Elsevier Editorial System(tm) for The Veterinary Journal
Manuscript Draft

Manuscript Number: YTVJL-D-16-00767R4

Title: Dog erythrocyte antigens (DEA) 1, 4, 7 and suspected naturally occurring anti-DEA 7 antibodies in Italian Corso dogs

Article Type: Original article

Keywords: Canine blood group; Dog erythrocyte antigen; Italy; Transfusion medicine; Transfusion reaction.

Corresponding Author: Dr. Eva Spada, DVM, Ph.D., Researcher

Corresponding Author's Institution: University of Milan

First Author: Eva Spada

Order of Authors: Eva Spada; Daniela Proverbio, Associate Professor; Vito Priolo; Dorotea Ippolito; Luciana Baggiani; Roberta Perego; Maria Grazia Pennisi

Abstract: We sought to determine the prevalence of dog erythrocyte antigen (DEA) 1, 4 and 7 and naturally occurring anti-DEA7 antibodies in Italian Corso dogs. In addition, we correlated DEAs with different epidemiologic variables, compared the prevalence of DEAs against other canine populations and assessed the risk of sensitisation and transfusion reactions (TRs) following unmatched transfusion. Blood samples from 100 Corso dogs were evaluated for DEA1, 4, 7 and naturally occurring anti-DEA 7 antibodies.

Seventy-one percent of samples were DEA 1-negative, 100% tested DEA 4-positive, and 95% tested DEA 7-negative. Suspected anti-DEA7 antibodies were found in 32% dogs. The DEA 1 and 7-negative phenotype was significantly more common than in most canine populations. When a previously tested Italian canine population was considered as blood donors for Corso dogs, the risk of DEA1 sensitisation using DEA 1 untyped blood was 29%, and of acute haemolytic TRs after a second untyped DEA 1-incompatible transfusion was 8%. The potential for delayed TRs between DEA 7-negative Corso dogs with suspected naturally occurring anti-DEA7 antibodies receiving untyped DEA 7-positive blood was 11%. Conversely, when Corso dogs were blood donors for the same population, the risk of DEA1 sensitisation was 17%, and the risk of an acute haemolytic TR after a second DEA 1-incompatible blood transfusion was 3%. Corso dogs can be suitable blood donors. Additional studies are needed to clarify whether the high prevalence of naturally occurring anti-DEA7 antibodies in this breed could increase their risk of delayed TRs when they are blood recipients.

Original Article

Dog erythrocyte antigens (DEA) 1, 4, 7 and suspected naturally occurring anti-DEA 7 antibodies in Italian Corso dogs

E. Spada [a, *], D. Proverbio [a], V. Priolo [b], D. Ippolito [b], L. Baggiani [a], R. Perego [a] , M. G. Pennisi [b]

[a] *Veterinary Transfusion Research Laboratory (REVLab), Department of Veterinary Medicine (DIMEVET), University of Milan, via G. Celoria, 10 -20133 Milan, Italy*
[b] *Department of Veterinary Sciences, University of Messina, Polo Universitario dell'Annunziata - 98168 Messina, Italy*

* Corresponding author: Tel.: +39 0250318188
E-mail address: eva.spada@unimi.it

Abstract

We sought to determine the prevalence of dog erythrocyte antigen (DEA) 1, 4 and 7 and naturally occurring anti-DEA7 antibodies in Italian Corso dogs. In addition, we correlated DEAs with different epidemiologic variables, compared the prevalence of DEAs against other canine populations and assessed the risk of sensitisation and transfusion reactions (TRs) following unmatched transfusion. Blood samples from 100 Corso dogs were evaluated for DEA1, 4, 7 and naturally occurring anti-DEA 7 antibodies.

Seventy-one percent of samples were DEA 1-negative, 100% tested DEA 4-positive, and 95% tested DEA 7-negative. Suspected anti-DEA7 antibodies were found in 32% dogs. The DEA 1 and 7-negative phenotype was significantly more common than in most canine populations. When a previously tested Italian canine population was considered as blood donors for Corso dogs, the risk of DEA1 sensitisation using DEA 1 untyped blood was 29%, and of acute haemolytic TRs after a second untyped DEA 1-incompatible transfusion was 8%. The potential for delayed TRs between DEA 7-negative Corso dogs with suspected naturally occurring anti-DEA7 antibodies receiving untyped DEA 7-positive blood was 11%. Conversely, when Corso dogs were blood donors for the same population, the risk of DEA1 sensitisation was 17%, and the risk of an acute haemolytic TR after a second DEA 1-incompatible blood transfusion was 3%. Corso dogs can be suitable blood donors. Additional studies are needed to clarify whether the high prevalence of naturally occurring anti-DEA7 antibodies in this breed could increase their risk of delayed TRs when they are blood recipients.

