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Review Article

Clinical and laboratorial description of the differential diagnoses of hemostatic disorders in the horse

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Abstract

The process of fibrin clot formation is a series of complex and well-regulated reactions involving blood vessels, platelets, procoagulant plasma proteins, natural inhibitors, and fibrinolytic enzymes. Vasculitis can be caused by a variety of different agents as bacteria, viruses, protozoal, rickettsial organisms, toxic, drugs, medications, and neoplasms. The most common cause of vasculitis is the purpura hemorrhagica, which is associated with exposure to *Streptococcus equi* ssp. *equi* or less commonly, equine influenza. Deficiencies or defects of the hemostatic components may result in bleeding and/or thrombosis. Inherited alterations of primary hemostasis (von Willebrand disease: vWD and Glanzmann's thrombasthenia: GT) and of secondary hemostasis (hemophilia A and prekallikrein: PK deficiency) are scarcely reported in equine clinic. On the contrary, acquired alterations of primary and secondary hemostasis are commonly found. They include thrombocytopenia, platelet dysfunction due to the administration of some drugs and targeted antiplatelet agents, decreased factor synthesis (liver disease or deficiency of vitamin K), release of inactive factors, inhibition of factor activity, or excessive consumption and depletion of factors (platelets, coagulation factors, and anticoagulants factors as antithrombin (AT) and protein C). Disseminated intravascular coagulation (DIC) is the most common and complex hemostatic disorder in horses and appears to be associated with sepsis, inflammatory and ischemic gastrointestinal tract disorders and other systemic severe diseases. These alterations are commonly found in patients in intensive care units.

Key words: Hemostatic disorders, Horse

Introduction

Clinical signs suggestive of primary hemostatic defects, i.e. platelet and von Willebrand factor (vWF) disorders include petechiae, ecchymoses, and mucosal hemorrhages, such as epistaxis and melena. Secondary hemostatic defects or coagulopathies typically cause hematoma formation, hemarthrosis, and intracavitary bleeding. However, clinical signs alone do not reliably differentiate specific disorders. Severe bleeding diatheses often cause spontaneous hemorrhage, whereas mild to moderate forms may become apparent only after surgery or trauma (Brooks, 2008).

Abnormal bleeding can result from disorders of the platelets, blood vessels, coagulation factors or a combination of them. Tables 1 and 2 show the

Table 1: Laboratory tests to diagnose alterations of primary hemostasis (Brooks, 2008; Epstein, 2014)

Test	Evaluated parameters
Platelets count	Platelet count and morphology
TBT PFA-100 closure time	Platelet adhesion, aggregation, and vWF function
Platelet aggregometry	Platelet aggregation in response to agonists (ADP, collagen, PAF)
VWF assays: VWF:Ag, VWF:CBA, and VWF:RCo	VWF:Ag: Quantitative measure of vWF concentration VWF:CBA: VWF functional activity based on collagen binding VWF:RCo: VWF functional activity based on platelet agglutination VWF agglutination
Flow cytometry	Platelet membrane glycoprotein-IIb/IIIa and activation markers (bound fibrinogen, P-selectin, PS exposure, and microparticle release

TBT: Template bleeding time, ADP: Adenosine diphosphate, PAF: Platelet activating factor, VWF: von Willebrand factor, VWF:Ag: von Willebrand factor antigen, VWF:CBA: von Willebrand factor collagen binding activity, VWF:RCo: von Willenbrand factor ristocetin cofactor activity, and PS: Phosphatidylserine

Table 2: Laboratory tests to diagnose alterations of secondary hemostasis (Brooks, 2008; Epstein, 2014)

Test	Evaluated parameters
Activated clotting time	Coagulation factor activity (intrinsic and common pathway, including fibrinogen)
Coagulation screening tests: aPTT, PT, TCT, and fibrinogen	aPTT: Activity of intrinsic and common pathway factors including fibrinogen PT: Activity of extrinsic and common pathway factors including fibrinogen TCT: Fibrinogen concentration and function Fibrinogen: Quantitative and functionality
AT	AT inhibitory activity (inhibition of F IIa or Xa)
FDP	Measure products of degradation of fibrin and fibrinogen
D-dimer	Measure products of degradation of cross-linked fibrin (fibrin that was part of a clot)
Coagulation factor assays	Specific activity of coagulation factors (F II, V, VII, VIII, IX, X, XI, and XII)

aPTT: Activated partial thromboplastin time, PT: Prothrombin time, TCT: Thrombin clotting time, AT: Antithrombin, and FDP: Fibrinogen derived products

