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, pigmentation, pale and icteric mucous membranes, lethargy and inappetence; 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 falling 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.

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, grippophiyphosa 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

Abbreviations:

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

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The paper was not presented at any meetings.

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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 12.5 mL/kg IV, followed by 2.2 mL/kg/h), and minocycline (4 mg/kg PO). Antimicrobial treatment was discontinued the next day after receiving negative *Leptospira* gene PCR. The urine was positive for pyrogallol at 90 mg/L (reference: 0 mg/L). 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 cytoclogic examination.

Nitrate and nitrite were not detected in serum and urine *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 horse 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* results. Activated charcoal c (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.

A 10-year-old Quarter Horse gelding was presented to Arizona Equine Medical and Surgical Center for evaluation and treatment of 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*, *P. terebinthus*, and *P. chinesis*. 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. chinesis* 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 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 with Case 3.
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 nation, the gelding was obtunded, icteric, slightly

A rubrum

include wilted red maple, sugar maple, and silver maple known to cause intravascular hemolysis in horses reported to cause oxidative damage, also yielded anemia.7

cells, resulting in methemoglobinemia and hemolytic cause severe oxidative damage to equine red blood

lethargy, icterus, pigmenturia, and even unexpected

412 Bozorgmanesh et al

than actual

in extract-free control erythrocytes, but caused less increased methemoglobin concentration, compared to

increased methemoglobin formation has been identified as gallic acid. An amount of gallic acid equiva-
lent 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 forma-
tion 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.11

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 one of the possible forms of metabolizing 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 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 eccentricytosis 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

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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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**Conflict of Interest Declaration:** Authors disclose no conflict of interest.

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

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