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ORIGINAL ARTICLE

Prevalence of dog erythrocyte antigens 1 and 7 in eleven canine breeds in the Republic of Korea

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Blood type in dogs is based on the antigen present on the red blood cell surface. Dog erythrocyte antigen 1 is a crucial red blood cell antigen in dogs, whereas the dog erythrocyte antigen 7 has been studied in limited dog breeds worldwide. To assess the prevalence of dog erythrocyte antigens 1 and 7 in 11 breeds in the Republic of Korea, 624 dog blood samples were examined for antigen detection. Overall, 520 dogs (83.3%) showed dog erythrocyte antigen 1 expression. The distribution varied from 50.0～100.0% according to the breed. Dog erythrocyte antigen 1-positive blood type was the highest in Chihuahua (100%), followed by Jindo dog (98.5%), and Sapsaree (95.3%). Dog erythrocyte antigen 7 was positive in 125 dogs (20.0%), and the positivity varied from 5.0～42.9% according to the breed. Dog erythrocyte antigen 7-positive blood type was the highest in Beagle (42.9%), followed by Chihuahua (37.5%), and Jindo dog (27.8%). The high prevalence of dog erythrocyte antigen 1 is because of the high proportion of Jindo dog and Sapsaree breeds that were mostly positive for the antigen. The high abundance of these breeds could be due to inbreeding and local breeding in the Republic of Korea. To our best knowledge, this study is the first to report on the prevalence of dog erythrocyte antigens 1 and 7 among various canine breeds in the Republic of Korea. The prevalence data obtained from this study may contribute to baseline information on veterinary transfusion medicine in small animal practice.

Key Words: Dog erythrocyte antigen 1, Dog erythrocyte antigen 7, Jindo dog, Sapsaree, Beagle, Chihuahua

INTRODUCTION

Blood group antigens are genetic markers present on the surface of red blood cells (RBC). Multiple alleles at a specific gene locus comprise the canine blood group system (Hohenhaus, 2004), which can independently be either positive or negative for most of the dog erythrocyte antigens (DEAs) (Kessler et al, 2010). There are seven internationally standardized RBC antigens in dogs, which are categorized as follows: DEA 1, 3, 4, 5, 6, 7, and 8 (Hale, 1995; Hohenhaus, 2004; Medina et al, 2017). Additionally, three new RBC antigens—Dal, Kai 1, and Kai 2—were identified recently (Blais et al, 2007; Euler et al, 2016).

DEA 1 is known as the most immunogenic of all antigens (Giger et al, 1995; van der Merwe et al, 2002; Ekiz et al, 2011; Ferreira et al, 2011; Mesa-Sanchez et al, 2014). A DEA 1-negative dog that receives the first blood transfusion from a DEA 1-positive dog becomes sensitized, which results in the production of alloantibodies against DEA 1 within 4～14 days after transfusion (Giger et al, 1995; Kessler et al, 2010). A second transfusion of DEA 1-incompatible blood to a DEA 1-sensitized dog could lead to acute transfusion reactions (TRs)

(Giger et al, 1995; Hale, 1995; Hohenhaus, 2004). Blood typing to check the DEA 1 antigen status before transfusion is recommended. Previous studies have reported that the prevalence of DEA 1 varies by breed and geographical region (Giger et al, 1995; van der Merwe et al, 2002; Iazbik et al, 2010; Ekiz et al, 2011; Ferreira et al, 2011; Riond et al, 2011; Mesa-Sanchez et al, 2014).

DEA 7 is not a true erythrocyte antigen. It is produced in the tissue or elsewhere in the body as a soluble form, secreted into the plasma, and eventually adsorbed onto the erythrocyte membrane (Corato et al, 1997; Hohenhaus, 2004; Goy-Thollot et al, 2017; Spada et al, 2017). Transfusion of DEA 7-positive RBC into previously sensitized DEA 7-negative recipients could result in delayed TRs, such as immunological clearance of the transfused RBC and reduction in the lifespan of the transfused RBCs (Hale, 1995; Hohenhaus, 2004). The prevalence of DEA 7 in dogs has been reported to vary from 5~71.7% according to the breed (Arikan et al, 2009; Iazbik et al, 2010; Spada et al, 2015; Spada et al, 2016a; Spada et al, 2016b; Spada et al, 2017).

