

## Standard Article

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Prevalence and Mode of Inheritance of the *Dal* Blood Group in Dogs in North AmericaS. Goulet , U. Giger, J. Arsenaault, A. Abrams-Ogg, C.C. Euler, and M.-C. Blais

**Background:** The *Dal* blood group system was identified a decade ago by the accidental sensitization of a *Dal*– Dalmatian with a *Dal*+ blood transfusion. Similar *Dal*-related blood incompatibilities have been suspected in other Dalmatians, Doberman Pinschers, and other breeds.

**Objectives:** To determine the prevalence and mode of inheritance of the *Dal* antigen expression in dogs.

**Animals:** A total of 1130 dogs including 128 Dalmatians, 432 Doberman Pinschers, 21 Shih Tzus, and 549 dogs of other breeds including 228 blood donors were recruited from North America between 2008 and 2015.

**Methods:** Prospectively, dogs were blood typed for *Dal* applying a gel column technique using polyclonal canine anti-*Dal* sera. Pedigrees from 8 typed families were analyzed.

**Results:** The prevalence of the *Dal*+ blood type varied between 85.6 and 100% in Dalmatians and 43.3–78.6% in Doberman Pinschers depending on geographical area. *Dal*– dogs were identified mostly in Dalmatians (15/128; 11.7%), Doberman Pinschers (183/432; 42.4%), and Shih Tzus (12/21; 57.1%), and sporadically in mixed-breed dogs (3/122; 2.5%), Lhasa Apsos (1/6) and Bichon Frises (1/3). Only 6/245 (2.4%) blood donors were found to be *Dal*–, including 5 Doberman Pinschers. The mode of inheritance of the *Dal*+ phenotype was determined to be autosomal dominant.

**Conclusions and Clinical Importance:** The high percentage of *Dal*– Doberman Pinschers, Dalmatians and Shih Tzus increases their risk of being sensitized by a blood transfusion from the common *Dal*+ donor. Extended *Dal* typing is recommended in those breeds and in dogs when blood incompatibility problems arise after initial transfusions.

**Key words:** Blood typing; Dog erythrocyte antigen; Hemolysis; Transfusion.

Many blood group systems have been described in dogs using immunohematological studies. Among these, 7 have been recognized internationally and are referred to as dog erythrocyte antigen (DEA) 1, 3, 4, 5, 6, 7, and 8.<sup>1–4</sup> Dogs do not possess clinically important naturally occurring alloantibodies.<sup>3,5,6</sup> Thus, a first mismatched transfusion is not expected to lead to an acute hemolytic transfusion reaction. However, alloantibodies produced after sensitization by mismatched blood can result in ineffective transfusion or acute hemolytic

## Abbreviations:

AKC	American Kennel Club
CHUV	Centre Hospitalier Universitaire Vétérinaire
DEA	dog erythrocyte antigen
EDTA	ethylene-diaminetetraacetic acid
FMV	Faculté de médecine vétérinaire
OVC	Ontario Veterinary College
RBC	red blood cell
vWD	von Willebrand disease

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PennGen and the Small Animal Blood Bank of the FMV offer blood typing and compatibility testing services.

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transfusion reaction, if a second transfusion is administered to a previously sensitized dog.<sup>7–11</sup>

A decade ago, the erythrocytic *Dal* antigen was described after accidental sensitization of an anemic Dalmatian dog by a DEA 1 matched transfusion. Four other *Dal*– Dalmatians were identified.<sup>12</sup> However, a high prevalence of *Dal*+ was seen among blood donor dogs, which suggested that *Dal* might be a high-frequency red blood cell (RBC) antigen rather than a blood group system similar to DEA 4,<sup>9,10</sup> but a larger survey in dogs was lacking. *Dal*– anemic dogs will likely be sensitized by their first transfusion with blood from the common *Dal*+ blood type dog,<sup>12</sup> and if requiring further blood transfusions, compatible *Dal*– blood donors might be difficult to find.

The objectives of this study were to determine the mode of inheritance of the *Dal* antigen expression and to define the prevalence of *Dal*+ and *Dal*– blood in Dalmatians, Doberman Pinschers, and some other breeds of dogs including blood donors in North America.

## Materials and Methods

## Animals

From 2008 to 2015, Dalmatians, Doberman Pinschers, and dogs of other breeds, including canine blood donors, were recruited

from the veterinary clinics of 3 Universities (University of Guelph/Ontario Veterinary College [OVC], University of Montreal/Centre Hospitalier Universitaire Vétérinaire [CHUV], and University of Pennsylvania [PennVet]), dog shows, and private blood banks in Canada and the United States. Dogs recruited from the veterinary clinics were either sick dogs or healthy dogs admitted for screening of subclinical disorders or routine health evaluation.

