Von Willebrand's disease in the German shepherd dog

R G Lobetti[a] and T Dippenaar[a]

ABSTRACT

Two litters of German shepherd dogs were evaluated for a haemorrhagic tendency that was characterised by excessive bleeding from the umbilicus at birth, haemorrhage and haematoma formation at vaccination, excessive bruising, and lameness due to haemarthrosis. Platelet counts, clotting times and Von Willebrand's factor (VWF) assays were assessed in all dogs. Factor VIII determination was performed in 1 puppy and its parents. Based on the clotting times and VWF assay, 6 puppies (4 male and 2 female) showed type I Von Willebrand's disease (VWD), 5 (4 male and 1 female) possible type II VWD, and 4 were unaffected. One puppy with possible type II VWD had very low factor VIII activity; its sire had a normal factor activity, whereas the dam was in the low-normal range. This article reports type I and possible type II VWD in 2 related litters of German shepherd dogs, the latter being rare in German shepherd dogs.

Key words: factor VIII, German shepherd dogs, haemorrhagic tendency, Von Willebrand's disease, Von Willebrand's factor assay.

Lobetti R G, Dippenaar T **Von Willebrand's disease in the German shepherd dog**. *Journal of the South African Veterinary Association* (2000) 71(2): 118–121 (En.). Department of Companion Animal Medicine, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

INTRODUCTION

Von Willebrand's disease (VWD) is a heterogeneous bleeding disorder caused by quantitative and/or qualitative abnormalities of Von Willebrand factor (VWF)[1,13,14]. Von Willebrand factor is a plasma glycoprotein required for platelet adhesion to the blood vessel wall during the formation of the primary haemostatic plug[3,7,11,17]. High morbidity and low mortality are usual in this disease. Physical, emotional and physiological stress or hormonal imbalances, especially hypothyroidism and concomitant disease, can affect the laboratory and clinical manifestation of VWD[11]. There are significant inter- and intra-breed differences in the clinical and laboratory expression of the disorder, which suggests that VWD actually represents a group of different abnormalities with a common end-point, namely the deficiency of a functional active VWF protein[8].

Von Willebrand factor is a large protein ranging from 0.5×10^6 to over 12×10^6 daltons consisting of a multimeric structure made up of identical 0.27×10^6 dalton sub-unit polymers[8,10,16]. Von Willebrand factor is found in plasma, alpha granules of platelets, megakarocytes, vascular endothelial cells and in the sub-endothelial matrices of blood vessel walls[10,16]. It is synthesised by the endothelial cells and megakarocytes[7]. In comparison to human platelets, canine platelets contain little or no VWF[7]. Endothelial cells release VWF from a constitutive pathway directly into the circulating blood or the sub-endothelium, bypassing the need for storage in granules. Release of stored VWF is stimulated by a variety of physiological substances, including histamine, thrombin, fibrin and oestrogen[16]. The major physiological role of VWF is in primary haemostasis, specifically in the adhesion of platelets at the site of vascular injury to the sub-endothelial structures of damaged blood vessels and in platelet-to-platelet interactions[8]. When a vessel is injured, VWF binds the components of the exposed sub-endothelium to a specific platelet receptor, forming a bridge between sub-endothelium and platelets, thus facilitating platelet adhesion under high shear-rate conditions[10].

Von Willebrand factor circulates as a non-convalenently bound complex with factor VIII, which can physiologically be separated by thrombin and phospholipid. This cleavage is essential to allow factor VIII to participate in secondary haemostasis, which results in the generation of fibrin and the formation of a stable thrombus[2,15]. Von Willebrand factor prolongs the half-life of factor VIII in circulation and hence decreased VWF may be associated with low factor VIII activity[13]. The binding of factor VIII in plasma prevents its proteolytic digestion[7,14]. Since VWF plays a dual role in haemostasis by mediating platelet adhesion to the sub-endothelium and protecting factor VIII from proteolytic inactivation, the disease is characterised by defective platelet adhesion, prolonged bleeding time, and often an accompanying deficiency in factor VIII[7,14,16,17].

Von Willebrand's disease is the most commonly occurring inherited bleeding disorder of man and dogs[12,13,17], having been reported in more than 54 different dog breeds[8,11], with a prevalence in the Doberman pinscher, German shepherd dog, golden retriever, miniature schnauzer, Scottish terrier and Pembroke Welsh corgi[5]. Isolated cases have also been diagnosed in other breeds as well as in cross-bred dogs[5].