The distribution of DEA 1 and 7-positive blood types may differ according to canine breed. This study aimed to determine the prevalence of DEA 1 and 7 in a variety of domestic dog breeds in the Republic of Korea (ROK) and to compare the results to those in other countries.

MATERIALS AND METHODS

Animals and Blood Samples

In total, 624 dogs that had never been transfused previously were examined to determine their blood types. The dogs examined in this study were as follows: Jindo dog (n=194); Sapsaree (n=171); Maltese (n=67); Beagle (n=42); Miniature Poodle (n=37); Mongrel (n=29); Shih Tzu (n=28); Yorkshire Terrier (n=20); Pomeranian (n=15); Miniature Schnauzer (n=13), and Chihuahua (n=8). Among them, 365 dogs, including Jindo dogs and Sapsarees, were raised for preserving the Korean native purebred, and the other 259 dogs were companion dogs. Approximately 3 mL of whole blood was collected intravenously into ethylenediaminetetraacetic acid (EDTA)-coated blood collection tubes (BD Vacutainer K2 EDTA; Becton Dickinson, Bedford, MA, USA) from Jindo dogs and Sapsarees for regular health check-up. Additionally, the leftover blood samples of the other breeds after hematological test were obtained from seven veterinary clinics and three organizations located in five provinces in the ROK from May 19, 2017, to May 14, 2019. All experimental procedures were performed in accordance with the Guidelines for the Care and Use and approved by Institutional Animal Care and Use Committee of the National Institute of Animal Science, Rural Development Administration in the ROK (Approval No.: 2017-257).

DEA 1 Blood Typing

The DEA 1 blood type was determined using a commercial immunochromatographic strip assay kit with monoclonal antibodies (Quick test DEA 1; Alvedia, Limonest, France) according to the manufacturer's instructions. Based on the results, the blood types of dogs were classified as DEA 1-positive or DEA 1-negative. DEA 1-positive results included visibly positive bands with weak, mild, and strong intensities.

DEA 7 Blood Typing

DEA 7 blood types were determined using gel column agglutination with polyclonal anti-DEA 7 antisera from Animal Blood Resources International (MI, USA). RBC suspension (0.8%) was prepared by adding 10 μL of RBCs obtained from each dog tested in this study to 1 mL of modified low ionic strength saline (LISS, ID-Diluent 2; Bio-Rad, Hercules, CA, USA). Thereafter, 50 μL of 0.8% RBC-LISS suspension was added to the test gel column (ID-Card NaCl enzyme test and cold agglutinins; Bio-Rad, USA), mixed with 25 μL of polyclonal anti-DEA 7 antisera in the gel column reaction card and incubated at 37℃ for 15 minutes. The card was then centrifuged at 80 g for 10 minutes and the agglutination reaction was

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visually interpreted and scored from 0 to 4+ according to the manufacturer's instructions—agglutination reaction score 0, all RBCs were at the bottom of the column; 1+, very few RBC agglutinates were dispersed in the gel; 2+, all RBCs were agglutinated and dispersed in the gel; 3+, some RBC agglutinates were dispersed in the upper part of the gel and most of the RBCs formed a red line on the on top of the gel; 4+, all RBCs formed a red line on top of the gel (Giger et al, 1995; Spada et al, 2017). The positive results included RBC agglutination reaction scores of +1 to +4, and samples with a score of 0 were interpreted as negative.

Statistical Analysis

The prevalence of DEA 1 and 7 blood types among 11 breeds were calculated and compared. The data generated in this study were arranged in spreadsheets using Excel 2010 software (Microsoft®, Redmond, WA, USA) and statistically analyzed using the SPSS software (version 22.0; IBM, Armonk, NY, US). The one-way analysis of variance test with Dunnett T3 or Scheffe *post hoc* test was used to assess the significant differences of the prevalence of DEA 1 and 7 among the different breeds tested in this study; moreover, the 95% confidence interval (CI) was also calculated. A P-value<0.05 was considered statistically significant.

RESULTS

The prevalence of DEA 1 and 7 in a total of 624 dogs is described in Table 1. Overall, 520 dogs (83.3%) were positive and 104 dogs (16.7%) were negative for DEA 1. The prevalence of DEA 1 varied from 50.0~100.0% among the different breeds. DEA 1 was the most prevalent in Chihuahua (100%, 8/8), followed by the Jindo dog (98.5%, 191/194), Sapsaree (95.3%, 163/171), Miniature Schnauzer (92.3%, 12/13), and Pomeranian (80.0%, 12/15). The result of statistical analysis showed that the prevalence of DEA 1-positive blood type was significantly different (P<0.05) among the eight breeds examined, including the Jindo dog, Sapsaree, Maltese, Miniature Poodle, Shih Tzu, Beagle, Miniature Schnauzer, and Chihuahua. In contrast, no significant difference was found among the three breeds—Mongrel, Yorkshire terrier, and Pomeranian—with any other breed (P>0.05).