### ***Dalmatians and Doberman Pinschers***

The majority of dogs recruited from OVC were asymptomatic Doberman Pinschers enrolled in a study of dilated cardiomyopathy. In addition to dogs recruited from the veterinary clinics, breeders were approached at dog shows to obtain blood samples from their Dalmatians and Doberman Pinschers. In order to establish the mode of inheritance of the *Dal* blood group system, some breeders were also asked to submit samples from families including dam, sire, and offspring. Most Dalmatians in the USA were recruited at the Dalmatian Club of America National Specialty show in Huron, Ohio, in 2015.

**Shih Tzus.** After recognizing a blood incompatibility in a *Dal*– Shih Tzu at OVC, additional Shih Tzu dogs were recruited from that clinic.

**Dogs of other breeds and blood donors.** Canine blood donors (>23 kg, 2–8 years old) were recruited from the 3 university blood banks listed above as well as from private blood banks in Canada and the United States. Dogs of other breeds that were not blood donors were recruited from the 3 universities and represented either dogs in need of a blood transfusion or dogs with blood incompatibility after a prior transfusion, or left-over blood samples from PennVet or CHUV.

Information including the medical history, breed, color, sex, and age of dog was obtained, in addition to the DEA 1 type, official registration name and number, and geographical location of dog at time of collection when available. Dalmatian and Doberman Pinscher owners were asked whether their dog was already enrolled in a blood donor program: if so, the information was used for descriptive data of the blood donor group, but the individual dog remained within its specific breed group for statistical purposes. The study was approved by the institutional animal care and use committees of the universities of Guelph, Montreal, and Pennsylvania.

### ***Blood Samples***

Ethylene-diaminetetraacetic acid (EDTA)-anticoagulated blood samples were collected from the jugular or cephalic vein with owner consent, or represented left-over samples submitted to the universities' clinical laboratories.

### ***Dal Blood Typing***

*Dal* blood typing was performed using neutral buffered gel column cards from Diamed<sup>d</sup> and Ortho Clinical Diagnostic.<sup>b,12,13</sup> Sera containing polyclonal anti-*Dal* antibody obtained after an accidental sensitization of a *Dal*– Doberman Pinscher from Tufts University in 2007<sup>c</sup> and of a sensitized *Dal*– research Beagle from the University of Montreal in 2013 were used undiluted.<sup>14</sup> Both sera were similarly active against the *Dal* antigen when compared to the serum from the original index *Dal*– Dalmatian.<sup>12</sup>

Briefly, a 0.8% RBC suspension was prepared by adding 10  $\mu$ L of packed and washed RBCs to 1 mL of low ionic strength saline solution.<sup>d,e,12</sup> Fifty  $\mu$ L of this RBC suspension was placed in the chamber on top of the gel column in addition to 25  $\mu$ L of anti-*Dal* serum, and then, the card was incubated at 37°C for 15 minutes.<sup>f</sup> The gel column card was then centrifuged<sup>g</sup> for 10 minutes, and the

degree of agglutination was read.<sup>12</sup> If the RBCs migrated to the bottom of the gel column, the result was negative, and the dog was typed as *Dal*–. If the RBCs were trapped on top or within the gel column, the results were positive and graded from 1+ to 4+.<sup>15,16</sup>

### ***DEA 1 Blood Typing***

If the DEA 1 information was not already available from the medical record, dogs were also blood typed for DEA 1 using either commercially available gel column tests<sup>h</sup> or, when they became unavailable in 2011, immunochromatographic strips,<sup>i</sup> according to the manufacturer's instructions.<sup>17</sup> Both techniques use the same monoclonal anti-DEA 1 antibody.<sup>18</sup>

### ***Detection of Dal Alloantibodies***

Plasma samples from *Dal*– dogs identified at PennVet, and from a *Dal*– Shih Tzu from OVC with transfusion-related incompatibilities, were examined for anti-*Dal* antibodies using the gel column technology and known *Dal*+ RBCs.<sup>13,18</sup>

### ***Statistical Analysis***

The prevalence of the *Dal*+ blood type with a 95% confidence interval was estimated for selected breed groups with larger sample size and for which *Dal*– dogs were identified (i.e., Dalmatians, Doberman Pinschers, Shih Tzus, and other breeds) per geographical areas (Ontario [Canada], Quebec [Canada], and USA). For Dalmatians and Doberman Pinschers, 1 puppy per litter was randomly selected (using a pseudorandom number generator in SAS v.9.4)<sup>j</sup> for prevalence estimations, and statistical adjustments were made for potential clustering of dogs within breeders and geographical regions.<sup>k</sup> Therefore, 24 Dalmatian puppies from 5 litters and 63 Doberman Pinscher puppies from 14 litters were excluded from estimation of geographical prevalences. Because of its clinical importance, risk factors were evaluated for the *Dal*– phenotype. Multivariate exact logistic regressions<sup>19</sup> were used to determine whether the *Dal*– blood type was significantly associated with sex, age, coat color, DEA 1 type, and geographical area and were performed separately for each breed with a sufficient sample size and in which *Dal*– dogs were detected. One dog per breeder was randomly selected for inclusion in these analyses. A forward selection procedure was used to build each model, using a  $P < .05$  as criteria for inclusion of variables (exact test).<sup>1</sup> Pedigree analysis was built based on the *Dal*+ or *Dal*– type and was analyzed for simple Mendelian inheritance patterns.<sup>20</sup>

