Diagnosis of EDTA-dependent pseudothrombocytopenia in a horse

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- When thrombocytopenia is diagnosed in horses that do not have clinical evidence of a bleeding tendency, EDTA-dependent pseudothrombocytopenia should be suspected.
- This type of thrombocytopenia is a result of platelet clumping in blood that contains EDTA.
- The diagnosis of EDTA-dependent thrombocytopenia can be confirmed by screening blood films for platelet clumps and by comparing platelet counts of paired blood samples—one sample containing EDTA and the other sample containing heparin.

A 14-year-old Thoroughbred gelding was examined because of pyrexia, muscle fasciculation, and intermittent excessive sweating of 18 hours’ duration. The horse had been treated with procaine penicillin G, streptomycin, flunixin meglumine, and dimethyl sulfoxide. On initial examination, rectal temperature was 38.9°C, pulse rate was 36 beats/min, and respiratory rate was 24 breaths/min. Results of physical examination were unremarkable, except the horse’s urine was noticeably red. Plasma total protein concentration was 7.3 g/dl; results of a CBC were normal, except for a low platelet count (37 × 10^3/μl; normal, 100 to 300 × 10^3/μl). The plasma was pale red. Results of serum biochemical analyses were within reference ranges with the exception of potassium concentration (2.4 mEq/L; normal, 3.0 to 4.5 mEq/L) and total bilirubin concentration (3.0 mg/dl; normal, 0 to 1.2 mg/dl). Serum concentration of bile acids was within reference range. Results of analysis of peritoneal fluid were normal. Urinalysis, including examination of urine sediment, was performed. There was a strong reaction for blood, but RBC were not seen in the urine. Results of a Coombs’ test and agar gel immunodiffusion for equine infectious anemia were negative. One-stage prothrombin time and activated partial thromboplastin time were normal. The plasma fibrinogen concentration was 471 mg/dl (normal, 100 to 300 mg/dl). Our diagnoses at that time were fever of unknown origin, hemolysis secondary to iv administration of dimethyl sulfoxide, and thrombocytopenia. The horse was treated with only polyionic fluids; it did not receive any other pharmacologic agents. The horse’s fever and hypokalemia resolved over the next 36 hours, and the hyperbilirubinemia resolved within 5 days. Subsequently, acute and convalescent serum samples were tested serologically for equine arteritis virus, equine herpesvirus-1, and equine influenza types A-1 and A-2. Changes in titer were not clinically significant. The immunofluorescent antibody titer for Ehrlichia risticii was 1:20 and was considered consistent with vaccination, but not active infection. Serum IgM concentration was 98 mg/dl (normal, 60 to 180 mg/dl).

Platelet counts in blood collected over the first 8 days after admission were consistently low (Fig 1); however, the horse did not have evidence of a bleeding tendency during this time. Examination of a bone marrow aspirate obtained 1 week after admission revealed particles of marrow of normal cellularity, a myeloid:erythroid ratio of 0.84, and normal maturation of cell lines. On the basis of a subjective assessment of bone marrow cellularity, there appeared to be more megakaryocytes than

Figure 1—Platelet count in blood samples containing EDTA (●―●) or heparin (■―■) and collected from a horse with EDTA-dependent thrombocytopenia. Dexamethasone (0.08 mg/kg of body weight, iv, q 24 h) was administered from day 9 to day 13.
normal. Dexamethasone (0.08 mg/kg of body weight, IM, q 24 h) was administered for 4 days. Administration of dexamethasone was discontinued when the platelet count was 4 × 10^7/μL and the gelding developed signs consistent with laminitis.

We considered EDTA-dependent pseudothrombocytopenia as a possible cause of the thrombocytopenia in this horse, because of the lack of evidence of spontaneous hemorrhage, the fluctuations in platelet count, and the lack of response to immunosuppressive treatment. The diagnosis of EDTA-dependent pseudothrombocytopenia can be confirmed by comparing platelet counts from a blood sample containing EDTA as an anticoagulant with platelet counts from a blood sample containing heparin as an anticoagulant. Consequentially, 5 days after the last dose of dexamethasone, a blood sample was collected into a plastic syringe. Immediately, half the sample was transferred to a glass tube containing tripotassium EDTA and the other half was transferred to a glass tube containing sodium heparin. The platelet count for blood containing EDTA was 8 × 10^7/μL, whereas that for heparinized blood was 167 × 10^7/μL. Platelet counts for blood collected the next day were similar (Fig 1). Platelet counts determined manually (by use of phase contrast microscopy and a hemocytometer) were similar. Platelet clumps were not detected in the hemocytometer. However, review of blood smears revealed large clumps of platelets at the head and feathered edges. A sample of this horse’s plasma (harvested from a blood sample containing EDTA) was added to an equal volume of plasma obtained from a horse that had a normal platelet count for blood containing EDTA, and incubated at 37 C for 15 minutes. The plasma of the gelding did not affect the platelet count in the plasma of the normal horse. The final diagnosis was EDTA-dependent pseudothrombocytopenia.

Thrombocytopenia in horses may be idiopathic or secondary to chronic infectious or inflammatory diseases (eg, equine infectious anemia, lymphosarcoma), drug administration, bone marrow depression, myelophthisic disease, or disseminated intravascular coagulation. It has been suggested that EDTA-dependent pseudothrombocytopenia may occur in horses. Spuriously low platelet counts in blood containing EDTA (EDTA-dependent pseudothrombocytopenia) have been reported in 0.9 to 1.9% of human patients. This type of pseudothrombocytopenia has not been associated with any particular disease, with splenomegaly, or with markers of autoimmune disease in human beings. It has been reported in apparently healthy human beings, but appears more frequently in severely ill human beings with autoimmune, neoplastic, atherosclerosis-related, and liver diseases. As in the horse of this report, morbidity associated with EDTA-dependent pseudothrombocytopenia in human beings is more often a result of therapeutic interventions for the incorrectly diagnosed thrombocytopenia, rather than a result of any platelet dysfunction.

This pseudothrombocytopenia in human beings is a result of platelet clumping in blood containing EDTA. The mechanism likely involves a reaction between platelets and platelet-specific antibodies in the presence of EDTA. Immunoglobulins of the IgG, IgM, and IgA classes may be involved, and the reaction occurs with either disodium or tripotassium EDTA. It is suggested that chelation and the depletion of membrane calcium changes the surface properties of the platelet, thereby exposing neoantigens that react with agglutinins. Plasma of human patients with EDTA-dependent pseudothrombocytopenia agglutinates EDTA-treated platelets of normal donors.

The mechanism of the pseudothrombocytopenia in this horse was not elucidated. However, addition of plasma from this horse to platelet-rich, EDTA-treated plasma from a donor horse failed to induce platelet aggregation, suggesting that the mechanism may not be identical to that in human beings. This is consistent with a report of EDTA-dependent pseudothrombocytopenia in a miniature pig, in which neither platelet antibodies nor other serum factors could be demonstrated.

When thrombocytopenia is diagnosed in horses without clinical evidence of a bleeding tendency, EDTA-dependent pseudothrombocytopenia should be considered. The diagnosis can be confirmed simply by screening blood films for platelet clumps and by comparing platelet counts of paired blood samples—i.e., one containing EDTA and the other containing heparin.