Table 1. Prevalence of dog erythrocyte antigen 1 and 7 in 624 dogs of 11 breeds

Breed	Number of dogs examined *n*	DEA 1		DEA 7	
		Positive *n* (%)	95% CI (%)	Positive *n* (%)	95% CI (%)
Jindo dog	194	191 (98.5)^{‡,§,ǁ,††}	96.7~100.2	54 (27.8)‡,††,‡‡	21.5~34.2
Sapsaree	171	163 (95.3)‡,§,††	92.1~98.5	25 (14.6)	9.3~20.0
Maltese	67	37 (55.2)*^{,†,ǁǁ}***	43.0~67.4	5 (7.5)*,§	1.0~13.9
Beagle	42	23 (54.8)*,†***	39.1~70.5	18 (42.9)‡,††,‡‡	27.2~58.5
Miniature Poodle	37	26 (70.3)****	54.8~85.7	8 (21.6)	7.7~35.5
Mongrel	29	22 (75.9)	59.3~92.4	6 (20.7)	5.0~36.4
Shih Tzu	28	14 (50.0)*,†***	30.3~69.7	2 (7.1)*,§	0.0~17.3
Yorkshire terrier	20	12 (60.0)	36.5~83.5	1 (5.0)*,§	0.0~15.5
Pomeranian	15	12 (80.0)	57.1~102.9	2 (13.3)	0.0~32.8
Miniature Schnauzer	13	12 (92.3)‡	75.5~109.1	1 (7.7)	0.0~24.5
Chihuahua	8	8 (100.0)^{‡,§,ǁ,††}	100.0~100.0	3 (37.5)	0.0~80.8
Total	624	520 (83.3)	80.4~86.3	125 (20.0)	16.9~23.2

Superscript lowercase letters indicate significant differences between groups (P<0.05, One-way analysis of variance with Dunnett T3 or Scheffe *post hoc* test).
*Jindo dog, †Sapsaree, ‡Maltese, §Beagle, ǁMiniature Poodle, **Mongrel, ††Shih Tzu, ‡‡Yorkshire terrier, §§Pomeranian, ǁǁMiniature Schnauzer, ***Chihuahua.
DEA, dog erythrocyte antigen; CI, confidence interval.

A total of 125 dogs (20.0%) were positive and 499 dogs (80.0%) were negative for DEA 7. The prevalence of DEA 7 varied from 5.0~42.9% among the different breeds examined. DEA 7 was the most prevalent in Beagle (42.9%, 18/42), followed by Chihuahua (37.5%, 3/8), Jindo dog (27.8%, 54/194), and Mongrel (20.7%, 22/29). The result of statistical analysis showed that the prevalence of DEA 7-positive blood type was significantly different ($P<0.05$) among five breeds, including the Jindo dog, Maltese, Shih Tzu, Beagle, and Yorkshire terrier. In contrast, no significant difference was found among six breeds—Sapsaree, Miniature Poodle, Mongrel, Pomeranian, Miniature Schnauzer, and Chihuahua—with any other breed ($P>0.05$).