## **Results**

### ***Breeds and Demographics***

From 2008 to 2015, 1130 dogs were typed for *Dal* including 128 Dalmatians, 432 Doberman Pinschers, 21 Shih Tzus, and 549 dogs of other breeds. This included 228 non-Dalmatian and non-Doberman Pinscher blood donors from volunteer blood donor programs: CHUV and 2 associated local private blood banks ( $n = 69$ ), OVC ( $n = 98$ ), and PennVet, which also received samples from blood donors at Hemopet<sup>m</sup> and the Ohio State University<sup>n</sup> ( $n = 61$ ). The 321 nonblood donor dogs represented various breeds in need of blood transfusions or from which left-over samples were available at PennVet or CHUV. The ages of all dogs considered together ranged from 1 month to 15 years. The description of the selected population for the prevalence study

after exclusion of randomly selected puppies is detailed in Table 1.

### Dal Type Gel Column Technique and Prevalence

All *Dal* blood typing results using the gel column technology yielded easily interpretable agglutination reactions, with either no (grade 0) or strongly positive agglutination reactions (3+ or 4+) (Fig 1).

The prevalence of the *Dal*<sup>+</sup> phenotype varied among breeds and geographical area (Tables 1 and 2). *Dal*<sup>-</sup> dogs were identified mostly in Dalmatians (12%), Doberman Pinschers (42%), Shih Tzus (57%), and few other breeds. All other purebred dogs were *Dal*<sup>+</sup> except for 1 Bichon Frise and 1 Lhasa Apso. Three *Dal*<sup>-</sup> dogs were detected among 122 mixed-breed dogs (Table 2).

**Table 1.** Prevalence with 95% confidence intervals (C.I.) of the *Dal*<sup>+</sup> blood type in Dalmatians, Doberman Pinschers, Shih Tzus, and other breeds from various areas of North America.

Origin	N	Year of sample submission	Mean age	<i>Dal</i> <sup>+</sup> Prevalence (%)	
				Estimate	95% C.I.
<b>Dalmatians<sup>d</sup></b>					
<i>Quebec, Canada</i>					
CHUV	5	2014–2015	4.4	100.0	47.8–100.0
Breeder	7 <sup>a</sup>	2013–2015	2.6	100.0	59.0–100.0
<i>Ontario, Canada</i>					
OVC	2	2010–2015	3.0	100.0	15.8–100.0
<i>USA</i>					
Breeder	90 <sup>b</sup>	2015	3.9	85.6	76.4–94.7
<b>Doberman Pinschers<sup>c</sup></b>					
<i>Quebec, Canada</i>					
CHUV	93	2013–2015	4.5	59.1	48.5–69.2
Breeder	104 <sup>c</sup>	2013–2015	3.3	43.3	28.9–57.6
<i>Ontario, Canada</i>					
OVC	158	2008–2013	5.5	72.2	64.5–79.0
<i>USA</i>					
PennVet	14	2015	5.4	78.6	49.2–95.3
<b>Shih Tzus</b>					
<i>Quebec, Canada</i>					
CHUV	2	2014–2015	11.5	100.0	15.8–100.0
<i>Ontario, Canada</i>					
OVC	14	2008–2014	9.0	21.4	4.7–50.8
<i>USA</i>					
PennVet	5	2015	9.1	80.0	28.4–99.5
<b>Other breeds</b>					
<i>Quebec, Canada</i>					
Blood donors	69	2013–2015	3.6	100.0	94.8–100.0
CHUV	27	2013–2015	4.9	100.0	87.2–100.0
<i>Ontario, Canada</i>					
Blood donors	98	2008–2013	4.2	99.0	94.5–100.0
OVC	13	2008–2015	4.3	100.0	75.3–100.0
<i>USA</i>					
Blood donors	61	2015	4.2	100.0	94.1–100.0
PennVet	281	2016	8.1	98.6	96.4–99.6

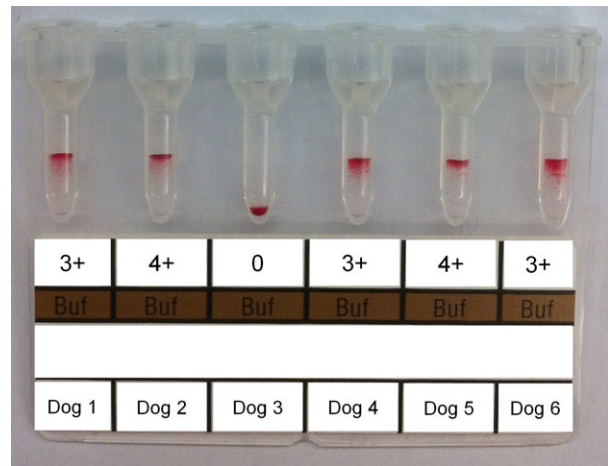
<sup>a</sup>From 6 breeders.

