Flow cytometric assessment of activation of peripheral blood platelets in dogs with normal platelet count and asymptomatic thrombocytopenia

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Abstract
Platelets play a crucial role in hemostasis. Their activation has not yet been evaluated in healthy dogs with a normal and low platelet count. The aim of this study was to determine the influence of activators on platelet activation in dogs with a normal platelet count and asymptomatic thrombocytopenia. 72 clinically healthy dogs were enrolled. Patients were allocated into three groups. Group 1 consisted of 30 dogs with a normal platelet count, group 2 included 22 dogs with a platelet count between 100 and 200x10⁹/l and group 3 consisted of 20 dogs with a platelet count lower than 100x10⁹/l. Platelet rich-plasma (PRP) was obtained from peripheral blood samples using tripotassium ethylenediaminetetraacetic acid (K₃-EDTA) as anticoagulant. Next, platelets were stimulated using phorbol-12-myristate-13-acetate or thrombin, stabilized using procaine or left unstimulated. The expression of CD51 and CD41/CD61 was evaluated. Co-expression of CD41/CD61 and Annexin V served as a marker of platelet activation. The expression of CD41/CD61 and CD51 did not differ between the 3 groups. Thrombin-stimulated platelets had a significantly higher activity in dogs with a normal platelet count than in dogs with asymptomatic thrombocytopenia. Procaine inhibited platelet activity in all groups. In conclusion, activation of platelets of healthy dogs in vitro varied depending on the platelet count and platelet activator.

Key words: asymptomatic thrombocytopenia, canine, flow cytometry, platelet activation

Introduction
Platelet activation in a response to a blood vessel wall damage plays a crucial role in the process of hemostasis. Platelets can become activated after adhesion to extracellular matrix in the site of endothelial injury and/or in response to the action of secondary mediators such as thrombin, adenosyno-5'-diposphor (ADP) and thromboxane A₂ (Boudreaux et al. 2010). The degree of platelet activation depends on the number and the density of surface receptors, composition of cytoplasmic granules and the concentration of