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ORIGINAL RESEARCH

Dog erythrocyte antigens 1.1, 1.2, 3, 4, 7, and *Dal* blood typing and cross-matching by gel column technique

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Background: Testing for canine blood types other than dog erythrocyte antigen 1.1 (DEA 1.1) is controversial and complicated by reagent availability and methodology.
Objectives: The objectives of this study were to use available gel column technology to develop an extended blood-typing method using polyclonal reagents for DEA 1.1, 1.2, 3, 4, 7, and *Dal* and to assess the use of gel columns for cross-matching.
Methods: Dogs (43–75) were typed for DEA 1.1, 1.2, 3, 4, 7, and *Dal*. Methods included tube agglutination (Tube) using polyclonal reagents, a commercially available DEA 1.1 gel column test kit (Standard-Gel) using monoclonal reagent, and multiple gel columns (Extended-Gel) using polyclonal reagents. Blood from 10 recipient and 15 donor dogs was typed as described above and cross-matched using the gel column technique.
Results: Of 43 dogs typed for DEA 1.1, 23, 25, and 20 dogs were positive using Standard-Gel, Extended-Gel, and Tube, respectively. Typing for DEA 1.2 was not achievable with Extended-Gel. For 75 dogs typed for DEA 3, 4, and 7, concordance of Extended-Gel with Tube was 94.7%, 100%, and 84%, respectively. *Dal*, determined only by Extended-Gel, was positive for all dogs. Post-transfusion major cross-matches were incompatible in 10 of 14 pairings, but none were associated with demonstrable blood type incompatibilities.
Conclusions: Gel column methodology can be adapted for use with polyclonal reagents for detecting DEA 1.1, 3, 4, 7, and *Dal*. Agglutination reactions are similar between Extended-Gel and Tube, but are more easily interpreted with Extended-Gel. When using gel columns for cross-matching, incompatible blood cross-matches can be detected following sensitization by transfusion, although in this study incompatibilities associated with any tested DEA or *Dal* antigens were not found.

Introduction

Transfusions in people and animals are associated with a number of inherent risks; of these acute hemolytic transfusion reactions can have the most severe consequences. Fortunately, pretransfusion screening and blood type-matched transfusion minimize sensitization. Based on serologic testing, >12 canine blood groups have been described,[1–5] and an international standardization committee has designated many as belonging to the dog erythrocyte antigen (DEA) system.[2,6,7] Whereas dogs are either positive (+) or negative (−) for most of the DEAs, the DEA 1 system contains 2 or more alleles: DEA 1.1, DEA 1.2, and possibly A3 (also referred to as DEA 1.3, but not yet committee-approved) and RBCs from individual dogs may express the genes of only one of the alleles or none of them.[1,2,5–10]

Acute hemolytic transfusion reactions due to a known blood group antigen mismatch have never been reported in a dog receiving its first transfusion. In contrast to many other species, dogs do not appear to have clinically important naturally occurring alloantibodies capable of causing acute hemolysis,

although weak anti-DEA 7 antibodies have been described and may result in shortened erythrocyte survival.[2,9,11–13] Moreover, unlike in people and horses, pregnancy has not been shown to induce alloantibodies in bitches, and neonatal isoerythrolysis has only been observed experimentally in neonatal puppies following ingestion of colostrum from bitches transfused with mismatched blood before delivery.[12,14]

DEA 1.1 mismatches can cause life-threatening transfusion reactions in sensitized dogs.[5,8,9,11] Approximately 50% of dogs are DEA 1.1+; therefore, typing for this antigen before transfusion has been recommended.[2,5,8,11,15] Available methods for typing DEA 1.1 antigen include typing cards (DMS RapidVet-H, DMS Laboratories Inc., Flemington, NJ, USA),[15–17] cartridge kits (Quick Test DEA1.1, Alvedia, Lyon, France),[17] tube agglutination (Animal Blood Resources International), and gel column agglutination within microtubes (ID-Gel Test Canine DEA 1.1, Dia-Med-Vet).[15,17] The practice of exclusively transfusing DEA 1.1- RBC products to DEA 1.1- dogs limits sensitization and the occurrence of acute hemolytic transfusion reactions.

Typing for other DEAs and other common RBC antigens has been difficult owing to the limited availability of typing reagents, cumbersome technology, and difficulty in interpreting agglutination results. Currently, tube agglutination is the only procedure used for testing DEA 1.2, 3, 4, 5, and 7, as these are the only antigens for which antisera currently exist and testing is mostly restricted to one laboratory (Animal Blood Resources International, Stockbridge, MI, USA). As DEA 4 and *Dal* are common or high-frequency RBC antigens, reports of reactions to either of these antigens are also rare, but the strength of the agglutination reaction of anti-*Dal* antibodies found in vitro suggests the potential for severe transfusion reactions in vivo.[3,18] Although clinical hemolytic transfusion reactions against DEA 1.2, 3, or 7 have not been documented, no surveys have extensively investigated the degree of sensitization of dogs against any nonself RBC antigen post-transfusion. These studies have been lacking likely owing to the difficulty of the extended canine blood-typing procedure, limited availability of typing reagents, and difficulty with patient follow-up.