<sup>b</sup>From 50 breeders.

<sup>c</sup>From 15 breeders.

<sup>d</sup>Including 1 blood donor.

<sup>e</sup>Including 16 blood donors.



**Fig 1.** *Dal* blood typing results using the gel column technology yielded easily interpretable agglutination reactions, with either no (grade 0) or strongly positive agglutination reactions (3+ or 4+). Dog 1, 2, 4, 5, and 6 are *Dal*<sup>+</sup> and Dog 3 is *Dal*<sup>-</sup>.

### Dal Type in Blood Donors

Of the 228 non-Dalmatian and non-Doberman Pinscher blood donors 227 were *Dal*<sup>+</sup>, including commonly recruited breeds such as Greyhounds (n = 73), Labradors Retrievers (n = 23), German Shepherd Dogs (n = 18), and Golden Retrievers (n = 14). All nonblood donor dogs of these same 4 breeds were also *Dal*<sup>+</sup> (Table 2). Only 1 *Dal*<sup>-</sup> mixed-breed blood donor was identified (at OVC).

Of all the Doberman Pinschers tested, 16 had been enrolled in a blood donor program, and 5 of them were *Dal*<sup>-</sup>. Only 1 Dalmatian was already part of a blood donor program and was *Dal*<sup>+</sup>.

### Evaluation for Anti-Dal Alloantibodies

No anti-*Dal* alloantibodies were detected in plasma of the 23 *Dal*<sup>-</sup> dogs tested without prior transfusion history. However, the plasma from the previously transfused *Dal*<sup>-</sup> Shih Tzu, found to be incompatible to regular donors, showed strong anti-*Dal* alloantibodies by the gel column method (4+).

### Risk Factor Analysis Related to Dal<sup>-</sup> Type

Risk factors for *Dal*<sup>-</sup> blood type were examined for Dalmatians and Doberman Pinschers (Table 3). No variables were significantly different for *Dal*<sup>+</sup> and *Dal*<sup>-</sup> Dalmatians. For Doberman Pinschers, *Dal*<sup>-</sup> blood type did not vary by sex, coat color, or DEA 1 status ( $P > .05$  for all analyses), but varied significantly by geographical region; the probability of *Dal*<sup>-</sup> was significantly greater in Quebec compared to Ontario, Canada (odds ratio of 2.01  $P = .01$ ).

### Mode of Inheritance

One Dalmatian and 7 Doberman Pinscher families comprised of 117 dogs (60 *Dal*<sup>-</sup> and 57 *Dal*<sup>+</sup>) were

**Table 2.** Percentage of *Dal+* dogs in breeds represented by  $\geq 20$  individuals or in which *Dal-* dogs were identified in North America.

Breed	Number of dogs			Percentage of <i>Dal+</i> (%)
	Total	<i>Dal+</i>	<i>Dal-</i>	
<b>Dalmatian</b>	<b>128</b>	<b>113</b>	<b>15</b>	<b>88.3</b>
<b>Doberman Pinscher</b>	<b>432</b>	<b>249</b>	<b>183</b>	<b>57.6</b>
<b>Shih Tzu</b>	<b>21</b>	<b>9</b>	<b>12</b>	<b>42.9</b>
<b>Bichon Frise</b>	<b>6</b>	<b>5</b>	<b>1</b>	<b>83.3</b>
<b>Lhasa Apso</b>	<b>3</b>	<b>2</b>	<b>1</b>	<b>66.7</b>
<b>Mixed-breed</b>	<b>122</b>	<b>119</b>	<b>3</b>	<b>97.5</b>
German Shepherd Dog	34	34	0	100
Golden Retriever	32	32	0	100
Greyhound	85	85	0	100
Labrador Retriever	47	47	0	100
Other <sup>a</sup>	220	207	0	100

Breeds in which *Dal-* individuals were identified are presented in bold lettering.