DISCUSSION

This study investigated and found that the frequencies of DEA 1 and 7 varied according to the canine breed in the ROK. DEA 1-positive blood type was present in 83.3% of the dogs tested in this study, which was relatively higher than the frequencies previously reported in canine population in Italy (61.2% to 62%) (Carli et al, 2017; Medina et al, 2017), North America (59.6%) (Euler et al, 2016), and India (61.6%) (Baranidharan et al, 2018), and was lower than that reported in Brazil (91.3%) (Novais et al, 1999). The relatively higher DEA 1 positivity in canine population in this study is due to the large proportion (58.5%, 365/625) of Jindo dog and Sapsaree breeds that mostly expressed DEA 1. The percentage of DEA 1-positive dogs varied from 50.0~100% according to the breed examined. Among them, 100% of the Chihuahua assessed had this antigen. In contrast, a lower frequency of DEA 1 (61%) was reported in the same breed in a study conducted in Italy (Medina et al, 2017). However, the small sample size of Chihuahua (n=8) tested in this study is not representative of this breed in the ROK. The prevalence of DEA 1 in the Korean native breeds, including Jindo dog (98.5%) and Sapsaree (95.3%), was much higher than in other purebreds (0~95.0%) previously reported, except Saint Bernard (100%) and Rottweiler (100%) (Iazbik et al, 2010; Ekiz et al, 2011; Ferreira et al, 2011; Riond et al, 2011; Mesa-Sanchez et al, 2014; Spada et al, 2015; Euler et al, 2016; Spada et al, 2016a; Spada et al, 2016b; Carli et al, 2017; Goy-Thollot et al, 2017; Medina et al, 2017; Spada et al, 2017; Baranidharan et al, 2018; Kim et al, 2018).

Pedigree studies (Ferreira et al, 2011; Polak et al, 2015; Carli et al, 2017) showed that DEA 1 blood group system is inherited in an autosomal dominant pattern; DEA 1-positive offspring can be produced when at least one parent is or both parents are DEA 1-positive. Because both the Jindo dog and Sapsaree are designated as natural monument animals and unique breeds of the ROK, they are strictly inbred for genetic preservation. Therefore, the high prevalence of DEA 1 in these two breeds could be the consequence of inbreeding (Polak et al, 2015; Medina et al, 2017). In addition, the prevalence of DEA 1 expression among Poodle, Maltese, Beagle, Yorkshire Terrier, and Shih Tzu were much higher than the same breed studied in Italy and Switzerland (Riond et al, 2011; Medina et al, 2017). In agreement with previous studies (Euler et al, 2016; Medina et al, 2017), these differences in DEA 1 frequencies are thought to be as a result of genetic drift derived from local breeding.

In this study, we found that the distribution of DEA 7 varied according to the breed. DEA 7 expression was prevalent in 20.0% of the dogs examined in this study, which was lower than what was previously reported in Spain and Italy (38.1%) (Spada et al, 2016a), and US (45%) (Hale, 1995), but higher than reported in Brazil (11% and 17%) (Esteves et al, 2011; Novais et al, 2015). The percentage of DEA 7-positive blood type varied from 5.0~42.9% according to the breed. Beagle (42.9%) and Chihuahua (37.5%) showed relatively higher DEA 7-positive blood type than other purebreds reported in the present and previous studies (Esteves et al, 2011; Spada et al, 2015; Spada et al, 2016b; Spada et al, 2017), except that reported in Turkish Kangal dogs (71.7%) (Arikan et al, 2009). In contrast, the prevalence of DEA 7 in Miniature Schnauzer (7.7%), Maltese (7.5%), Shih Tzu

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(7.1%), and Yorkshire (5.0%) was relatively lower than that in other purebreds previously reported, except that in Corso dogs (5.0%) and Argentine Dogo (0%) (Arikan et al, 2009; Esteves et al, 2011; Spada et al, 2015; Spada et al, 2016b; Spada et al, 2017).

DEA 7 is produced in tissue or elsewhere in the body and finally adsorbed onto the erythrocyte membrane. Thus, low levels of DEA 7 attached to RBCs may present difficulties in antigen typing because of weak or undetectable agglutination reaction between DEA 7 and the polyclonal anti-DEA 7 antisera reagent (Corato et al, 1997; Hohenhaus, 2004; Spada et al, 2016c; Goy-Thollot et al, 2017; Spada et al, 2017). In addition, Kessler et al evaluated that this technique and reagents show 100% specificity to DEA 7; however, the sensitivity can vary (Kessler et al, 2010; Spada et al, 2016c). Therefore, DEA 7 frequencies may be underestimated than the actual prevalence in the present and previous studies.

CONCLUSION

The prevalence of DEA 1 and 7 varied according to the breed examined. Most Korean native dogs, including the Jindo dog and Sapsaree, possessed DEA 1. This may be due to inbreeding strategies undertaken for the preservation of Korean native purebred dogs. The discrepancies between DEA 1 frequencies within the same breeds in the ROK and other countries were thought to be a result of genetic drift derived from local breeding. This study is expected to contribute to baseline information on veterinary transfusion medicine in small animal practice. However, further study is needed on the prevalence of DEA 7 according to breed, geography, and inheritance.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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