The goals of the present study were to investigate a laboratory method of extended typing beyond DEA 1.1 that minimizes the use of reagents while maximizing sensitivity, specificity, interpretability, and reproducibility and, in addition, to use gel column technology for cross-matching. In particular, we aimed to develop and standardize a laboratory method of extended typing using available polyclonal reagents and microtube gel columns, which have become standard in human blood-banking and have been adapted for canine DEA 1.1 and feline AB blood-typing owing to their ease of use, lack of any RBC washing steps, ease of gading and interpreting agglutination reactions, and ability to store and copy results as permanent records.[15,17,19,20]

Materials and Methods

Animals

Study animals included 75 client-owned dogs enrolled either as clinically healthy volunteer blood donors ($n = 47$) or dogs seen as patients at the Matthew J. Ryan Veterinary Hospital of the University of Pennsylvania (VHUP; $n = 28$). The following breeds were represented: Mixed breed (10), Greyhound (9), Labrador Retriever (9), Borzoi (8), Golden Retriever (5), German Shepherd (5), Weimeraner (2), Husky (2), Boxer (2), Bichon Frise (2), Belgian Malinois (2), Labradoodle (2), unknown (2), and 1 each of Standard Poodle, Chesapeake Bay Retriever, Presa Canario, Doberman, Old English Sheepdog, Scottish Terrier, Cocker Spaniel, Boston Terrier, Chihuahua, French Bulldog, Miniature Pinscher, Portuguese Water Dog, Soft Coated Wheaton Terrier, Pekinese, and Viszla. An additional 50 small breed dogs that were a mixture of inpatients and outpatients at VHUP were solely screened for DEA 4. The studies were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania.

Blood samples

EDTA-anticoagulated blood samples and/or segments of blood-collection tubing containing whole blood anticoagulated and preserved in citrate–phosphate–dextrose (Baxter Healthcare Corp, Fenwal Division, Deerfield, IL, USA) were used for blood typing and compatibility testing. All samples were tested for auto-agglutination, and a direct antiglobulin test (DAT or direct Coombs' test) was performed; only blood without evidence of auto-agglutination and that were Coombs' test-negative were included in the study.

Blood-typing

The specifics of the different blood-typing procedures are compared in Table 1. Some dogs were later transfused, but blood-typing was performed on pretransfusion samples.

Table 1. Comparison of procedures used for canine extended blood-typing.

			Test Method*		
Antigen	Procedure	Unit	Tube†	Extended-Gel‡	96 Well†
DEA 1.1 and 1.2	Incubation temperature	°C	37	37	37
	Incubation time	Min	15	15	15
	RBC suspension volume	µL	50	25	20
	Reagent volume	µL	50§	15¶	20§
DEA 3, 4, and 7	Incubation temperature	°C	4	4	4
	Incubation time	Min	30	30	30
	RBC suspension volume	µL	50	25	10
	Reagent volume	µL	50	25/15‖	10
Dal	Incubation temperature	°C	ND	37	ND
	Incubation time	Min	ND	15	ND
	RBC suspension volume	µL	ND	25	ND
	Reagent volume	µL	ND	15	ND

*Tube and Gel tests were done according to manufacturer instructions.
†For Tube and 96-well typing, 2–5% washed RBC suspensions were used.
‡For DEA 1.1 and DEA 1.X, DiaMed anti-canine globulin columns (Gel-Coombs) were used, whereas for DEA 3, 4, 7, and Dal, DiaMed saline columns were used.
§For Tube and 96-well typing, additional tests were run in parallel with samples incubated with 25 µL (for Tube) or 20 µL (for 96 W) Coombs' reagent diluted 1:10 (VMRD).
¶Dilution was 1:4 for DEA 1.1 and 1:64 for DEA 1.X.
‖25 µL of reagent were used for DEA 3 and DEA 7, whereas 15 µL were used for DEA 4.
ND, not done.

Standard-gel – DEA 1.1

Commercially available ID-Gel Test DEA 1.1 (Dia Med-Vet, Cressier sur Morat, Switzerland), which uses monoclonal antibodies to DEA 1.1, was performed according to the manufacturer's instructions and as described previously.[15,17] Briefly, RBCs were added to microtubes shaped as columns and filled with gel media that contain DEA 1.1 antibody. Blood that is positive for DEA 1.1 forms a line of agglutination on the surface of the gel or diffuse agglutination within the gel; if negative for DEA 1.1 a red pellet forms at the bottom (Figure 1).