Keywords: Canine blood group; Dog erythrocyte antigen; Italy; Transfusion medicine; Transfusion reaction.

Introduction

Blood typing prior to canine blood transfusions minimises the risk of a transfusion reaction (TR) due to blood type incompatibility. Therefore, information on the prevalence of different blood types in various breeds helps in selection of blood donors for inclusion in a blood donor program.

In dogs there are seven internationally recognised blood groups in the dog erythrocyte antigen (DEA) system (DEA 1, 3, 4, 5, 6, 7, 8) (Vriesendorp et al., 1976). More recently three additional blood types have been recognised called Dal (Blais et al., 2007), Kai 1 and Kai 2 (Euler et al., 2016). However, little is known about many of these blood types and for some, such as DEA 6 and 8, typing sera no longer exist.

DEA 1 is the most studied blood type. The prevalence of DEA 1-positive dogs varies both geographically and among breeds from 100% to <10%, but has been estimated at ~50% overall internationally (Giger et al., 1995; Novais et al., 1999; Hale et al., 2008; Iazbik et al., 2010; Sinnott Esteve et al., 2011; Ergul Ekiz et al., 2011; Spada et al., 2015a, 2015b, 2016c; Euler et al., 2016). DEA 1 is the most antigenic blood type for which naturally occurring antibodies do not exist (Giger et al., 1995; Hale and Wefelmann, 2006; Blais et al., 2009; Euler et al., 2016) . However, following a DEA 1-incompatible transfusion, the blood recipient becomes sensitised, and a second DEA 1-incompatible transfusion can result in an acute, and potentially fatal, haemolytic transfusion reaction (HTR; Giger et al., 1995).

The DEA 4 blood type has a prevalence of nearly100% in the canine population (Hale et al., 2008; Iazbik et al., 2010; Sinnott Esteve et al., 2011; Spada et al., 2015a, 2015b, 2016c), but in the rare DEA 4-negative dogs, repeated transfusion with incompatible DEA 4 blood can lead to a TR (Melzer et al., 2003).

DEA 7 is not integral to the canine red cell membrane. It is produced elsewhere in the body in soluble form, secreted into the plasma, and adsorbed onto the cell membrane. The reported prevalence of DEA 7 varies between 6% and 82% in various canine populations ((Giger et al., 1995; Hale et al., 2008; Blais et al., 2009; Iazbik et al., 2010; Kessler et al., 2010; Sinnott Esteve et al., 2011; Spada et al., 2015a, 2015b, 2016c). Naturally occurring anti–DEA 7 antibodies have been identified in up to 50% of all DEA 7–negative dogs that have never received transfusions (Giger et al., 1995; Hale and Wefelmann, 2006; Blais et al., 2009; Spada et al., 2016b). The clinical significance of naturally occurring anti-DEA 7 antibodies is unclear as clinical TRs against DEA 7 have not been clearly documented; nor has there been extensive investigation of the post-transfusion survival of DEA 7 incompatible RBCs in DEA 7-negative dogs with naturally occurring anti-DEA 7 antibodies. The paucity of these studies might be explained by the difficulty of the extended canine blood-typing procedure (limited availability of typing reagents, lack of simple and standardised typing techniques) and the complexity in administering labelled DEA 7-positive RBCs (e.g., radiolabeled antigen-positive red cells) to DEA 7-negative recipients and following survival of these cells in the recipient.

The Corso dog is an ancient Italian medium-to-large size breed. The breed was officially recognised by the American kennel club (AKC) in 2010 and is now the 36th most popular dog breed in America[1]. In Europe, the Fédération Cynologique Internationale (FCI) definitively classified this breed in 2007 in Group 2, Pinscher and Schnauzer type, section 2,

[1] See: American Kennel Club, www.akc.org (Accessed 20 February 2017).

