Case Report

Idiopathic thrombocytopenic purpura and anaemia in a pregnant mare

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Keywords: horse; anaemia; bleeding; pregnancy; purpura; thrombocytopenia

Introduction

In horses, clinical signs suggestive of thrombocytopenia reflect abnormal primary haemostasis. Horses presenting with petechial and ecchymotic haemorrhages of mucous membranes, prolonged bleeding after venipuncture, epistaxis, melena and/or hyphema have all been reported previously (Byars and Greene 1982; Larson et al. 1983; Morris and Whitlock 1983; Sockey et al. 1987; Humber et al. 1991; Cohen and Carter 1991; Fey and Sasse 1998). Thrombocytopenia may be caused by platelet production failure, increased platelet utilisation or destruction, or sequestration of platelets in the spleen (Sellon and Grindem 1994; Sellon 1998).

Thrombocytopenia caused by increased destruction of platelets is of the immune-mediated type, which includes all conditions caused by disintegration of platelets as a consequence of antibody-dependent cytotoxicity. This includes idiopathic thrombocytopenic purpura (ITP) or autoimmune thrombocytopenia. Immune-mediated thrombocytopenias can be divided into ITP with an unknown mechanism triggering the disease, and the so-called secondary immune-mediated thrombocytopenias, in which there is a specific underlying cause (Morris 1998; Sellon 1998).

Idiopathic thrombocytopenic purpura in the horse is, therefore, a coagulation disorder characterised by an insufficient number of circulating platelets in the absence of other recognisable haemostatic dysfunction. It is thought to be due to immune-mediated destruction of platelets (Morris 1998). The life span of platelets is shortened either by removal by reticuloendothelial organs when specific antibodies are directed against them, or by indirect involvement in an immune reaction (Sellon 1998). Horses of any age, sex and breed may be affected by ITP, although the disease seems to be more prevalent among young adult Thoroughbreds (Morris 1998). The treatment of choice for ITP is immunosuppressive therapy (Berchtold and McMillan 1989; George et al. 1996). Most horses with suspected ITP show an increase in platelet count following administration of dexamethasone 0.05–0.20 mg/kg bwt i.m. or i.v. within 4–7 days (Morris 1988). A dose of prednisolone 1 mg/kg bwt i.m. b.i.d. may be used in lieu of dexamethasone (Morris 1998; Sellon 1998). In this article, we report a case of a 6-year-old pregnant Thoroughbred mare with idiopathic thrombocytopenic purpura and severe anaemia.

Case details

History

A 6-year-old Thoroughbred mare in the sixth month of pregnancy was presented to the Equine Clinic of the University of Veterinary and Pharmaceutical Sciences in Brno with a history of intermittent unilateral epistaxis of 12 days’ duration. Swelling in all 4 limbs was observed several days after onset of epistaxis. Increased body temperature (39.0–39.8°C) was recorded during the 5 days before admission to the clinic. The referring veterinarian initiated therapy with penicillin immediately after onset of pyrexia. As the clinical signs (pyrexia, limb oedema and epistaxis) persisted, penicillin was replaced with kanamycin.

Clinical findings

On admission, the mare was alert and responsive but in poor body condition and examination revealed swelling in the extremities and ventral abdomen. Rectal temperature was 37.8°C and heart rate 55 beats/min. Auscultation of the lungs was unremarkable, but a holosystolic heart murmur (maximum intensity over the mitral valve area) was present. Oral and nasal mucous membranes were pale, slightly

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icteric and showed numerous petechial and ecchymotic haemorrhages. Sanguinous fluid dripped continuously from the left nostril. Endoscopy of the upper respiratory tract, trachea and gullet pouches showed no abnormalities except numerous petechial haemorrhages on the nasal, pharyngeal and tracheal mucosa. Rectal examination was normal and a live fetus was palpated in the uterus.

**Laboratory findings and diagnosis**

Haematology indicated severe microcytic anaemia with a haemoglobin concentration of 48 g/l, packed cell volume (PCV) 0.10 l/l, red cell count 2.77 x 10^{12} cells/l and mean cell volume 36 fl. Schistocytes were not found in peripheral blood smears. Platelet count was 55 x 10^9/l and white blood cell count and differential count were within reference ranges for our laboratory. Prothrombin time (PT), activated partial thromboplastin time (APTT), thrombin time, D-dimers and fibrinogen/fibrin degradation products were also within reference ranges for our laboratory. Antithrombin III and fibrinogen/fibrin degradation products were also within reference ranges for our laboratory. Antithrombin III and fibrinogen (4.2 g/l) levels were slightly elevated. There was hypoproteinaemia (total protein 39 g/l) with hypoalbuminaemia (20 g/l). Total serum bilirubin level was slightly elevated (79 µmol/l). Serum urea, creatinine and liver and muscle enzyme levels were within reference ranges. Eggs of intestinal parasites were not found on coprology.