Tube agglutination (Tube) – DEA 1.1, 1.2, 3, 4, and 7

Fifty microliters of a 2–5% suspension of washed RBCs were added to 50 µL of canine polyclonal antisera against DEA 1.1, DEA 1.X, DEA 3, DEA 4, or DEA 7 (Midwest Animal Blood Services Inc., Stockbridge, MI, USA) in a 3 mL glass test tube.[11,15] DEA 1.X antiserum reacts positively with RBCs that are positive for DEA 1.1, DEA 1.2, or A3. After briefly mixing and incubating at either 4°C for 30 minutes (DEA 3, 4, and 7) or 37°C for 15 minutes (DEA 1.1, 1.X), tubes were centrifuged at 1000*g* for 15 seconds and the degree of agglutination was scored as previously described and according to the manufacturer's instructions.[3,11,12,15] Results were interpreted as negative if scored as "no agglutination" or "1+ agglutination," whereas ≥2+ agglutination reactions were considered positive. All samples tested with DEA 1.1 or 1.X antisera were also incubated with a 1:10 dilution of polyclonal canine

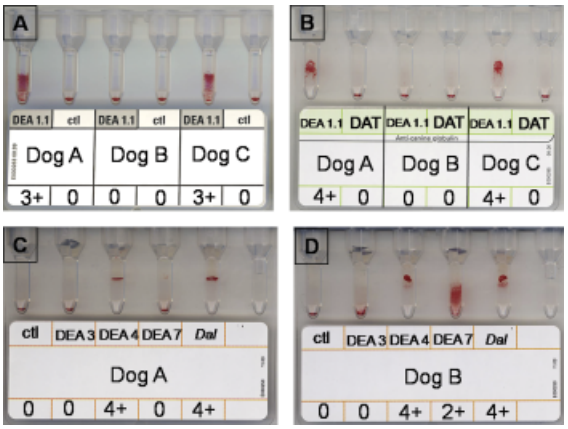

Figure 1. Dog erythrocyte antigen (DEA) profiles of 3 dogs. The reaction strengths are recorded at the bottom and are graded from negative (0) to 4+ with strengths ≥2+ considered positive. Blood types and controls are recorded directly below the reaction wells. (**A**) Standard-Gel showing that Dogs A and C are DEA 1.1+ and Dog B is DEA 1.1−; associated saline controls (ctl) are negative. (**B**) Extended-Gel (Gel-Coombs) showing that Dogs A and C are DEA 1.1+ and Dog B is DEA 1.1−; associated direct antiglobulin tests (DAT) are negative. (**C**) Extended-Gel showing the extended DEA and *Dal* profile of Dog A; associated control (ctl) is negative and dog is positive for DEA 4 and *Dal*. (**D**) Extended-Gel showing the extended DEA and *Dal* profile of Dog B; associated ctl is negative and dog is positive for DEA 4, 7, and *Dal*.

Coombs' reagent (Canine Coombs' Reagent, VMRD, Pullman, WA, USA) to enhance the strength of the reaction, according to protocol, with reporting of the highest level of agglutination (Midwest Animal Blood Services Inc., typing procedure for DEA, package insert).[11,15] As DEA 1.1+ dogs will show agglutination with both DEA 1.1 and 1.X antisera, dogs were considered positive for DEA 1.2, if they had a negative reaction with DEA 1.1 antisera but a positive reaction with DEA 1.X antisera.

Extended typing by gel column (Extended-Gel) – DEA 1.1, 1.2, 3, 4, 7, and Dal

Volumes of reagents, extrapolated from the gel column cross-matching procedure according to the manufacturer's instructions (DiaMed-Vet), were 50 μL of an 0.8% RBC suspension and 25 μL serum/plasma. The 0.8% RBC solution was made by suspending 10 μL of the RBC pellet in 1 mL of low ionic strength salt solution (RBC-LISS). Using a panel of known-positive cells and canine polyclonal antisera (Midwest Animal Blood Services), a series of dilutions was used for titration to determine the smallest volume of RBC suspension and polyclonal reagent in which predictable positive and negative agglutinations were obtained. Final volumes for the study were selected (Table 1), and agglutination reactions were interpreted similarly to those for the Standard-Gel. Each antiserum was added to adjacent gel columns, along with a saline control (Figure 1).

For DEA 1.1 and DEA 1.2, procedures were performed using commercially available anti-canine immunoglobulin-impregnated gel columns (DiaMed-Vet ID-Card, "Anti-canine globulin," DiaMed-Vet), hereafter termed Gel-Coombs. Initial experiments with plain saline gel columns (DiaMed-Vet ID-Card, "NaCl, enzyme test and cold agglutinins," DiaMed Vet) did not demonstrate any repeatable agglutination using any reagent concentration or combination of Coombs' reagent (Canine Coombs' Reagent, VMRD), DEA 1.1 antiserum, or DEA 1.X antiserum. Incubation times and temperatures for these antigens were identical to those for Tube (Table 1). Initial optimization studies using DEA 1.1+ and DEA 1.1 − RBCs revealed required dilutions of 1:4 for DEA 1.1 antisera and 1:64 dilution for DEA 1.X antisera to yield predictable results (data not shown).