Table 3: Interpretation of coagulation profile in alterations of primary and secondary hemostasis in the horse (Brooks, 2008; Epstein, 2014)

aPTT	PT	TCT	TBT	Platelets	CT	Specific parameters	D-dimer	Alteration
			↑	Normal	↑ CT			Platelets dysfunction (uremia, aspirin, non-steroid anti-inflammatory drugs …)
			↑	↓	Normal			Thrombocytopenia
			↑	Normal	↑ CT			Glanzmann thrombasthenia
Normal or ↑			↑	Normal	↑ CT	↓ Slight FVIII:C ↓ VWF:Ag and vWF:RCo		Type 1 von Willebrand disease
Normal or ↑			↑	Normal	↑ CT	↓ Slight FVIII:C ↓ VWF:Ag ↓↓ VWF:Rco		Type 2 von Willebrand disease
↑	Normal	Normal				↓ Slight FVIII:C. Severe form: <10-15% ↓ PK (10-35%)		Intrinsic pathway defect: factors VIII (hemophilia A), IX (hemophilia B), XI Contact deficiencies: factor XII, PK/HMWK
Normal	Normal	↑						Fibrinogen deficiency or dysfunction or low concentration of inhibitors
↑	↑	Normal						Common pathway deficiency: factors II (thrombin), V and X Multiple deficiency: Vitamin K-dependent factors: II, VII, IX, and X
↑	↑							Hepatic diseases:
	↑	↑		↓			↑	Early stage End stage
↑	↑	↑						Severe hypofibrinogenemia/high concentration inhibitors Combined deficiency: fibrinogen, intrinsic, extrinsic, and common pathway factors
↑	↑	↑	↑	↓		↓ Fibrinogen ↓ AT activity	↑	Disseminated intravascular coagulation (DIC)

aPTT: Activated partial thromboplastin time, PT: Prothrombin time, TCT: Thrombin clotting time, TBT: Template bleeding time, CT: Closure time, PK: Prekallikrein, FVIII:C: Factor VIII clotting activity, VWF:Ag: von Willebrand factor antigen, VWF:RCo: von Willenbrand factor ristocetin cofactor activity, HMWK: High molecular weight kininogen, and AT: Antithrombin

hemostatic tests that are clinically used to evaluate primary and secondary hemostasis in horses with suspected coagulopathies. Factor activities lower than 30% or activated partial thromboplastin time (aPTT) and thromboplastine time (TT) values exceeding 120% of the upper end of normal reference range are considered abnormal. Commercially available analyzer platelet function, viscoelastic tests include dynamic viscoelastic coagulometry, thromboelastometry, and thromboelastography provide a holistic view of the time to clot formation, the strength of the clot formed, clot retraction, and clot lysis (Brooks, 2008; Epstein, 2014). Table 3 summarizes the primary and secondary hemostatic alterations for the diagnosis of coagulopathies in the horse. Table 4 includes reference values for hemostatic parameters in the horse.

Table 4: Reference values of hemostatic parameters in the horse (Brooks, 2008; Muñoz *et al*., 2011; Satué *et al*., 2012, 2017)

Tests (unit)	Reference ranges
Platelets count (/μL)	100-350.000
MPV (fl)	5.3-7.8
PDW (%)	26-74
PCT (%)	0.05-0.22
TBT (min)	2-6
CT (min)	60-116
VWF:Ag (%)	65-165
FVIII:C (%)	50-200
VWF:RCof (%)	>50
PT (s)	8-10
aPTT (s)	30-45
ACT (min, s)	2 min, 38 s
TCT (s)	5-9
Fibrinogen (mg/dL)	<400
AT (%)	180-220
Protein C (%)	100-120
D-dimer (ng/ml)	200-400