Von Willebrand's disease is classified into 3 major types based on the clinical severity, inheritance pattern and quantitative function and structure of the VWF protein[11]. Type I is the most common, in which the concentration of VWF is low but the multimeric pattern is normal; in type II, the large mulitmers of VWF are reduced or not detectable, and may be qualitatively altered, and in type III the entire VWF molecule is not detectable[2,8,10,14].

We report type I and possible type II VWD in a group of related German shepherd dogs, the latter type being rare in the German shepherd dog.

MATERIALS AND METHODS

Animals

Two litters from the same dam but different sires were evaluated for a haemorrhagic tendency that was characterised by excessive bleeding from the umbilicus at birth, haemorrhage and haematoma formation at vaccination, excessive bruising, and lameness as a result of haemarthrosis. Each litter consisted of 8 puppies (5 males and 3 females in each). At evaluation, the puppies were approximately 6–8 weeks of age.

Blood collection

Blood was collected from the jugular vein, using a 22-gauge needle with a

[a]Department of Companion Animal Medicine, Faculty of Veterinary Science, University of Pretoria, Private Bag X04, Onderstepoort, 0110 South Africa.

Received: January 2000. Accepted: May 2000.

holder into citrated (9 parts of blood to 1 part sodium citrate) and EDTA vacuum tubes (Vacutainer, SA Scientific) from each dog. Within 30 minutes the citrated samples were centrifuged at 3000 rpm for 15 minutes. The plasma was stored at –20 °C.

Haemostasis assays

Platelet counts were determined using an automated cell counter (Cell Dyn 3500, Abbott Laboratories) by means of impedance counting. The prothrombin time (PT) was determined on the freshly harvested plasma using simplastin reagent (Organon Teknika Corporation). The partial thromboplastin time (PTT) was determined on the freshly harvested plasma using an automated APTT reagent (Organon Teknika Corporation). Von Willebrand factor was measured in the stored plasma using a commercial antigen assay test kit (VWF Zymtec test kit, DMS Laboratories), which is an enzyme-linked immunosolvent assay (ELISA). Assay for Factor VIII was done in 1 puppy, its sire and its dam by the Haematology and Clinical Chemistry section of the Animal Health Trust, Newmarket, Suffolk, England.

RESULTS

In total, 16 puppies, their dam, the dam's sister, brother and mother, and 1 sire were evaluated. Haemostasis results are tabulated in Table 1.

In all dogs, the platelet counts were within normal limits. The PT activity was also normal, whereas 6 of the puppies (5 male, 1 female) showed prolonged PTT activity. Von Willebrand factor was normal (>70 %) in 6 dogs, borderline in 1 and abnormal (<50 %) in 13. One dog was euthanased prior to VWF testing.

Based on the clotting times and VWF assay, 6 puppies (4 male and 2 female) showed type I VWD, 5 (4 male and 1 female) possible type II VWD, and 4 were unaffected (Fig. 1; Table 2). From the clinical signs and prolonged PTT, the euthanased puppy most probably also had type II VWD. The dam and 1 sire had type I VWD. The dam's mother was a carrier and the dam's littermates were normal. All the dogs with possible type II VWD showed severe clinical signs of haemorrhage (excessive bleeding from the umbilical cords, subcutaneous haematomas, and haemarthrosis) and had severely prolonged PTTs. One puppy with possible type II VWD disease had very low factor VIII activity. The factor VIII activity in the sire was normal, whereas the dam had low-normal factor activity.

Table 1: **Haemostasis parameters of the 2 litters of German shepherd dogs affected with Von Willebrand's disease.**

Dog type	Sex	PT %[a]	PTT %[a]	VWF %	Disease
1	M	95	122	100	Normal
2	M	98	177	49	Possible II
3	M	95	217	49	Possible II
4	M	101	227	ND	?
5	M	98	227	36	Possible II
6	F	97	134	78	Normal
7	F	97	118	80	Normal
8	F	87	120	73	Normal
9	M	99	265	32	Possible II
10	M	108	147	30	I
11	M	111	145	45	I
12	M	101	129	32	I
13	M	102	134	39	I
14	F	102	152	28	Possible II
15	F	99	141	23	I
16	F	105	136	42	I
Dam	F	97	114	38	I
Sire of one litter	M	98	86	45	I
Dam's brother	M	ND[b]	ND	100	Normal
Dam's sister	F	ND	ND	100	Normal
Dam's mother	F	ND	ND	55	Carrier

[a]PT = prothrombin time; PTT = partial thromboplastin time. PT/PTT >150 % is abnormal.
[b]Not done.