For DEA 3, 4, and 7 typing, plain saline gel columns (DiaMed-Vet ID-Card "NaCl, enzyme test and cold agglutinins") were used (Table 1). For *Dal* typing, 15 μL of thawed frozen serum from the sentinel *Dal*–dog[3] was added to 25 μL of RBC-LISS suspension in plain saline gel columns and incubated similarly to the cross-matching procedure previously described for this antigen, but using smaller volumes that result in a ratio of serum to RBC-LISS suspension of 3:5, rather than 1:2 described previously.[3] Although this ratio uses slightly more plasma than RBCs, preliminary titration studies (results not reported) indicated repeatable and reliable results.

96-well test (96W) – DEA 1.1, 1.2, 3, 4, and 7

Round-bottomed 96W plates (Linbro, Flow Laboratories Inc., McLean, VA, USA) were used with volumes of antisera and washed RBCs extrapolated from the Tube typing procedure (Table 1).

Cross-matching

When applicable, cross-matching was performed on samples from typed dogs using Gel-Coombs according to the manufacturer's instructions (DiaMed-Vet ID-Card "Anti-canine globulin"). Absence of agglutination was scored as "compatible," whereas any agglutination $\geq 1+$ was considered "incompatible." The test was run in duplicate using saline gel columns to check for autoagglutination, and an auto-control was run on both Gel-Coombs and saline gels for all cross-matches performed. DAT were also run for all samples. Results reported are those of the Gel-Coombs.

Statistical analysis

Each set of results was analyzed using a 2×2 table method comparing "true positive" and "true negative" to "test positive" and "test negative." For DEA 1.1 typing, the gold standard was considered to be Standard-Gel results where "test" was either Extended-Gel Coombs or Tube, whereas for DEA 1.2, 3, 4, and 7, the gold standard was considered Tube, where the "test" was either Extended-Gel Coombs (DEA 1.2) or plain saline Extended-Gel (DEA 3, 4, and 7). For each test, sensitivity and specificity were calculated. Concordance was calculated as the number of dogs with concordant test results divided by the total number of dogs tested.

Results

Blood-typing

DEA 1.1 and 1.2

A total of 43 dogs were typed for DEA 1.1 and 1.2 using Tube, Extended-Gel, and Standard-Gel (Table 2). As expected, all samples from 20 dogs that tested DEA 1.1+ by Tube were also DEA 1.X+ by Tube. All 20 dogs that typed DEA 1.1+ by Tube were also positive with

Table 2. DEA 1.1, 3, 4, 7, and *Dal* results with Tube, Extended-Gel, and Standard-Gel techniques.

		Positive			Negative		
	Number of Dogs	Tube	Extended-Gel*	Standard-Gel	Tube	Extended-Gel*	Standard-Gel
DEA 1.1	43	20	25	23	23	18	20
DEA 3	75	8	10	—	67	65	—
DEA 4	75	75	75	—	0	0	—
DEA 7	75	17	9	—	58	66	—
Dal †	63	—	63	—	—	0	—

*DiaMed anti-canine globulin columns were used for DEA 1.1, whereas DiaMed plain saline columns were used for other antigens.
†Dalmatians were not tested in either the blood donor or the patient population.

Standard-Gel, whereas an additional 3 dogs that were DEA 1.1− by Tube were found to be DEA 1.1+ by Standard-Gel. Interestingly, all 3 of these samples showed 1+ agglutination by Tube using DEA 1.1 reagent, and all were DEA 1.X+ by Tube (≥2+ agglutination) using DEA 1.X reagent. All dogs that were DEA 1.1+ by Standard-Gel were also found to be DEA 1.1+ using Extended-Gel Coombs. Two dogs were DEA 1.1+ only by Extended-Gel Coombs (Table 2). These results were reproducible using the same blood sample. In addition, 4 dogs tested DEA 1.X+ by Tube but DEA 1.1− by Tube and Standard-Gel, implying they were DEA 1.2+.

It was not possible to use Extended-Gel for DEA 1.2 typing as we were unable to identify a dilution of DEA 1.X antisera in which a confirmed DEA 1.1−/DEA 1.2+ sample showed positive agglutination and a confirmed DEA 1.1−/DEA 1.2− sample had a negative reaction. Thus, this procedure was not pursued (results not shown). The addition of Coombs' reagent for the DEA 1.1 and 1.X typing by Tube did not always enhance agglutination reactions, and sometimes made them less strong (eg, 2+ reaction became 1+ after addition of Coombs' reagent), as observed previously.[15]

DEA 3

Of 75 dogs tested 8 were positive for DEA 3 by Tube and 10 were positive with Extended-Gel (Table 2, Figure 1) These results were reproducible when this procedure was repeated with the same samples. Of the 8 dogs that tested positive for DEA 3 by Tube 7 were positive by Extended-Gel; an additional 3 dogs were positive for DEA 3 by Extended-Gel but not by Tube. One of these had a 1+ agglutination reaction by Tube. All positive results by either method had either 2+ or 3+ agglutination reactions.

DEA 4

All dogs were positive for DEA 4 by both Tube and Extended-Gel (Table 3). All positive results by either method had either 3+ or 4+ agglutination reactions. An additional 50 dogs were screened for DEA 4 by Extended-Gel; again all dogs tested positive for DEA 4.