<sup>a</sup>Breeds (n) represented by less than 20 individuals and without any *Dal-* dog identified included the following: Great Dane (13), Standard Poodle (9), Maltese Terrier (9), Yorkshire Terrier (8), English Bulldog (7), Havanese (7), Siberian Husky (7), Rottweiler (7), Boxer (6), Miniature Schnauzer (6), West Highland White Terrier (6), Bernese Mountain Dog (5), Chihuahua (5), American Cocker Spaniel (5), Dachshund (5), Newfoundland Retriever (5), Unknown (5), Airedale Terrier (4), American Pitbull Terrier (4), Australian Shepherd Dog (4), Hound (4), Australian Cattle Dog (3), Bouvier des Flandres (3), Bull Mastiff (3), Jack Russell Terrier (3), Samoyed (3), Beagle (2), Cavalier King Charles Spaniel (2), Flat Coated Retriever (2), Great Pyrenees (2), Irish Wolfhound (2), Kuvasz (2), Mastiff (2), Miniature Dachshund (2), Pitt Bull (2), Pomeranian (2), Pug (2), Redbone Coonhound (2), Saint Bernard (2), Shar Pei (2), Tibetan Terrier (2), Vizsla (2), Weimaraner (2) and 1 each of Afghan Hound, Akita, Anatolian Shepherd Dog, Beauceron, Bloodhound, Border Collie, Borzoi, Bull Terrier, Bulldog, Cairn Terrier, Catahoula Leopard, Chinese Crested, Chow Chow, Collie, Dogue de Bordeaux, English Cocker Spaniel, English Mastiff, English Shepherd Dog, English Springer Spaniel, Griffon Korthal, Italian Greyhound, Japanese Mastiff, Labradoodle, Leonberger, Miniature Pinscher, Norwich Terrier, Old English Sheepdog, Papillon, Pekingese, Pembroke Welsh Corgi, Red Tick Hound, Rhodesian Ridgeback, Saluki, Schipperke, Schnauzer, Shetland Sheepdog, Soft Coated Wheaton Terrier, Spanish Water Dog, Staffordshire Bull Terrier, Whippet.

studied (Table 4 and Fig 2). As the proportion of *Dal+* phenotype did not vary significantly by sex in these families, or within the breeds (Table 3), an X-chromosomal mode of inheritance could be excluded. When both parents were *Dal-*, only *Dal-* offspring were found. *Dal+* dogs bred with *Dal-* dogs produced more *Dal+* than *Dal-* puppies (ratio 19:14). If both parents were *Dal+*, they produced all *Dal+* offspring, except for 2 litters where 3 *Dal-* puppies were identified among 12 offspring. These observations support an autosomal dominant mode of inheritance for *Dal* antigen expression and the *Dal+* type. In addition to these families, 4 litters including 12 offspring but missing 1 or both parents for the pedigree analysis showed distributions consistent with an autosomal dominant mode of inheritance.

**Table 3.** Descriptive statistics of evaluated risk factors, including sex, age, coat color, DEA 1 status, and region, for *Dal-* status. For each breed, 1 dog was randomly selected per breeder. For some dogs, the information was not available.

Evaluated Risk Factor	Dalmatian		Doberman Pinschers	
	n	% <i>Dal-</i>	n	% <i>Dal-</i>
Sex				
Male	31	9.7	158	34.2
Female	28	3.6	116	32.8
Age				
<1 year	13	23.1	18	50.0
1–5 years	37	5.4	140	32.1
>5 years	12	0.0	122	33.6
Coat color				
Black white	46	8.7	NA	NA
Liver white	13	7.7	NA	NA
Black	NA	NA	63	47.6
Red	NA	NA	27	51.9
Isabella	NA	NA	5	40.0
Blue	NA	NA	2	0.0
DEA 1				
Positive	59	8.5	52	36.4
Negative	1	0.0	111	60.4
Region				
Ontario (Can)	2	0.0	158	27.8
Quebec (Can)	11	0.0	108	44.4
USA	49	10.2	14	21.4

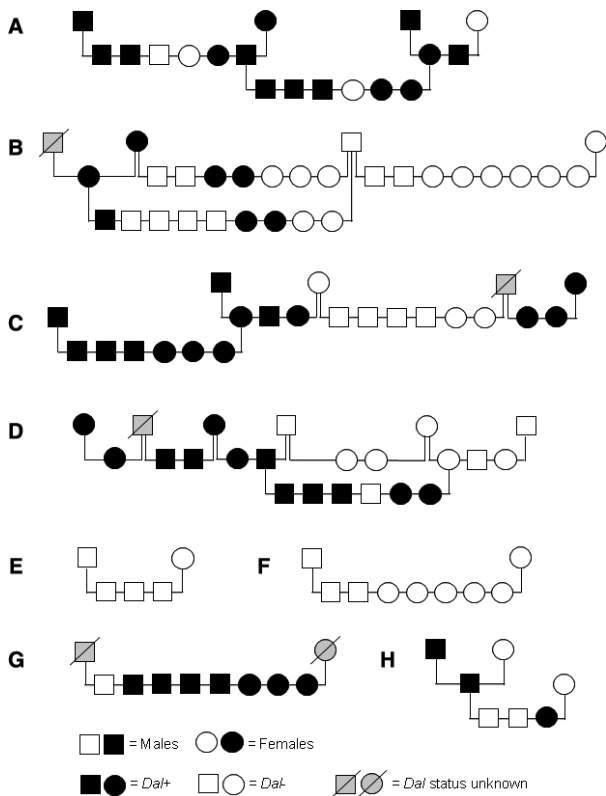
NA, not applicable.

