

## WHY DO GREYHOUNDS BLEED?

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With the increasing popularity of retired rescued Greyhounds, veterinarians are likely to evaluate dogs of this breed more frequently in their practice. It is estimated that approximately 120,000 Greyhounds lived in homes as pets, compared to 55,000 Greyhounds in racetracks. In the past few years, private Greyhound adoptions ranged from 15,000 to 18,000/year (Gary Guccione, National Greyhound Association, personal communication).

Therefore, it is important that they recognize the physiological peculiarities of this breed. For instance, mean packed cell volume (PCV), hemoglobin concentration, red blood cell count, and whole blood viscosity are higher, while white blood cell, neutrophil, and platelet counts are lower in Greyhounds than in other breeds<sup>1-3</sup>. In addition, serum creatinine concentrations and liver transaminase activities are higher than in non-Greyhound dogs<sup>1,4</sup>.

In the Greyhound community, the term "Greyhound bleeder" is typically used for dogs that bleed either spontaneously or after minor trauma or a simple surgical procedure. An extensive literature search using a combination of "Greyhound and bleed, bleeder, hemorrhage, bleeding diathesis, platelets, and/or coagulopathies", did not yield any specific articles on the syndrome. However, the speaker (who recently founded Veterinarians for Retired Racing Greyhounds) conducted a survey of 30 veterinarians involved in Greyhound rescue, inquiring about bleeding complications after surgery in the breed. Twenty veterinarians returned the survey, and the consensus opinion is that 10 to 15% of Greyhounds bleed profusely (frequently requiring a blood transfusion as a life-saving measure) 1 to 4 days after simple surgical procedures such as spays, neuters, dewclaw removals, or laparotomies. A retrospective evaluation of the medical records of the last 10 Greyhounds who had a limb amputated for primary bone tumors at OSU revealed that most of them had severe postoperative bleeding (9/10); the bleeding was diffuse, in the ventral abdomen and distal limbs, and frequently required transfusion of blood components. In contrast, only 1/10 non-Greyhound dogs that underwent amputation during the same period required a transfusion due to local bleeding (ligature failure).

At OSU we receive 2 to 6 consults a month from practicing veterinarians about "bleeder Greyhounds". A website that evaluates causes of death in Greyhounds in the UK ([www.gurk.demon.co.uk/ghsurvey](http://www.gurk.demon.co.uk/ghsurvey)) lists "bleeding" and "stroke/blood clots" as the cause of death in 3% of the dogs. Finally, a survey of Greyhound rescue websites revealed that "bleeders" are prominently listed in the health care manual for most rescue groups. Most of the dogs with bleeding diathesis evaluated by practicing veterinarians have normal one-stage prothrombin time (OSPT), activated partial thromboplastin time (APTT), and platelet counts.

Greyhounds are not one of the breeds with a reported high prevalence of von Willebrand disease (VWD). However, in a recent 2-year review period (7/2002 to 7/2004) approximately 10% (22 of 216) of the Greyhounds screened at the Comparative Coagulation Section had plasma VWF concentration of  $\leq 30\%$ . Therefore, it is possible that VWD is responsible for the perioperative bleeding in some of the Greyhounds.

The buccal mucosa bleeding time has been used historically to screen for VWD in dogs; however, this test has marked inter- and intraobserver variability, and is not highly reliable on the clinical setting<sup>5</sup>. On the other hand, the PFA-100® is an objective and sensitive instrument to detect low concentrations (or abnormal structure) of VWF in people<sup>6</sup> and dogs<sup>7-10</sup>. Therefore, we will use the PFA-100® as a rapid screening method for VWD; results of the platelet aggregation assays will be correlated with the VWF concentration and collagen binding assays (CBA)<sup>6,11</sup>.

Greyhounds are also one of the few breeds in which a hemolytic uremic- or thrombotic thrombocytopenic purpura-like syndrome has been described<sup>12-13</sup>. It is referred to as "cutaneous and renal vasculopathy of Greyhounds", and it has been described only in racing Greyhounds, who are typically fed a raw meat-based diet<sup>13</sup>. In children, *E.coli* O157:H7 infection may cause a rapidly progressive thrombotic microangiopathy referred to as hemolytic uremic syndrome (HUS); this syndrome is typically preceded by hemorrhagic gastroenteritis, and culminates in embolic acute renal failure that frequently necessitates dialysis<sup>14</sup>. Thrombotic thrombocytopenic purpura (TTP) is another thrombotic microangiopathy that occurs primarily during pregnancy (or

postpartum) and results in embolization of the brain or other tissues<sup>14</sup>. Some authors believe that HUS and TTP are difficult to differentiate, and that they represent different manifestations of a broader spectrum of microangiopathic thromboses<sup>15</sup>. Renal and vascular lesions in affected Greyhounds are similar to those of the TTP/HUS in people<sup>12</sup>.

A high proportion of human patients with TTP have unusually large multimers of VWF in plasma, and lack a metalloprotease (ADAMTS 13) that cleaves the large multimers directly from the surface of endothelial cells<sup>14</sup>. These large multimers result in adhesion and aggregation of platelets to the endothelium, and thrombotic microangiopathy. Shiga toxins (produced by *Shigella dysenteriae* or *E. coli* O157:H7) induce production of high-molecular weight VWF multimers in the endothelium, thus resulting in a similar effect in people<sup>14</sup>.

Recently, it was shown that racing Greyhounds with or without diarrhea have a high fecal load of *E. coli* Shiga toxins 1 and 2<sup>15</sup>; moreover, experimental injection of Shiga toxin to Greyhounds results in a syndrome indistinguishable from TTP/HUS in people<sup>16</sup>. Because this mechanism can play a role in the development of thrombocytopenia and subsequent bleeding in Greyhounds, a CBA to detect high-molecular weight VWF multimers will be used in the study.

We recently documented that 67% of retired racing Greyhounds have a soft, 1/6 systolic basilar murmur associated with high aortic velocity<sup>18</sup>. Humans with aortic endocarditis frequently have severe gastrointestinal (GI) bleeding<sup>19</sup>; this is due to the fact that blood flow through a stenotic valve depletes VWF high-molecular weight multimers from circulation, resulting in a type 2A acquired von Willebrand syndrome and bleeding from preexisting GI angiodysplasia. The severe GI bleeding and depletion of high-molecular weight VWF multimers are corrected by valve replacement therapy<sup>19-20</sup>.

A similar situation (i.e.; platelet dysfunction in patients with aortic stenosis) was recently documented in dogs by Tarnow et al (2004) using the PFA-100™ platelet function analyzer<sup>21</sup>. Evaluation of high-molecular weight multimers of VWF by the CBA and platelet function using the PFA-100® will shed light on this potential mechanism of bleeding in Greyhounds.

In summary, we are currently evaluating Greyhounds pre- and postoperatively for hemostatic abnormalities including platelet counts, platelet function using the PFA-100®, OSPT, APTT, fibrinogen, plasminogen, antiplasmin, D-dimer, VWF, VWF-CBA, factor XIII, and antithrombin. Results will be presented at the conference.

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