DEA 7

Of 75 dogs tested 17 were positive for DEA 7 by Tube and 9 dogs tested positive by Extended-Gel (Table 2, Figure 1). These results were reproducible when this procedure was repeated with the same samples. All positive results by either method had either 2+ or 3+ agglutination reactions. By Extended-Gel for DEA 7 typing, 1+ agglutination reactions were not observed.

Dal

Dal was determined by Extended-Gel method only. All dogs tested positive and had either 3+ or 4+ agglutination reactions.

DEA 3, 4, and 7 concordance

Extended-Gel was compared with Tube as the gold standard using descriptive statistics, and sensitivity and specificity were calculated (Table 3). There were 16 sets of discordant results across 15 dogs and 2 DEA types (Table 4). Four samples had discordant results for DEA 3, 3 of which were Extended-Gel, positive, and Tube-negative. Two of 4 samples with discordant DEA 3 typing results were from the only 2 Weimeraners in the study. There were 12 discordant results for DEA 7. Two were Extended-Gel, positive, and Tube-negative, whereas 10 were Extended-Gel, negative, and Tube-positive. Three of these discordant DEA 7 results were

Table 3. Sensitivity, specificity, and concordance of Extended-Gel for detecting DEA 3, 4, and 7 compared with Tube.

Blood Type	Total Number of Dogs	Sensitivity %	Specificity %	Concordance %
DEA 3	75	100	97	95
DEA 4	75	100	NA	100
DEA 7	75	53	100	84

NA, not applicable (no negative results obtained).

Table 4. Individual discordant results in detecting DEA 3 and 7.

Dog	Breed (Total Number Tested)	Discordant Type	Agglutination Results*	
			Tube	Extended-Gel
1	Doberman (1)	DEA 3	0	3+
2	Weimeraner (2)	DEA 3	1+	3+
3		DEA 3	0	2+
4	Unknown (2)	DEA 3, 7	2+, 3+	0, 0
5	Belgian Malinois (2)	DEA 7	1+	2+
6	Borzoi (8)	DEA 7	2+	0
7	Chihuahua (1)	DEA 7	2+	0
8	French Bulldog (1)	DEA 7	2+	0
9	German Shepherd (5)	DEA 7	3+	0
10	Golden Retriever (5)	DEA 7	3+	0
11		DEA 7	2+	0
12		DEA 7	2+	0
13	Greyhound (9)	DEA 7	2+	0
14	Husky (2)	DEA 7	0	3+
15	Mixed Breed (10)	DEA 7	2+	0

*Agglutination results: 0 or 1+ = negative; 2+, 3+, or 4+ = positive.

from 3 of 5 Golden Retrievers tested (all blood donors for the Penn Animal Blood Bank), all of which were positive for DEA 7 by Tube but negative by Extended-Gel. Of the other 2 Golden Retrievers tested, one was DEA 7 −, and the last was DEA 7+ by both methods. Only 1 dog had discordant results for both DEA 3 and DEA 7.

96W assay

The results of the 96W test for DEA 3 and 7 typing were generally weak and inconsistently positive (data not shown). All canine samples tested positive for DEA 4 using the 96W method. Consistent or repeatable results were not obtained using the 96W method for DEA 1.1 and DEA 1.X typing, with or without the addition of Coombs' reagent, and are not reported.

Cross-matching

Of the 75 dogs typed, 10 dogs received blood transfusions from 15 donors. All 10 dogs were cross-matched either at the time of their transfusion (day 0; $n = 1$), at a follow-up time ($n = 9$), or at both day 0 and a follow-up time ($n = 5$), for a total of 15 series of cross-matches performed (Table 5). All cross-matched samples were negative by Coombs' test, negative for auto-agglutination, and negative by auto-control.

Pretransfusion blood samples (Day 0) from all 5 dogs that were cross-matched to the initial red-cell product were compatible, and none of the dogs had been transfused previously. Six recipient–donor pairings for 4 dogs (one each for dogs 1, 2, and 7 and 3 for dog 8) were negative for a DEA that was positive on the donor RBCs and that could potentially result in sensitization (Table 5). Four of these 6 pairings became

Table 5. Major cross-match results for 10 dogs before (day 0) and following transfusion with 15 donors.