**Table 4.** Proportion of *Dal+* and *Dal-* offspring depending on the *Dal* status of matings (*Dal+* × *Dal+*, *Dal+* × *Dal-* or *Dal-* × *Dal-*).

Matings	n	Number of offspring (n)	
		<i>Dal+</i>	<i>Dal-</i>
<i>Dal</i> blood type of parents			
<i>Dal+</i> × <i>Dal+</i>	3	15	3
<i>Dal+</i> × <i>Dal-</i>	8	19	14
<i>Dal-</i> × <i>Dal-</i>	5	0	23
Total	16	34	40

## Discussion

The *Dal* blood group was initially described in an anemic *Dal-* Dalmatian that was incompatible with other dogs except to several Dalmatians, but a larger survey was required to establish its prevalence in Dalmatians as well as in other breeds.<sup>12</sup> In the present study, using a gel column technology and polyclonal anti-*Dal* antibodies, 1130 dogs from North America were blood typed. Only strong *Dal+* of *Dal-* typing reactions, and no naturally occurring anti-*Dal* antibodies, in nontransfused *Dal-* dogs, were detected. This study identified *Dal-* dogs in breeds other than Dalmatians and research Beagles,<sup>12,14</sup> notably in Doberman Pinschers and Shih Tzus, but also in Lhasa Apsos,



**Fig 2.** Pedigree analysis of the *Dal* phenotype in Dalmatian (A) and Doberman Pinscher families (B–H). Two *Dal*<sup>-</sup> parents always give birth to *Dal*<sup>-</sup> puppies as shown in subfigures B, D–F. In matings of *Dal*<sup>+</sup> with *Dal*<sup>-</sup>, both phenotypes were observed in different proportions as shown in A–D, H. Two *Dal*<sup>+</sup> parents give birth to a majority of *Dal*<sup>+</sup> puppies, and in some rare instance to *Dal*<sup>-</sup> puppies (see subfigure A), which confirms an autosomal dominant mode of inheritance of the *Dal*<sup>+</sup> phenotype.

Bichon Frises, and mixed-breed dogs. The *Dal* was initially thought to be a high-incidence antigen,<sup>12</sup> but based on this study, it should be actually classified as a blood group system where *Dal*<sup>+</sup> is dominantly inherited over *Dal*<sup>-</sup>. *Dal*<sup>-</sup> dogs are not exclusive to the Dalmatian breed and the *Dal* type should be investigated when facing transfusion incompatibility in any breed.

The proportion of *Dal*<sup>+</sup> and *Dal*<sup>-</sup> individuals did not vary significantly by sex. In addition, *Dal*<sup>-</sup> parents produced only *Dal*<sup>-</sup> offspring, and *Dal*<sup>-</sup> offspring were rare if both parents were *Dal*<sup>+</sup>. Consequently, the pedigree analyses revealed an autosomal dominant mode of inheritance of the *Dal*<sup>+</sup> phenotype similar to what has been found with other canine blood groups investigated to date.<sup>6,20–22</sup> In 1953, inheritance of canine blood types A, B, C, and D (which likely correspond to DEA 1, 3, 4, and 5, respectively, using the current international nomenclature) was studied, which showed that blood groups are inherited as simple Mendelian dominants with no evidence of association.<sup>1,21</sup> Thereafter, a dominant inheritance was suggested for several other canine RBC antigens for which relations were not established with internationally recognized DEA, but no formal pedigree analyses were presented.<sup>22</sup> Most recently, a

multiallelic ( $\geq 4$ ) autosomal dominant blood group system for DEA 1 was reported, after a heritable pattern of varied antigenic expression was observed, ranging from a complete lack (DEA 1-) to various degrees of positivity (agglutination reactions ranging from 2+ to 4+).<sup>20</sup> In the current study, all *Dal* typing results yielded either a clear negative (0) or a strong positive (3+ or 4+) agglutination reaction, supporting a simpler 2 allele model with the *Dal*<sup>+</sup> allele being dominant over the *Dal*<sup>-</sup> allele.

This study identified numerous *Dal*<sup>-</sup> dogs in Dalmatians as well as in Doberman Pinschers. Moreover, we also discovered several *Dal*<sup>-</sup> Shih Tzus while investigating a blood incompatibility in a recently transfused anemic dog. Although only a small number of Lhasa Apsos and Bichon Frises were typed, 1 *Dal*<sup>-</sup> of each breed were identified, which deserves further prevalence investigation. According to the International Federation of Cynology,<sup>9</sup> both Shih Tzus and Lhasa Apsos originate from Tibet and likely share some common ancestry, but the others have no close ancestors and thus the *Dal*<sup>-</sup> blood type might be far more wide spread. In addition, 2 *Dal*<sup>-</sup> dog were identified after screening 100 research Beagles.<sup>14</sup> These findings suggest a more ancestral mutation, and thereby, more breeds with *Dal*<sup>-</sup> dogs are likely to be found.