DISCUSSION

Von Willebrand's disease is a highly prevalent haemorrhagic diathesis with high morbidity and low mortality[4], caused by insufficient concentration or a functional abnormality of VWF[5]. VWD was first recognised in dogs in a closely related family of German shepherd dogs in 1970[7]. The diagnosis was based on life-long bleeding tendencies with decreased factor VIII activity in related males and females, decreased platelet adhesion to glass bead columns, prolonged bleeding times, and a delayed post-transfusion rise in factor VIII activity. It was also shown that the dogs had moderately reduced plasma concentrations of VWF antigen.

Although a deficiency or dysfunction of VWF is usually associated with abnormal primary haemostasis, abnormal secondary haemostasis may also occur[2]. Clinical signs include skin bruising, mucosal bleeding, haematuria, epistaxis, protracted haemorrhage after injury or surgery (tail docking, ear cropping or dew claw removal), eosinophilic panostitis, stillbirth or neonatal deaths, excessive umbilical cord bleeding at birth and joint bleeds[4,5,7,12,13,17]. Affected dogs described in

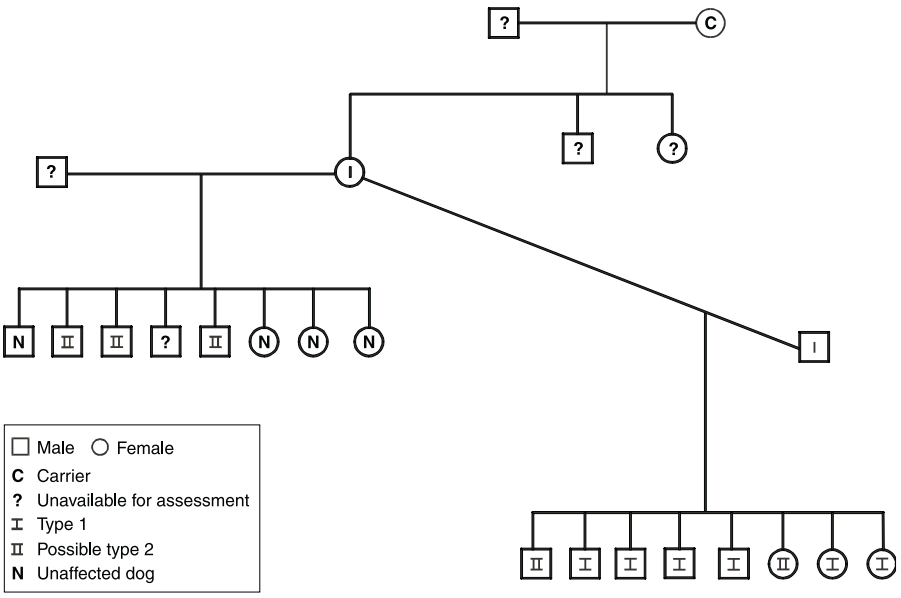

Fig. 1: **Pedigree evaluation of the German shepherd dog families affected with Von Willebrand's disease.**

Table 2: **Diagnosis of Von Willebrand's disease[a].**

PTT[b]	VWF factor (%)	Interpretation
Normal	70 or greater	Normal dog
Normal	70–79	Lower end of normal
Normal	50–69	Borderline normal (equivocal results) or heterozygous carrier of VWD gene
Normal	<50	Type I VWD. This is the most common form of VWD. Heterozygous carrier of VWD
Mildly elevated	<50	Type I VWD. Exhibited some bleeding problems (haematuria, epistaxis, melaena, post-surgical bleeding)
Elevated	<50	Type II VWD. Clinically affected animals exhibit severe bleeding signs

[a]Modified from VWF Zymtec, DMS Laboratories.
[b]Partial thromboplastin time.

this report showed similar clinical signs. Severely affected dogs may bleed to death from surgical procedures[4]. Clinically severe bleeding diathesis has been described in the German wirehaired pointer, Scottish terrier, Shetland sheepdog, Chesapeake Bay retriever and German shorthaired pointer[2]. In the Scottish terrier the animals are usually young, show bleeding from multiple mucosal sites, and death attributable to uncontrolled haemorrhage[1]. The puppies described in this report showed excessive bleeding from the umbilical cords, subcutaneous haematomas and haemarthrosis, whereas the adult dogs were asymptomatic, which is similar to the situation described in the related German shepherd dogs[7]. In those animals the disease manifested either as a rare and severe form (frequent serious or fatal bleeding episodes and markedly abnormal laboratory test results) or the more common moderate or mild form (infrequent bleeding episodes and moderately reduced or near-normal test results). Affected dogs described in this report showed both forms of the disease.