Dog	Recipient Blood Type*	Donor Blood Type*	Major Cross-Match Results				
			Day 0	1st Follow-Up		2nd Follow-Up	
			Cross-Match Results	Day	Agglutination Strength†	Day	Agglutination Strength
1	DEA 1.1, 4, *Dal*	DEA 4, 7, *Dal*	Compatible	13	1+	50	Compatible
2	DEA 4, *Dal*	DEA 4, *Dal*	Compatible	32	Compatible	ND	ND
		DEA 4, 7, *Dal* ‡	Compatible	32	Compatible	ND	ND
3	DEA 4, *Dal*	DEA 4, *Dal*	Compatible	56	Compatible	ND	ND
4	DEA 1.1, 4, 7, *Dal* ‡	DEA 4, *Dal*	Compatible	ND	ND	ND	ND
5	DEA 4, 7, *Dal* ‡	DEA 4, *Dal*	ND	18	1+	ND	ND
6	DEA 1.1, 4, *Dal*	DEA 4, *Dal*	ND	29	3+	99	2+
7§	DEA 1.1, 4, *Dal*	DEA 1.1, 3, 4, *Dal*	ND	28	4+	42	2+
8	DEA 1.1, 4, *Dal*	DEA 1.1, 4, *Dal*	ND	15	Compatible	23	1+
		DEA 1.1, 4, *Dal*	ND	15	Compatible	23	2+
		DEA 1.1, 3, 4, *Dal*	ND	15	Compatible	23	4+
		DEA 1.1, 4, 7, *Dal* ‡	ND	ND	ND	22	3+
		DEA 1.1, 4, 7, *Dal*	ND	ND	ND	21	3+
9	DEA 1.1, 4, *Dal*	DEA 1.1, 4, *Dal*	ND	14	2+	ND	ND
10	DEA 4, *Dal*	DEA 4, *Dal*	Compatible	28	Compatible	ND	ND

*Only antigens for which there was positive agglutination are reported.

†Agglutination strength of > 0 was considered incompatible.

‡DEA 7 positive by Tube only.

§Additional studies performed by Animal Blood Resources International, including antibody screen, failed to yield results consistent with the production of an antibody to any DEA antigen capable of being typed.

ND, not determined.

incompatible by major cross-matching at later times (the 1 match for dog 7 and the 3 matches for dog 8). Blood from dog 1 was 1+ incompatible initially, but later became compatible. Dog 2 remained compatible. On the other hand, several pairings not predicted to become incompatible, based on extended DEA typing, developed major cross-match incompatibilities (dogs 5, 6, and 9 and 2 pairings for dog 8).

Discussion

Acute hemolytic transfusion reactions from blood group incompatibility between recipient and donor are the most dangerous reactions, but they are also preventable in human and veterinary patients. Despite the description of more than a dozen canine blood groups, approximately half of which have been internationally standardized as DEA, acute hemolytic transfusion reactions have rarely been reported and only a few times have they been related to incompatibility with any of the known canine blood types.[11,18] The infrequency of these reactions may have several explanations: (1) Dogs do not appear to have clinically relevant naturally occurring alloantibodies to other blood types unless sensitized; (2) dogs typically receive blood transfusions once or over a short period of time (within 1 week) and are only rarely chronically transfused; (3) dogs are typically cross-matched if they receive blood more than 1 week after the first transfusion to select an in vitro compatible unit; (4) before transfusion dogs are typically blood-typed for DEA 1.1, which is considered the most antigenic and clinically relevant blood type, and dogs that are DEA 1.1− receive only DEA 1.1− blood; (5) acute hemolytic transfusion reactions may not be detected or may be falsely attributed to the underlying disease; (6) transfusion reactions are rarely investigated beyond DEA 1.1 incompatibilities; and (7) there is a lack of readily available typing reagents, as well as simple and standardized typing (other than DEA 1.1 typing) and cross-matching techniques.

In this study we show the application of a simple and standardized gel column typing method for DEA 3, 4, 7 and *Dal*, which provided results similar to those obtaining using the classic tube assay. As a result, these novel applications may make extended canine blood typing more readily available in veterinary clinical pathology laboratories. The few discrepancies observed between typing techniques could not be resolved owing to the lack of a 100% accurate typing method, although the Tube method has been historically accepted as the closest to a "gold standard" currently available for typing DEA 3, 4, 5, and 7. Moreover, none of the blood cross-match incompatibilities observed could be associated with any identifiable tested blood groups, suggesting that there are other canine blood types that have yet to be characterized. In contrast, the additional attempt to semi-automate the process by performing the typing procedures in round-bottomed 96W plates proved difficult owing to weak agglutination reactions for any antigen other than DEA 4. Similarly, Extended-Gel could not be adapted to differentiate between dogs positive and negative for DEA 1.2, which proves to be a weak RBC antigen.

The blood type distribution of the 43–125 donor and recipient dogs typed in the present study, including the additional 50 dogs tested for DEA 4 with Extended-Gel, was similar to that in previously published surveys and was independent of the typing method used.[2,4,11,18] In this study 43–58% of the dogs were DEA 1.1+ compared with 33–45% in other studies[2,4,11,12]; thus, there seems to be a fairly equal distribution of DEA 1.1+ and DEA 1.1− dogs. The recommendation and need for general screening of donors and recipients for this strongly antigenic blood type and the use of either DEA 1.1− matched RBC products or the exclusive use of DEA 1.1− donors, therefore, cannot be underestimated. The RBC antigens DEA 1.2, 3, and 7 were found in $< 23\%$ of the population tested in this and previous studies, classifying them as RBC antigens of lower frequency.[2,4,8,11] Conversely, all dogs in this study and $\geq 98\%$ in other surveys were positive for DEA 4 and *Dal*, which are now recognized as RBC antigens of high frequency.[2–4,8,11]