Molossoid[2]. Many characteristics of Corso dogs make them good blood donors: they are large (>25 kg bodyweight), good-tempered, easy to train, breed and handle, and have readily accessible jugular veins. To date, no studies have evaluated the prevalence of blood types in this breed.

We sought to determine the prevalence of DEA 1, 4 and 7 and naturally occurring anti-DEA7 antibodies in Italian Corso dogs, to correlate blood types with different epidemiologic variables, to compare prevalence with DEAs in other canine populations tested worldwide, and to assess the potential risk of sensitisation and TRs following random, DEA 1- and DEA 7-unmatched transfusions.

Material and methods

Blood samples

The study used left-over EDTA blood from samples collected from 100 clinically healthy purebred active blood donors Corso dogs, in Northern and potential blood donors Corso dogs in Southern Italy during Spring 2016. The blood had originally been collected during routine blood sampling to test for *Leishmania infantum* or *Dirofilaria immitis* infections, which are endemic in Italy. Blood-typing and alloantibodies studies were performed at the Veterinary Transfusion Research Laboratory (Laboratorio di Ricerca di Medicina Emotrasfusionale Veterinaria, REVLab), University of Milan, Milan, Italy. Based on University of Milan animal use regulations, formal ethical approval was not needed as dogs were sampled with the informed consent of the owners during routine visits for prophylactic reasons and owners gave their consent for the use of excess blood after routine testing be used in further studies. Data on gender, age, origin (Northern or Southern Italy),

[2] See: Fédération Cynologique Internationale, www.fci.be (Accessed 20 February 2017).

the breeder from which they were sourced, coat and eye colour, and transfusion history were collected for each dog sampled.

Blood typing

Blood types were assessed on fresh blood or on blood stored at 4-6 °C within 7 days of blood collection (Brechter, 2005).

DEA 1 blood type was determined with an immunochromatographic strip technique using monoclonal antibody (Lab Test DEA 1, Alvedia) following the manufacturer's guidelines.

DEA 4 and DEA 7 blood types were determined with polyclonal antiserum using agglutination on gel columns as previously described (Kessler et al., 2010; Spada et al., 2015b, 2016c). The polyclonal anti–DEA 4 and 7 antiserum used in the study were purchased from ABRI (Animal Blood Resources International) and imported to the University of Milan with the authorisation of the Italian Health Minister (protocol authorisation No. 0024135-23/09/2015- DGSAF-COD_UO-P).

Briefly, 25 µL of DEA 7 antisera or 15 µL of DEA 4 antisera were mixed with 25 µL of a 0.8% RBC suspension (made by suspending 10 µL of the RBC pellet in 1 mL of low ionic strength solution, LISS, ID-Diluent 2, DiaMed) in the reaction chamber of saline gel columns (ID-Card NaCl enzyme test and cold agglutinins, DiaMed). For all samples, a negative control column containing the RBC suspension of interest and saline was included. The gel columns were incubated at 4 °C for 30 min and thereafter were centrifuged in a special gel column card centrifuge (ID-Centrifuge 24 S, DiaMed) at 80 x *g* for 10 min.

Finally, the gel column cards were evaluated for the presence and strength of agglutination. The gel cards were visually interpreted and agglutination reactions graded from 0 to 4+ in accordance with the manufacturer's instructions as follows (Harmening, 2012): 0, negative, all RBCs were at the bottom of the column; 1+, very few RBC agglutinates were dispersed in the lower part of the gel, with most RBCs at the bottom of the tube; 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 surface of the gel; and 4+, all RBCs formed a red line on top of the gel. Results were interpreted as negative if no agglutination or 1+ agglutination was present, whereas ≥2+ agglutination reactions were considered positive (Kessler et al., 2010; Euler et al., 2016). Results were considered valid if the control was negative. In preliminary tests, all samples were negative for autoagglutination, so no auto-control (i.e., reaction between dog's RBC solution and its own plasma) was performed.

The prevalence of DEAs in Corso dogs was compared with the prevalence of DEAs in other canine breeds and populations previously typed for DEA 1, 4 and 7, i.e. Italian blood donors (Spada et al., 2015a), Brazilian blood donors (Sinnott Esteve et al., 2011), US canine population (Hale et al., 2008) and populations of different breeds such as Greyhounds (Iazbik et al., 2010), Spanish Greyhounds (Spada et al., 2015b) and Ibizan Hounds (Spada et al., 2016c).