MPV: Mean platelet volume, PDW: Platelet distribution width, PCT: Plateletcrit, TBT: Template bleeding time, CT: Closure time, VWF:Ag: von Willebrand factor antigen, FVIII:C: Factor VIII clotting activity, VWF:RCo: von Willenbrand factor ristocetin cofactor activity, PT: Prothrombine time, aPTT: Activated partial thromboplastin time, ACT: Activated clotting time, TCT: Thrombin clotting time, and AT: Antithrombin

Vasculitis

Vasculitis can be caused by a variety of different conditions, but it is usually precipitated by a reaction of immune system against bacteria (*Streptococcus equi*), virus (equine infectious anemia, equine viral arteritis), protozoal (*Babesia caballi* and *Theilleria equi*), rickettsial organisms (*Anaplasma phagocytophilum*), toxic, drugs, medications and neoplasms, or for unknown reasons (Kaese *et al*., 2005; Balasuriya, 2014; Wilson *et al*., 2019). One of the more common causes of vasculitis is the purpura hemorrhagica, which is associated with exposure to *S. equi* ssp. *equi*, causal agent of strangles, or less commonly, to equine influenza. Other systemic diseases that provide sources of antigen are bronchopneumonia, cholangiohepatitis, ganulomatous enteritis and colitis (Pusterla *et al*., 2003). Horses with these diseases would develop complications including purpura hemorrhagica due to the antibodies combine with the bacteria or virus that are deposited in the walls of the blood vessels (Pusterla *et al*., 2003). Consequently, it is an immune-mediated vasculitis, that leads to inflammation and leakage of fluid, pain, hemorrhage, thrombosis and ischemic tissue damage (Brooks, 2008; Epstein, 2014).

Hereditary defects of primary hemostasis

Inherited platelet function disorders, alterations in the intrinsic contact system, and qualitative and quantitative defects in vWF all have been rarely identified in horses. A hereditary hemostatic defect implies a mutation within the gene encoding a specific hemostatic protein, leading to impaired synthesis or function of that protein and altering normal coagulation (Norton *et al*., 2016).

Hereditary platelet dysfunction

Hereditary platelet function defects or thrombopathies include abnormalities of platelet membrane receptors, signal-transduction pathways, granule secretion, or membrane phospholipid (Cattaneo, 2003). Glanzmann thrombasthenia (GT) is an inherited, intrinsic quantitative or qualitative defect in the heterodimer platelet membrane receptor αIIbβ3, which acts as fibrinogen receptor on the platelet membrane and consequently, it is essential for normal platelet aggregation (Nurden *et al*., 2012). This disorder has been described in Peruvian Paso horses, Quarter horses, Thoroughbred and Oldenburg horses (Livesey *et al*., 2005; Christopherson *et al*., 2006; Macieira e*t al*., 2007, 2011; Sanz *et al*., 2011). Mutational analysis performed in the Thoroughbred and Oldenburg horses identified that both horses were homozygous for a missense mutation, that led to a predicted amino acid change from arginine to proline in exon gene encoding αIIb (Christopherson *et al*., 2006; Macieira *et al*., 2011). Mutational analysis in the Peruvian Paso horse identified a homozygous ten base pair deletion encompassing the last three base pairs of exon 11 and the first 7 base pairs of intron 11 of the gene encoding the glycoprotein αIIb. The mutation was predicted to affect normal splicing of intron 11 (Sanz *et al*., 2011). A compound heterozygote possessing both of the above described mutations has been described in the Quarter horse (Livesey *et al*., 2005; Christopherson *et al*., 2006).

The most common clinical signs of GT are associated with exaggerated bleeding manifesting as purpura, epistaxis, gingival bleeding, and prolonged hemorrhage after trauma or surgery. In most of the published reports, epistaxis was the main complaint (Livesey *et al*., 2005; Christopherson *et al*., 2006; Macieira *et al*., 2007; Sanz *et al*., 2011). Glanzmann's thrombasthenia was suspected in the Oldenburg filly due to hematoma formation and excessive bleeding after arthroscopy and venipuncture (Macieira *et al*., 2007).