The standard laboratory tube technique is cumbersome and hard to standardize among even well-trained blood-banking personnel.[21] Therefore, much effort has been made to simplify and standardize typing and similar immunohematologic techniques in people and animals. In human medicine, the gel column technique has been established as 1 standard method for blood-typing, Coombs' testing, and cross-matching since its establishment in the 1990s and has proven to be both sensitive and reliable.[22–24] In particular, its use in human medicine to identify weak alloantibodies against Rh and other blood groups as well as cross-match incompatibilities (with and without the addition of human antiglobulin [Coombs'] reagent) has improved antibody recognition and titer strength determination when using the gel column compared with the traditional tube.[25–27] In people, gel-typing uses mostly monoclonal reagents and is commercially available for various blood types. Similarly, this gel column technology has been recently introduced for DEA 1.1 and feline

AB typing using monoclonal antibodies,[15,17,19] and in the present study its application for extended typing for DEA 3, 4, 7, and *Dal* as well as cross-matching was investigated and found to be useful.

As the polyclonal reagents used in the present study were generated by alloimmunization between purposely selected mismatched canine donor–recipient pairs, typing (both Tube and Extended-Gel) using polyclonal reagent is essentially a cross-match or Coombs' test procedure. Use of gel columns with polyclonal reagents produced similar strengths of agglutination reactions compared with Tube, although gels were easier to read and had the added advantages of not requiring RBC washing, stability of the reaction for hours to days, and the ability to digitally record or photocopy results. Interestingly, positive agglutination reactions against DEA 4 and *Dal* (4+ agglutination) were much stronger than those against DEA 1.1, 1.2, 3, and 7 (2+ to 3+ agglutination), presumably a result of the titer and affinity of polyclonal antibodies to the different RBC antigens.

With respect to DEA 1, the commercial availability of a number of reliable laboratory and in-clinic typing assays for DEA 1.1 that use monoclonal antibodies makes the use of a gel column procedure with polyclonal DEA 1.1 antibody unnecessary. The fact that 3 dogs tested DEA 1.1− by Tube (actual result was a 1+ agglutination reaction with DEA 1.1 reagent and ≥2+ using DEA 1.X reagent), but were positive for DEA 1.1 by Standard-Gel and Extended-Gel techniques suggests that Gel methods are more sensitive. The 2 DEA 1.1 samples that were positive by Extended-Gel but not Standard-Gel may be the result of different specificity of the polyclonal antibody compared with monoclonal antibody, as variation in strength and antigen spectrum of polyclonal antibodies has been recognized.[15] The importance of DEA 1.2 remains unclear as no clinical transfusion reactions have ever been related to a DEA 1.2 mismatch. Despite our failure to adapt the blood-typing procedure for this antigen to Extended-Gel, we were able to identify 3 DEA 1.1+ dogs by Standard-Gel that were DEA 1.2+ by Tube; this may suggest either a spectrum of agglutination from DEA 1.1+ to both DEA 1.1− and DEA 1.2− or cross-reaction of the monoclonal DEA 1.1 antibody with DEA 1.2; indeed DEA 1.2 and A3 (DEA 1.3) may represent a weaker density of DEA 1.1 on canine RBCs.[10,15] Clearly, studies are needed to further define the DEA 1 blood group system and its antigen(s). Such studies will be facilitated by the availability of the full canine genome sequence. Molecular genetic blood-typing methods are not currently available for dogs.

Extended-Gel was easily adapted to typing for DEA 4. Dogs negative for DEA 4 were not found among the study population, which was not surprising for this high-frequency RBC antigen.[2,4,11,18] In the only case report of an acute hemolytic transfusion reaction from DEA 4 mismatch, the potential for DEA 4 sensitization was calculated at ~1.5%.[17] As < 10% of previously transfused dogs are transfused a second time after ≥4 days have passed (unpublished observation),[11] the probability of a DEA 4-related acute hemolytic transfusion reaction is likely < 0.15% for donors and repeatedly transfused recipients with unknown DEA 4 blood type. Whereas less data on the frequency of *Dal* antigen are available and polyclonal *Dal* antisera are likewise limited in availability, the likelihood of a transfusion reaction may be similar to that for DEA 4. Nevertheless, it may be prudent to type any dog for DEA 4 and any Dalmatian for *Dal* if these dogs are expected to be chronically transfused, as in vitro and in vivo reactions to these antigens are quite severe.[3,18] It will be extremely difficult, however, to identify any DEA 4− or *Dal*− donors for DEA 4− or *Dal*− dogs requiring transfusion, as exemplified by the lack of finding such dogs in the present study population.