Whereas all Dalmatians recruited in Canada were *Dal*<sup>+</sup> (n = 26), the prevalence of the *Dal*<sup>+</sup> phenotype in Dalmatians (n = 90) in the USA was 85.6%. In comparison, the original *Dal* survey conducted in 2005 showed a similar ratio with 5 *Dal*<sup>-</sup> of 26 Dalmatians (80.8%) recruited from various geographical areas in the USA (Pennsylvania, New York, Illinois, and Texas) and some dogs were known to be related.<sup>12</sup>

The clinical importance of *Dal* in Doberman Pinschers might be considerable given that 42% were *Dal*<sup>-</sup> in this study sample. In addition, the high prevalence of von Willebrand disease (vWD) in Doberman Pinschers<sup>23</sup> creates a particular challenge for this breed. This primary hemostatic disorder puts Doberman Pinschers at higher risk of bleeding which can lead to the need for multiple transfusions, including fresh whole blood, packed RBC, fresh-frozen plasma, or cryoprecipitate,<sup>24,25</sup> increasing their risk of being sensitized to the *Dal* antigen. Unfortunately for *Dal*<sup>-</sup> dogs, there is a scarcity of *Dal*<sup>-</sup> blood donors (1 mixed-breed dog and 5 Doberman Pinschers among all typed donors) based upon this survey of blood donors and the original publication on *Dal*.<sup>12</sup>

Doberman Pinschers and Dalmatians are not commonly recruited as blood donors.<sup>12,26</sup> In the original *Dal* report, of the 55 privately owned canine blood donors, only 1 was a Doberman Pinscher and none were Dalmatians.<sup>12</sup> Similarly, in a study including 66 non-Greyhounds screened as potential blood donors, only 1 Doberman Pinscher and no Dalmatians were investigated.<sup>26</sup> In addition to Greyhounds, the most common purebred dogs were Borzois, German Shepherd Dogs, Golden Retrievers, and Labrador Retrievers.<sup>12,26</sup> In the current study, only 1 Dalmatian and 16 Doberman Pinschers were enrolled active blood donors. The most common purebred dogs recruited as blood donors were

Greyhounds, Labrador Retrievers, German Shepherd dogs, and Golden Retrievers, all of which were *Dal+* (as were all dogs of these same breeds which were not blood donors). The near absence of Dalmatians as blood donors might be due to their weight generally being borderline for recruitment criteria (>23 kg recommended when using standard collection bags)<sup>27</sup> and their perceived apprehensive demeanor in veterinary clinics. The low number of Doberman Pinschers as blood donors might be due to the high prevalence of vWD, the associated cost of screening, and the potential occult cardiomyopathy in the breed. The scarcity of Doberman Pinschers and Dalmatians as blood donors amplifies the rarity of *Dal-* blood in blood banks leading to the almost certain sensitization of *Dal-* dogs and consequently risk of acute hemolytic transfusion reactions if subsequent transfusions are required. Thus, the specific recruitment of *Dal-* Doberman Pinschers and Dalmatians as blood donors might be desirable.

When discovered, *Dal* was thought to be a high-incidence antigen, as the incidence of *Dal+* was >90% in the population tested and seemed to be missing in only 1 breed and in only a few individuals of that breed.<sup>12</sup> According to the International Society of Blood Transfusion (ISBT) in human medicine, the criteria for inclusion as a high-incidence antigen are as follows: (1) an incidence of >90% in most populations tested, but usually >99%; (2) distinction from all other high-incidence specificities; and (3) demonstration that the antigen is lacking in at least 2 siblings, giving evidence that the negative phenotype is genetically determined.<sup>28</sup> Studies identifying high-incidence antigens usually report only a few individuals lacking the antigen.<sup>29–31</sup> In this study, the prevalence of the *Dal-* minor allele was >10% in several breeds and thus the *Dal* antigen seems to be a true blood group system rather than a high-incidence antigen.

Given the dominant inheritance pattern and the scarcity of *Dal-* dogs within the general population of dogs, the most promising sources of compatible blood for a *Dal-* recipient would be littermates, family members, or dogs from the same breed, in that order.<sup>9,12</sup> Indeed, in the family studies reported herein, if 1 *Dal-* dog was detected in a litter, there was a 60% chance that another randomly selected littermate was also *Dal-* (based on 78 dogs from 13 litters with at least 1 *Dal-* dog). If needed, compatible smaller breed dogs, for instance Shih Tzu, Lhasa Apso, and Bichon Frise, could also serve as blood donors by collecting an appropriate volume of blood similar to standard blood donation in cats.<sup>27</sup> When considering such *Dal-* dogs as donors, following blood banking standards regarding general health status and infectious disease screening remains of the outmost importance.<sup>32,33</sup>

Canine blood groups have been reported to vary geographically.<sup>8,26</sup> In this study, the prevalence of *Dal-* varied significantly only between Ontario and Quebec, and in Doberman Pinschers. No risk factors (i.e., sex, age, coat color and DEA 1) for the *Dal-* phenotype within a breed were identified. DEA 1 blood typing has been recommended before any blood

transfusion in dogs for decades.<sup>8</sup> As there is no relationship between DEA 1 status and *Dal*, DEA 1 blood compatibility unfortunately does not predict compatibility for *Dal* or any other blood groups.<sup>12,18</sup> Because *Dal* testing is not routinely available as is DEA 1 testing, the cross-match (starting 4 days post-transfusion and for the animal's lifetime) remains currently the best alternative method to help rule out clinically relevant *Dal* incompatibility.