In type I VWD the bleeding tendency is mostly of minor importance, although severe bleeding may occur in some patients[7,16]. This was evident in some of the dogs that are described in this report. Type II has been reported in German shorthaired and wire-haired pointers and is associated with a severe bleeding tendency[6], which was also evident in some of the dogs described in this report. Type III has been described as an autosomal recessive trait in Scottish terriers and Chesapeake Bay retrievers, in which heterozygous carriers are asymptomatic but homozygous descendants of 2 carrier parents have severe bleeding tendencies and VWF cannot be detected in their plasma[1,2,11,14]. In humans, type II VWD is found in 10–20 % of affected people, whereas type III is a rare form of VWD[2]. However, based on the pattern of genetic transmission and quantitative structural or functional abnormalities of VWF, at least 25 sub-types of VWD have been classified in humans[14]. In the Shetland sheepdog, a combination of types I and III VWD has been reported[7,11].

Types I and II VWD are usually inherited as an autosomal dominant trait with variable clinical expression because of variable penetrance of the VWD gene[2,4,11,14], but recessive inheritance has recently been proven in the Doberman pinscher[6]. Affected individuals are compound heterozygotes with a different defect on each of the VWF alleles[10]. This type of inheritance is termed incompletely dominant, with both sexes equally affected in VWD, unlike haemophilia, which is an X-linked recessive trait that classically affects males[4]. A homozygous carrier for the gene can have 2 asymptomatic heterozygous or carrier parents, or there may be autosomal incompletely dominant expression (variable penetrance), in which both homozygous and heterozygotes can manifest a bleeding tendency. A recessive form of VWD has been recognised in Poland China swine, Scottish terriers and Chesapeake Bay retrievers[4]. The inheritance pattern of type I in humans is often autosomal dominant, but in some families inheritance can be recessive[10]. In the Doberman pinscher, matings between parents with low- or mid-range plasma VWF concentrations produced some offspring that had mid-range or low concentrations. Matings between parents with normal VWF concentrations produced only normal offspring and matings between parents with low VWF concentrations produced only offspring with low plasma VWF concentrations[10]. Severely affected parents are more likely to produce severely affected puppies. A similar trend was evident in the German shepherd dogs described in this report.

Von Willebrand's disease can be confused with haemophilia A, as both diseases can exhibit a low factor VIII activity and prolonged PTT. However, dogs with VWD have abnormal or low levels of VWF protein, whereas haemophiliacs do not[4]. This was evident in 1 of the dogs described in this report for which factor VIII was measured. A marked secondary decrease in factor VIII coagulant activity to 5 % or less, resulting in a prolonged PTT, can occur in some human families with VWD. By contrast, factor VIII seldom drops below 20 % in comparable canine VWD families. These dogs have either normal or only slightly prolonged PTTs, thus the PTT assay has limited diagnostic value in the dog[7]. All the dogs that had a prolonged PTT probably had type II VWD. It is highly unlikely that these dogs also suffered from haemophilia, as both males and females were affected.

Bleeding time assays give the best indication of the haemostatic status of individuals with VWD, but these assays are not specific, as many conditions can interfere with primary haemostasis and produce prolonged bleeding times[7,9]. Bleeding time assays were not performed in the dogs described in this report, as sedation would have been required to adequately perform the test.

The diagnosis of VWD has most often been based on subnormal VWF antigen concentration in plasma, but this concentration is not always a good indicator of the severity of the haemorrhagic defect[7]. VWF in dogs can be quantified by the Laurell electroimmunoassay, which measures the ability of plasma protein to cross-react with antibodies specific for a VWF protein, or by use of rocket electroimmunodiffusion in agarose[12]. A more sensitive assay for canine VWF is an enzyme-linked immunosorbent assay (ELISA) test[11]. The advantage of the ELISA test is its relatively technical simplicity, low cost, sensitivity, accuracy, and suitability for the screening of large series of test plasma[12]. This test was used in this study. The test kit can be used to identify dogs likely to be afflicted with VWD and to identify asymptomatic carriers of the VWD trait. The test provides a quantitative measure of VWF based on the protein's antigenic properties. Owing to the test's sensitivity, it can measure extremely low levels of VWF, such as those seen with homozygous type III VWD disease.