Examination of the faeces for occult intestinal bleeding (Haemoccult) was negative on the 19th day and positive on Day 14. Examination via polymerase chain reaction of the aborted fetus for EHV-1 was negative. Petechial haemorrhages and prolonged bleeding after injection persisted. Haemoglobin concentration was 55 g/l, PCV 0.18 l/l and platelet count decreased to 14 x 10^9/l. Azathioprine was administered at an initial dose of 2.0 mg/kg bwt per os then 1.0 mg/kg bwt on subsequent days, and concurrent prednisolone treatment (0.7 mg/kg bwt) instituted. Trimethoprim/sulphadoxine (20 mg/kg bwt i.v. s.i.d.) was administered concurrently because of the immunosuppressive effect of the drugs chosen. Bleeding from the anus was noted on Day 33.

Since an improvement in clinical signs and haematology parameters was not observed, it was decided on Day 35 to increase the azathioprine dose gradually to 3 mg/kg bwt/day per os and maintain prednisolone administration (0.5 mg/kg bwt i.m.). Despite this treatment, platelet count was 17 x 10^9/l and PCV 0.10 l/l on Day 39. The mare died on Day 40.

**Post mortem evaluation**

Sternal aspiration was performed immediately after death. Bone marrow aspirate revealed adequate cellularity, numerous macrophagocytes and erythroid hyperplasia (60% of all nucleated cells were erythroid precursors). A pathological cell population was not found.

Gross pathological abnormalities noted during necropsy included mucosal anaemia, numerous mucosal haemorrhages, intestinal and lung bleeding and subcapsular haematoma of the spleen. Histopathology revealed bone marrow hyperplasia with increased red blood precursor cells and erythroid precursors, centroleubullary miliary necrosis of the liver and swelling of the colon.

**Discussion**

Various types of thrombocytopenic purpura in horses have been reported previously (Sellon and Grindem 1994; Sellon 1998). Thrombocytopenia can develop in equine infectious anaemia (Cohen and Carter 1991) and consumption of thrombocytes and clotting proteins occurs in disseminated intravascular coagulation (DIC) (Morris 1998). Thrombocytopenia in the absence of DIC or infection can be induced by certain drugs, bacterial and viral infections or develop secondary to neoplastic diseases (Sellon 1998). In the case reported here, clinical and laboratory signs of infection or DIC were not found and neoplasia was found.
neither clinically nor on necropsy. Clinical evidence of small blood vessel haemorrhage and severe thrombocytopenia in a horse with normal coagulation times (PT, APTT) and neutrophil count are suggestive of ITP (Morris 1998). We did not find any underlying disease indicating secondary immune-mediated thrombocytopenia. Diagnosis was supported in this case by the results of bone marrow examination. The number of megakaryocytes is usually normal to increased in immune-mediated and platelet consumptive processes (Sellon 1998). Megakaryocytes were slightly increased in our case.

A definitive diagnosis of ITP requires demonstration of increased platelet-associated Ig or C3. However, these methods are not available for horses (Morris 1998; Sellon 1998). Antiplatelet antibody levels can be estimated by the platelet factor 3 (PF-3) immunoinjury technique (Cohen and Carter 1991), but this test is nonspecific, poorly standardised and difficult to interpret (Morris and Whitlock 1983; Sellon 1998).

Anaemia can develop due to blood loss, haemolysis and decreased erythrocyte production or any combination of these. In the case of blood loss and haemolysis, bone marrow responds by increasing erythropoiesis. The myeloid/erythroid ratio ranges from 0.5 to 1.5 in normal horses; therefore, a ratio of less than 0.5 classifies an anaemia as regenerative (Morris 1998). A definitive diagnosis of immune-mediated haemolytic disease is based upon the demonstration of autoantibodies against the patient’s own erythrocytes. This is most accurately proven with the direct Coombs’ test, which measures the presence of autoantibodies (IgG and IgM) and complement on the surface of red blood cells. However, a false negative test may occur immediately following a haemolytic crisis, when corticosteroid therapy has been initiated or when Coombs’ antisera is inappropriately diluted (Morris 1998). Direct confirmation of immune-mediated platelet and/or erythrocyte destruction is not practically possible in the horse (McClure 2000).