Diagnosis of GT is based on normal platelet count and morphology and prolonged bleeding time. Platelet function analyzer (PFA)-100 is highly sensitive for detecting GT. The PFA assay uses collagen + adenosine diphosphate (ADP) and collagen/ADP embedded cartridges to mimic a damaged vessel endothelium. As citrated whole blood flows at a high shear stress rate through these cartridges, platelets bind, creating a platelet plug (closure time-CT). Closure time is prolonged in patients with GT (Brooks, 2008). Platelet aggregation in response to various agonists was markedly impaired in the Quarter horse diagnosed with GT (Livesely *et al*., 2005).

A platelet function defect distinct from GT has been reported in Thoroughbreds (Norris *et al*., 2006, 2015). Affected horses demonstrated prolonged template bleeding time (TBT), abnormal platelet aggregation

response to certain agonists, and impaired fibrinogen binding by flow cytometric assay. The physiologic and molecular base of this defect is currently unknown.

A heritable bleeding diathesis associated with decreased thrombin generation by activated platelets was described in a 2 years old Thoroughbred mare. The mare showed platelet aggregation in response to thrombin and COL (Fry *et al*., 2005).

von Willebrand disease (vWD)

The von Willebrand disease includes quantitative and functional defects of vWF. Both inherited quantitative and functional vWF defects have been reported in horses (Brooks *et al*., 1991; Rathgeber *et al*., 2001; Laan *et al*., 2005).

The vWF is a high molecular weight glycoprotein synthesized by megakaryocytes and endothelial cells. It is found in platelets and endothelium and circulates in plasma bound to coagulation factor VIII. The functions of vWF are to stabilize and to protect circulating coagulation factor VIII from immediate degradation by protease inhibitors, and also provides a scaffold for platelet adherence and formation of the platelet plug after endothelial damage occurs (Mazurier and Meyer, 1996). Patients with vWD typically present spontaneous bleeding from mucosal surfaces or impaired hemostasis after trauma or surgery. Clinical variability in phenotype is dependent on the amount of functional vWF present. Diagnosis is based on assessment of circulating vWF antigen concentrations (VWF:Ag), vWF function (based on ristocetin cofactor activity or collagen-binding capacity), evaluation of multimeric forms of vWF, and comparison of VWF:Ag to activity ratio (Lillicrap, 2007).

Three distinct types of vWD have been described in people and dogs, but only two types have been reported in horses. Type 1 vWD is defined as a partial quantitative protein deficiency with diagnosis based on normal vWF multimeric structure and low levels of circulating VWF:Ag with a concomitant reduction in vWF function (Mazurier and Meyer, 1996). It has been reported in an Arabian filly and a Quarter horse colt (Laan *et al*., 2005), with multiple hematomas and hemarthrosis. Diagnosis of type 1 vWD is based on prolonged aPTT, decreased VWF:Ag activity (8%), reduced vWF function, and low-normal factor VIII:C activity. Maternal inheritance has been suspected (Laan *et al*., 2005). Type 2 vWD is defined as a qualitative defect in vWF and can be further divided into subtypes 2A, 2B, 2M, and 2N (Laan *et al*., 2005). Cases reports of type 2 vWD have been described on a Quarter Horse filly (Brooks *et al*., 1991) and on a Thoroughbred mare and her foal (Rathgeber *et al*., 2001). In these clinical cases, a diagnosis of vWD type 2A, consistent with a loss of platelet-dependent function due to abnormal multimers, was suspected (Brooks *et al*., 1991; Rathgeber *et al*., 2001). Diagnoses were made based on prolonged TBT, low plasma VWF:Ag with disproportionately severe loss of vWF function, and decreased concentrations of high molecular weight vWF multimers (Brooks *et al*., 1991; Rathgeber *et al*., 2001). Type 3 vWD is an autosomal recessive disease and represents a severe quantitative defect of vWF characterized by immeasurable or extremely low levels of vWF (Mazurier and Meyer, 1996). No clinical cases of type 3 vWD have been documented in horses until now.

Hereditary defects of secondary hemostasis

Inherited coagulation factor deficiencies

Inherited defects caused by coagulation factor deficiencies identified in horses include deficiencies in coagulation factors VIII (hemophilia A), IX (hemophilia B), and combined factor deficiencies (Henninger, 1988; Littlewood *et al*., 1991; Winfield and Brooks, 2014).