Extended-Gel was also useful for typing DEA 3 and 7. Using available polyclonal antibodies, these 2 antigens produced weaker reactions by Tube that were often equivocal (between 1+ and 2+ agglutination). The additional subjectivity of interpreting results likely affects whether a sample is considered positive (≥2+) or negative (< 1+). However, the distinction between 1+ and 2+ agglutination reaction is more marked and standardized with Extended-Gel, and thus easier to score consistently. Twelve dogs were found to have discordant results for DEA 7 (84% concordance), whereas 4 were discordant for DEA 3 (95% concordance). There was no obvious difference between concordant and discordant results in this small group of dogs between the recipient and donor populations or among breeds. Interestingly, samples from 3 of 5 Golden Retrievers produced discordant results for DEA 7 (all Tube-positive and Extended-Gel-negative), whereas 1 additional Golden Retriever was DEA 7+ and 1 was DEA 7− with both methods. One could expect negative Tube and positive Gel results with weaker polyclonal antibodies, as weak agglutination reactions in the Tube can accidentally be dispersed when reading the reaction.[21,23] This may also apply for DEA 3, as 3 of 4 discordant results were negative by Tube but positive by Extended-Gel. Although the discordant results were reproducible, human error and difficulty in reading weak reactions can play a role.

Moreover, the small sample size in this study prevents any conclusions from being drawn as to whether Golden Retrievers and Weimeraners have common DEA profiles within their respective breeds. In a survey of ~9500 dogs that were extended-typed using Tube, neither breed was characterized as being "universal donors," ie, only DEA 4+,[4] suggesting that they may be positive for additional antigens. Indeed, in the aforementioned study, 51% of 47 Weimeraners tested positive for DEA 3, whereas 27% of 411 Golden Retrievers tested positive for DEA 7.[4]

Some study dogs were transfused with extended-typed units, and 9 surviving dogs were followed for RBC sensitization by cross-match 2–4 weeks post-transfusion. In this study, 7 of 9 dogs transfused with DEA 1.1-matched packed RBC units became sensitized (had an agglutination result of $\geq$1+). This reflects the high risk of dogs becoming sensitized to RBC antigens following transfusion, although the detrimental effect of such alloantibodies was not determined. Although several dogs became incompatible with their donors, extended typing of donor and recipient failed to correlate these incompatibilities with any DEA antigen for which typing antiserum was available. This may have profound clinical applications as it suggests that having a full DEA type-matched transfusion (not only DEA 1.1) may not prevent sensitization. Indeed, 5 of the 15 donor–recipient pairs that developed incompatible cross-matches in this limited survey were seemingly extended type-compatible. It remains unclear how these in vitro incompatibilities correspond to in vivo RBC survival or the development of acute hemolytic transfusion reactions.

In dogs, the lack of alloantibodies that occur naturally and are clinically relevant may preclude the need for having extended type-specific blood available for a first transfusion; however, the risk of sensitization may have long-term impact on patient management if additional transfusions are required. Preexisting weak alloantibodies to the less common antigens, DEA 3, 5, and 7, have been reported at rates of 1.2–30%, 0.8–10%, and 9.8–50%, respectively.[2,11–13] However, they are difficult to recognize by routine cross-matching, and acute hemolytic transfusion reactions have not been attributed to any of these antigens or to DEA 1.2 following a first or subsequent transfusion. Instead, alloimmunization and acute hemolytic reactions with repeated transfusions $>$ 7 days to years after the first transfusion have been reported only for RBC antigens that have a prevalence of 40–99% prevalent: DEA 1.1, DEA 4, *Dal*, and still to be further characterized blood groups.[3,11,18,28] Most incompatible cross-matches are not investigated, but with Extended-Gel technique for extended canine typing and cross-matching, this should now be more feasible in clinical pathology laboratories. In order to prevent fatal antigen–antibody mismatches in the form of hemolysis, some have recommended that blood donors should be tested for all DEA antigens in order to achieve a "universal" donor that minimizes sensitization (DEA 1.1− and DEA 4+),[4] whereas others consider that cross-matching a dog before every transfusion (including the first) is a more appropriate approach.[7,29] Realistically and practically, the authors recommend always giving DEA 1.1− matched blood products and only cross-matching if $\geq$4 days have passed because the first transfusion or if a transfusion reaction had occurred or was suspected with a previous transfusion.

In conclusion, we found that there was good concordance between the Extended-Gel and Tube blood-typing assays, Extended-Gel was easy to adapt for most available polyclonal antisera, and that results were easy to interpret. Discrepancies were reproducible and occurred more frequently with DEA 7 than DEA 3, both of which had weaker agglutination reactions. Using Extended-Gel for DEA 4 or *Dal* screening may be particularly useful as these are high-frequency antigens and can be associated with acute hemolytic reactions. Although we have yet to see a cross-match incompatibility result from an alloantibody to DEA 1.2, 3, 5, or 7, the ability to perform the extended typing procedure in commercial and reference laboratories is invaluable in investigating cross-match incompatibilities or transfusion reactions. Conversely, as long as the ability of extended-typing in practice is limited, cross-matching of donor blood with blood from a multiply transfused recipient will identify incompatibilities owing to sensitization against known and yet to be determined RBC antigens, as was seen in our small survey. It is still recommended to have donors and recipients type-matched for DEA 1.1 before a first transfusion; however, until further studies of erythrocyte survival after mismatched DEA 1.2, 3, 4, 5, 7, or *Dal* transfusions are performed, the importance of typing for these antigens in donor–recipient pairs is unknown.

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