We could not find any evidence of naturally occurring anti-*Dal* alloantibody within the plasma of untransfused *Dal-* dogs, which is concordant with the original *Dal* study.<sup>12</sup> However, anti-*Dal* alloantibodies were found in the plasma of a *Dal-* Shih Tzu facing a transfusion incompatibility after being previously transfused, reinforcing that *Dal* is actually a blood group system. That being said, little is known about the clinical importance of anti-*Dal* alloantibodies in eliciting a hemolytic transfusion reaction in a previously transfused dog. The discovery of *Dal* was possible because of the production of anti-*Dal* alloantibodies in a clinically anemic dog, but thereafter, the dog was never transfused with incompatible blood. Similarly, the anemic Shih Tzu described above was not further transfused once the incompatibility was recognized. However, the Doberman Pinscher from Tufts University, whose serum was used for typing purpose in part of this study, was originally investigated in 2007 because of an acute hemolytic transfusion reaction attributed to *Dal*.

As with most blood typing in dogs, except for DEA 1, Kai 1, and Kai 2,<sup>18</sup> *Dal* blood typing remains dependent on the availability of polyclonal reagents produced by the sensitization of *Dal-* dogs and thus might remain limited in supply. For *Dal* typing, both tube and gel column techniques can be used.<sup>12,13</sup> The gel column technology is easier to interpret, but requires kits and equipment.<sup>34</sup> With DEA 1, the production of a monoclonal antibodies and commercial typing kits extended the availability and standardization of DEA 1 typing.<sup>17,35</sup> In that perspective, production of monoclonal antibodies could increase the availability of *Dal* blood typing in dogs and would facilitate its commercial availability.

## Conclusion

This study showed that the *Dal* blood type has an autosomal dominant mode of inheritance and identified several breeds with *Dal-* dogs in addition to the previously reported Dalmatians, notably Doberman Pinschers and Shih Tzus. Because most purebred breeds tested are *Dal+*, including all Greyhounds, Golden and Labrador Retrievers, and German Shepherd dogs, *Dal-* blood donors are rare and *Dal-* dogs are at high risk of transfusion incompatibility when in need of blood on more than 1 occasion. In addition to the current recommendation to cross-match when dogs have been previously transfused, extended *Dal* typing, although limited by its commercial availability, is recommended in Dalmatians, Doberman Pinschers, and Shih Tzus, particularly when requiring transfusions over more than a few days.

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## Footnotes

- <sup>a</sup> DiaMed AG, Cressier FR, Switzerland  
<sup>b</sup> ID-MTS Buffered Cards, Ortho Clinical Diagnostic, Ontario, Canada  
<sup>c</sup> Goulet S, Blais MC, Abrams-Ogg ACG. Prevalence of the Dal blood type in Doberman Pinschers and in canine blood donors. *J Vet Intern Med* 2014;28:1054  
<sup>d</sup> LISS ID-Diluent “Vet 1”, DiaMed AG, Switzerland  
<sup>e</sup> Antibody Enhancement Solution, Ortho Clinical Diagnostic, Ontario, Canada  
<sup>f</sup> ID-incubator 37S I, DiaMed Microtyping System, Switzerland  
<sup>g</sup> ID-centrifuge 12S II, DiaMed Microtyping System, Switzerland  
<sup>h</sup> DiaMed-Vet ID Card DEA 1.1, DiaMed AG, Switzerland  
<sup>i</sup> DEA 1 Alvedia lab test, Alvedia, Lyon, France  
<sup>j</sup> RANUNI function, SAS statistical software v.9.4, SAS Institute Inc. Cary, NC  
<sup>k</sup> SURVEYFREQ procedure, SAS statistical software v.9.4, SAS Institute Inc. Cary, NC  
<sup>l</sup> LOGISTIC procedure, SAS statistical software v.9.4, SAS Institute Inc. Cary, NC  
<sup>m</sup> Hemopet, Garden Grove, CA  
<sup>n</sup> Veterinary Medical Center, Columbus, OH  
<sup>o</sup> Federation Cynologique Internationale, <http://www.fci.be/en/>

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*Off-label Antimicrobial Declaration:* Authors declare no off-label use of antimicrobials.

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