Based on the ELISA test, dogs that are free of the VWD trait, when bred to a normal-testing mate, should only produce offspring of normal genotype with

VWF levels of 70 % or greater. Dogs in the borderline range (50–60 % VWF) cannot be accurately classified as free of the VWD trait or as carriers on the basis of a single measurement. It is recommended that dogs whose tests fall in this range be bred only to mates with levels well within the normal range. Their offspring should be tested to identify normal individuals and to further clarify the VWD status of the parents. Healthy dogs with abnormal levels (0–49 % VWF) are considered to be carriers of the VWD trait and can transmit the defect to some of their offspring, and therefore should not be used as breeding animals.

Based on the history, clinical findings and haemostatic tests it would appear that some of the dogs in this report possibly had type II VWD, although it could not be confirmed, as multimer analysis was not carried out.

ACKNOWLEDGEMENTS

We would like to thank Dr Stan Krawitz for referring the dogs, the dog breeders for providing the animals and some of the funding, and the Animal Health Trust for doing factor VIII assay on 1 dog.

REFERENCES

1. Brooks M, Dodds W J, Raymond S C 1992 Epidemiologic features of Von Willebrand's disease in Doberman pinschers, Scottish terriers, and Shetland sheepdogs: 260 cases (1984–1988). *Journal of the American Veterinary Medical Association* 200: 1123–1127
2. Brooks M, Raymond S, Catalfamo J 1996 Severe, recessive Von Willebrand's disease in German wirehaired pointers. *Journal of the American Veterinary Medical Association* 209: 926–929
3. Brooks M, Raymond S, Catalfamo J 1996 Plasma Von Willebrand factor antigen concentration as a predictor of Von Willebrand's disease status in German wirehaired pointers. *Journal of the American Veterinary Medical Association* 209: 930–933
4. Dodds W J 1984 Von Willebrand's disease in dogs. *Modern Veterinary Practice* 63: 681–686
5. Johnson G S, Lees G E, Benson R E, Rosborough T K, Dodds W J 1980 A bleeding disease (Von Willebrand's disease) in a Chesapeake Bay retriever. *Journal of the American Veterinary Medical Association* 176: 1261–1263
6. Johnson G S, Turrentine M A, Dodds W J 1987 Type II Von Willebrand's disease in German shorthaired pointer. *Veterinary Clinical Pathology* 16: 7
7. Johnson G S, Tussentine M A, Kraus K H 1988 Canine Von Willebrand's disease. a heterogeneous group of bleeding disorders. *Veterinary Clinic of North America: Small Animal Practice* 18: 195–229
8. Johnstone I B, Norris A M, Hirzer L 1993 Type III Von Willebrand's disease in Scottish terriers. a report of two cases. *Canadian Veterinary Journal* 34: 679–681
9. Meyers K M, Wardrop K J, Meinkoth J 1992 Canine Von Willebrand's disease: pathobiology, diagnosis and short-term treatment. *Compendium on Continuing Education for the Practicing Veterinarian* 14: 13–23
10. Moser J, Meyers K M, Russon R H 1996 Inheritance of Von Willebrand factor deficiency in Doberman pinschers. *Journal of the American Veterinary Medical Association* 209: 1103–1106
11. Raymond S C, Jones D W, Brooks M B, Dodds W J 1990 Clinical and laboratory feature of a severe form of Von Willebrand's disease in Shetland sheepdogs. *Journal of the American Veterinary Medical Association* 197: 1342–1346
12. Slappendel R J, Frielinck R A J, Mol J A, Noordzij A, Hamer R 1992 An enzyme-linked immunosorbent assay (ELISA) for Von Willebrand factor antigen (VWF-Ag) in canine plasma. *Veterinary Immunology and Immunopathology* 33: 145–154
13. Slappendel R J 1995 Von Willebrand's disease in Dutch kooiker dogs. *The Veterinary Quarterly* 17: 521–522
14. Slappendel R J, Beijer M, Van Leeuwen M 1998 Type III Von Willebrand's disease in Dutch kooiker dogs. *The Veterinary Quarterly* 20: 93–97
15. Stokol T, Parry B W, Mansell P D 1995 Factor VIII activity in canine Von Willebrand disease. *Veterinary Clinical Pathology* 24: 81–90
16. Thomas J J 1996 Von Willebrand's disease in the dog and cat. *Veterinary Clinics of North America: Small Animal Practice* 26: 1089–647
17. Turecek P L, Gritsch H, Piekler L, Auer W, Fischer B, Mitterer A, Mundt W, Schlokat U, Dorner F, Brinkman H J, Van Mourik J A, Schwarz H P 1997 In vivo characterization of recombinant Von Willebrand Factor in dogs with Von Willebrand's disease. *Blood* 90: 3555–3567