The genes encoding coagulation factors VIII and IX are located on the X chromosome. Clinical disease primarily affects males with mutations occurring either as *de novo* stochastic events or as an X-linked mode of inheritance from phenotypically normal females. Both syndromes can manifest as mild, moderate, or severe clinical bleeding based on the percentage of residual coagulation factor activity. Hemophilia A has been described as the most common inherited coagulopathy reported in Thoroughbreds, Standardbreds, Quarter, and Tennesse Walking horses and in Shetland ponies (Mills and Bolton, 1983; Henninger, 1988; Littlewood *et al*., 1991). In addition, Winfield and Brooks (2014) showed an acquired hemophilia A caused by inhibitory antibodies against factor VIII in a Thoroughbred mare. Bleeding tendencies after minor injury and recurrent hemarthrosis have been associated with a poor prognosis (Sabatini *et al*., 2012).

Diagnosis is based on clinical signs, coagulation panel findings (prolonged aPTT with a normal platelet count and prothrombin time (PT)), and decreased FVIII:C activity. The severity of disease varies inversely with the percentage of FVIII:C activity. Severe disease has been associated with a FVIII:C activity lower than 10-15% (Sabatino *et al*., 2012).

In an early report, Hinton *et al*. (1977) described a 2-month-old Arabian colt with clinical signs of epistaxis, hematoma formation, and excessive bleeding from injection sites. Coagulation profile revealed prolonged aPTT, decreased activity for VIII, IX, and XI factors and normal II, V, VII, and X factors. The colt was diagnosed with a multiple coagulation factor deficiency of the intrinsic pathway, although a single factor deficiency with secondary consumption of the remaining factors could not have been ruled out. Alterations in coagulation panels and coagulation factor activity were not found in the dam, sire, half-sister or brother of the affected colt.

Additional inherited intrinsic pathway defects include deficiencies in the contact activator prekallikrein (PK), described in specific families of miniature and Belgian horses (Geor *et al*., 1990). Prekallikrein is a glycoprotein which functions in conjunction with high molecular weight kininogen (HMWK) and coagulation factor XII to form the intrinsic contact system (Yarovaga *et al*., 2002). Prekallikrein circulates in plasma bound to HMWK.

Activation of coagulation factor XII occurs due to contact of the negatively charged surface of damaged subendothelium with the PK-HMWK complex. Small amounts of activated factor XII cleave PK to kallikrein and thus acts as a source of auto-activation for additional coagulation factor XII. Kallikrein also augments the cleavage of HMWK to form bradykinin, an inflammatory mediator, and the activation of the plasminogen activator pro-urokinase (Yarovaga *et al*., 2002). Deficiencies in PK have an autosomal recessive mode of inheritance and are diagnosed via a prolonged aPTT and decreased level of serum PK (10-35% activity) (Girolami *et al*., 2010) with normal HMWK and coagulation factor activity (Geor *et al*., 1990).

Acquired hemostatic disorders affecting primary hemostasis

Thrombocytopenia

Platelet counts lower than 30.000/µL are usually associated with clinical bleeding, including petechial and ecchymotic hemorrhages of mucosal membranes, epistaxis, increased bleeding after venipuncture, melena or hyphema. Thrombocytopenia may be due to a variety of mechanisms such as reduction of thrombopoiesis, increased peripheral destruction of platelets, sequestration of the spleen and loss of platelets by idiopathic origin (Sellon, 1998).

