of their traditional uses, phytochemistry, and pharmacology. Sci World J 2013;2013:219815.