The clinical and laboratory findings in our case were suggestive of not only peripheral platelet but also red cell destruction. Epistaxis lasting several days was considered to be the possible cause of anaemia immediately after admission of the mare to the clinic. This disappeared following transfusion and no massive bleeding was observed during the following 30 days. Neither melena nor discoloration of the urine were observed macroscopically and the Haemoccult test was negative on Day 19 (and positive on Day 32). We concluded that the aetiology of anaemia was combined haemorrhagic and haemolytic. Signs of haemolysis were present; serum bilirubin elevation, blood transfusion failure and microcytic anaemia were consistent with haemolysis, even though the direct Coombs’ test was negative. The regenerative anaemia and increased platelet production in this mare was confirmed post mortem by a sternal bone marrow aspirate and histopathology.

In man, ITP can be acute, chronic or recurrent. In the horse, acute (Larson et al. 1983), chronic (Cohen and Carter 1991) and recurrent (Morris and Whitlock 1983; Fey and Sasse 1998) thrombocytopenia has also been described. Cases of concomitant immune-mediated haemolytic anaemia and thrombocytopenia (Evans’ syndrome) were described in the foal by Sockett et al. (1987) and in a 3-year-old mare by Lubas et al. (1997). Immune-mediated haemolysis was confirmed by a positive Coombs’ test in both cases.

Idiopathic thrombocytopenic purpura has been treated successfully with dexamethasone at a dose of 0.1 mg/kg bwt i.v. b.i.d. with gradual reduction to 0.02 mg/kg bwt (Morris and Whitlock 1983). Larson et al. (1983) described 2 cases of ITP that responded favourably to dexamethasone at a dose of 0.1 mg/kg bwt i.m. s.i.d. However, the mechanism of therapeutic action of corticosteroids in patients with ITP is ill-defined. Corticosteroids improve the integrity of capillaries and reduce the phagocytic clearance of thrombocytes in reticuloendothelial organs; they may also diminish the production of antithromocyte antibody and impede thrombocyte-antibody interactions (George et al. 1996).

The increase in platelet count after institution of corticosteroid therapy and transfusion in our case was of a short duration (Days 4–8). This transient rise was probably caused by the transfusion. The effect of corticosteroid therapy is questionable, because further treatment was not successful. Cases of steroid-refractory ITP in the horse are rare. Two horses with ITP refractory to dexamethasone were successfully treated by oral azathioprine at a dose of 3 mg/kg bwt per os s.i.d. (Humber et al. 1991). A case of successful treatment of ITP with vincristine was described by Fey and Sasse (1998). An attempt to use azathioprine at a dose of 3 mg/kg per os failed in the present case.

A suckling foal suffering from ITP with concomitant haemolytic anaemia (Evans’ syndrome) was successfully treated with prednisolone at a dose of 2 mg/kg bwt i.m. s.i.d. then reduced to 1 mg/kg bwt i.m. (Sockett et al. 1987). Lubas et al. (1997) successfully used dexamethasone at a dose of 0.1 mg/kg i.v. bwt s.i.d. when treating Evans syndrome in a 3-year-old mare.

Treatment with splenectomy has been used previously in man and dogs with ITP resistant to corticosteroids. Splenectomy is recommended after 4–6 weeks of unsuccessful medical therapy (George et al. 1996). Azathioprine, cyclophosphamide and vinca alcaloids have also been used (Greene et al. 1982; George et al. 1996). The use of high-dose immunoglobulin, danazol, cyclosporine A, interferon-alpha and i.v. anti-D immunoglobulin has been described in man for management of acute and chronic ITP (Greene et al. 1982; George et al. 1996; Smith 1996). None of these therapeutic approaches have been described in horses as far as we are aware.

The platelet and red cell count of the mare in this report did not rise despite corticosteroid and azathioprine therapy. As far as we are aware, this is the first reported case of idiopathic thrombocytopenic purpura and anaemia in a horse unresponsive to immunosuppressive therapy.

Acknowledgement
This study was supported by the project VZ MSM 6215712403.

Manufacturer’s address

Röhm Pharma GmbH, Weiterstadt, Germany.
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