Myelosuppressive drugs such as phenylbutazone, chloramphenicol, estrogens, irradiation and bone marrow diseases, as myeloptisis, myelofibrosis, myeloproliferative disease, myelodysplasia and idiopathic medullary aplasia with pancytopenia have been associated with thrombocytopenia (Cian *et al*., 2013; Muñoz *et al*., 2013; Barrell *et al*., 2017; Cooper *et al*., 2018). Increased peripheral destruction of platelets have been found in primary and secondary immunomediated thrombocytopenia (IMT). The primary IMT platelets are removed by the action of the mononuclear phagocytic system in liver, spleen and bone marrow (McGurrin *et al*., 2004; Ouellette *et al*., 2004; Brooks *et al*., 2007; Satué *et al*., 2017). Neonatal alloimmune thrombocytopenia has been previously reported in foals (Buechner-Maxwell *et al*., 1997; Boudreaux and Humphries, 2013). Secondary IMT has been associated with virus (herpesvirus, influenza, equine infectious anemia), bacteria (endotoxemia, neonatal septicemia, *A. phagocytophilum*), protozoa (*Theileria equi* and *B. caballi*), neoplasia, immune-mediated hemolytic anemia (IMHA), glomerulonephritis, drugs (penicillin, heparin, trimethoprim-sulfadoxine, azathioprine), vasculitis and toxins (Sockett *et al*., 1987; Clabough *et al*., 1991; Humber *et al*., 1991; McGovern *et al*., 2011; Rodríguez *et al*., 2014; Cooper *et al*., 2018; Eberhardt *et al*., 2018; Wilson *et al*., 2019). Another cause of thrombocytopenia is severe combined immunodeficiency (SCID) in foals (Giguère and Polkes, 2005). Further, increased consumption and loss of platelets are observed in hemorrhage of different origin, complex coagulopathies as disseminated intravascular coagulation (DIC) and localized activation of coagulative and fibrinolytic processes. Thrombocytopenia are a common laboratorial finding in vasculitis, vascular neoplasms, as hemangiosarcoma, kidney diseases (hemolytic-uremic syndrome) and gastrointestinal or inflammatory diseases (Monreal *et al*., 2000; Johns *et al*., 2005; Dickinson *et al*., 2008). In addition, spleen disorders associated with storage capacity and with production of anti-megakaryocyte antibodies have been related to thrombocytopenia in horses in a limited number of cases (Satué *et al*., 2012, 2014, 2017). Recently, hypersplenism was suspected to be the cause of thrombocytopenia in a Miniature horse (Ruby *et al*., 2018).

Platelet dysfunction

Administration of some drugs can result in significant platelet function alterations. Drugs that impair platelet function include reserpine, aspirin, phenylbutazone and other nonsteroidal antiinflamatory drugs, sulfonamides, estrogens, chlorpromazine, halothane, and phenothiazines (Muñoz *et al*., 2011; Blond *et al*., 2013; Burkett *et al*., 2016; Gilbertie *et al*., 2018). Aspirin and other nonsteroidal anti-inflammatory drugs act by mean of inhibition of intraplatelet cyclooxygenase, resulting in impaired production of thromboxane and diminished platelet response to other agonists (Burkett *et al*., 2016). The effects of targeted antiplatelet agents on equine platelet activation response have been described, including fibrinogen receptor complex and ADP receptor blockade, in addition to altered cyclic adenosine monophosphate (cAMP) metabolism (Mateos-Trigos *et al*., 2002; Weiss and Evanson, 2004).

The production of elevated levels of immunoglobulins, specifically immunoglobulin M (IgM) in monoclonal gammopathies, impairs platelet function by protein coating of platelet surfaces (Zangani *et al*., 2007). Anemia, liver failure, uremia, paraproteinemia, and DIC alter intrinsic platelet metabolic pathways or cause extrinsic changes in blood viscosity (Shen and Frenkel, 2007).

Acquired hemostatic disorders affecting secondary hemostasis

Acquired coagulation factor deficiencies generally result from decreased factor synthesis, release of inactive factors, inhibition of factor activity, or excessive consumption and depletion of factors (Brooks, 2008). Acquired causes of coagulopathy include liver disease, vitamin K deficiency, drug or toxin exposure, and DIC. The liver is the primary site of synthesis and clearance of coagulation factors, and therefore, liver failure (hepatic necrosis, cirrhosis or acute hepatitis) can produce fibrinogen and factors deficiency and coagulopathies. Hemorrhage is an indicator of severe liver failure and a poor prognostic sign. Synthetic failure of liver disease may impair hemostasis by means of vitamin K deficiency, dysfibrinogenemia, platelet dysfunction, or abnormal clearance of fibrinolytic activators and fibrin degradation products (FDP) (McGorum *et al*., 1999;

Lisman and Leebeek, 2007; Johns and Sweeney, 2008; Satué *et al*., 2013, 2017).

Vitamin K is a lipid-soluble vitamin present in milk and green forage, synthesized by the intestinal microflora and absorbed from the intestine in the presence of bile salts. Vitamin K acts as a coenzyme that catalyzes posttranscriptional γ-carboxylation of glutamic acid residues on factors II, VII, IX, and X, allowing them to bind calcium and thereby localize on negatively charged phospholipid surfaces, leading to activation of the coagulation pathway. In the absence of this carboxylation, vitamin K-dependent clotting factors cannot bind to platelets and the coagulation process becomes defective. These processes are more common in human neonates that are relatively vitamin K deficient because of low vitamin K stores at birth, poor placental transfer of vitamin K, low concentrations of vitamin K in milk, and lack of production of vitamin K by the immature intestinal microflora (Clarke and Shearer, 2007; Burke, 2013). In adult humans, vitamin K deficiencies result from inadequate intestinal absorption or impaired intrahepatic recycling, biliary obstruction, intrahepatic cholestasis, chronic oral antibiotic administration, and infiltrative bowel disease may all reduce vitamin K absorption (Parker, 2013; Dong *et al*., 2018). Coumadin (warfarin), second-generation anticoagulant rodenticides (e.g., brodifacoum), and sweet clover contaminated with mold producing dicoumarol all act to block irreversibly the activity of vitamin K epoxide reductase, a critical vitamin K-processing enzyme. Coagulopathies attributable to vitamin K antagonism have been diagnosed in horses, ponies, and miniature donkeys (Ayala *et al*., 2007; McGorum *et al*., 2009).

Combined deficiencies of factors II, VII, IX, and X cause prolongation of the aPTT and PT screening tests. However, changes in plasma fibrinogen concentrations are not detected (Ayala *et al*., 2007; Epstein, 2014).

Disseminated intravascular coagulation

Disseminated intravascular coagulation (DIC) is a complex syndrome characterized by marked activation of the intravascular coagulation, resulting in massive thrombin activation, fibrin formation, and subsequent, widespread microvascular clot deposition. Marked activation of the coagulation can be mainly counteracted by downregulation and depletion of natural anticoagulant proteins (antithrombin: AT and protein C) and upregulation of fibrinolysis inhibitors. Subsequent platelet and coagulation protein depletion resulting from ongoing coagulation may induce severe consumption coagulopathy and, secondarily, severe hemorrhagic diathesis. When fibrin formation is severely increased, and fibrinolysis is reduced or suppressed, widespread fibrin formation and deposition may cause small and midsize blood vessel thrombosis, ischemic tissue lesions, and multiorgan dysfunction/failure syndrome (Cotovio *et al*., 2007, 2008; Cesarini *et al*., 2016).

Disseminated intravascular coagulation is the most common hemostatic disorder in horses and is always secondary to a severe clinical condition. It occurs in septic newborn foals, in horses with inflammatory and ischemic gastrointestinal tract disorders and other systemic conditions (Dolente *et al*., 2002; Pusterla *et al*., 2007; Armengou *et al*., 2008; Cotovio *et al*., 2008; Cesarini *et al*., 2016). This coagulopathy has a wide range of the clinical signs, and diagnosis is based on the presence of a predisposing disease and clinical pathology findings. Signs of coagulopathy may be nonexistent in case of subclinical DIC or can be consistent with systemic excessive thrombosis and multiorgan dysfunction or excessive bleeding as consumption occurs (Cotovio *et al*., 2008). Common signs of DIC are prolonged bleeding after minor surgery or wounds as well as petechiation and/or spontaneous hemorrhage as epistaxis and hemorrhagic diathesis from mucous membranes (Brooks, 2008). The thrombotic form of DIC might lead to multiorgan dysfunction and result in kidney, liver or respiratory failure, colic, heart arrhythmia, shock, and death (Monreal *et al*., 2000). Laboratory diagnosis of DIC include thrombocytopenia (usually lower than 100.000/µL), prolonged PT and aPTT, low fibrinogen concentrations (lower than 150 mg/dL), low AT activity (lower than 60-70%) and increased FDP or D-dimers (Monreal *et al*., 2000).

Conclusions

Disorders of primary and secondary coagulation are common clinical findings in horses, both as primary entities and as complications of many other diseases. Due to this high frequency of appearance, investigations are continuously developing on the diagnostic procedures and the pathophysiological mechanisms underlying the coagulopathies in equids. This information should be incorporated into the daily clinic. Future research should be aimed at early diagnosis and towards the development of new treatment strategies.

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