Alloantibody study

The presence of naturally occurring anti-DEA 7 alloantibodies in DEA 7-negative plasma samples was tested using an agglutination on gel technique as previously described (Blais et al., 2007; Euler et al., 2016; Spada et al., 2016a, 2016b). Briefly, 0.8% RBC-LISS suspensions were prepared from one DEA 7–positive, DEA 1–negative, and DEA 4–positive blood sample. Fifty μL of this 0.8% RBC-LISS suspension and 25 μL plasma from each DEA 7–negative dog were mixed in the reaction chamber of the gel column and incubated at 37°C for 15 min. Gel columns were centrifuged in the special column gel card centrifuge at 80 x *g* for 10 min and examined for agglutination strength. Similar to the grading for blood typing, positive agglutination reactions could be graded from 0 to 4+ according to the manufacturer's instructions (Harmening, 2012). Reaction ≥1+ identified samples with naturally occurring anti-DEA 7 antibodies (Blais et al., 2007; Euler et al., 2016; Spada et al., 2016a, 2016b).

In human transfusion medicine the specificity, or identity, of an anti-RBC antibody can be determined by testing a recipient's serum or plasma with a panel of RBC suspensions with a known antigenic composition. As a rule, serum or plasma that reacts with an RBC suspension that is positive for a given antigen, but not with a RBC suspension that is negative for that antigen, is suspected to contain antibodies against the given antigen (Shulman et al., 2001). Following this rule samples in which agglutination was detected when cross matched against DEA 7-positive RBCs were then cross-matched against RBCs from one DEA 7–negative dog. Samples that showed agglutination against the DEA 7-positive sample but not with the DEA 7-negative sample were identified as samples containing suspected anti–DEA 7 antibodies.

Sensitisation and transfusion reactions risk analysis

The potential probability of a dog becoming sensitised following the first transfusion of blood that was neither cross-matched nor typed for DEA 1 was calculated using the following formula as previously described (Novais et al., 1999; Ergul Ekiz et al., 2011; Spada et al., 2016c):

(% DEA 1-negative × % DEA 1-positive)/100.

The potential probability of the same dog developing an acute HTR with a second DEA 1-incompatible transfusion using untyped blood was calculated as:

([% DEA 1-negative ×% DEA 1-positive] × % sensitisation for the first transfusion) /10, 000.

The same calculations were made to estimate the potential risk of DTR due to untyped DEA 7-positive blood transfused in DEA 7-negative Corso dogs with suspected naturally occurring anti-DEA 7 antibodies.

Calculations were made considering the Corso dogs of this study and a population of Italian blood donors previously blood typed in which prevalence of DEA 1-positive and DEA 7-positive dogs was 40.5% and 33.3%, respectively (Spada et al., 2015a) as both blood donors and recipients. These were potential probability calculations only.

Statistical methods

Prevalence was calculated as the proportion of samples testing positive for the different DEAs and was reported as a percentage with 95% confidence intervals (95%CI).

The Pearson's chi-square test or Fisher's Exact Test were used for statistical comparison of prevalence of DEA 1, 4 and 7 blood types and suspected naturally occurring anti-DEA 7 antibodies among different variables i.e. origin (Southern or Northern Italy), the breeder from which they were sourced, gender, coat and eye colours and to compare the prevalence of DEAs in Corso dogs with the prevalence of DEAs in other canine breeds or populations previously typed for DEA 1, 4 and 7.

Statistical software (Medcalc Software, version 16.4.3) was used for all analysis and statistical significance was set at $P < 0.01$.

Results

Blood typing was performed on 20 privately owned active blood donors at University of Milan, Northern Italy and 80 potential blood donors from breeders and private owners of Italian Corso Dogs in Southern Italy. Median age was 1.6 years (range, 0.8-8.6 years), 63 were female and 37 were male. No dog had a history of blood transfusion.

The prevalence of DEA 1, DEA 4 and DEA 7-positive samples and strength of agglutination is reported in Table 1. Sixty-seven dogs (67.0%; 95% confidence intervals [CI], 51.9-85.0%) were negative for both DEA 1 and 7 (but DEA 4-positive).

Thirty plasma samples from 95 DEA 7-negative dogs (32%; 95% CI, 20.2-42.8%) contained detectable suspected alloantibodies against DEA 7 with a strength of agglutination on gel column of 1+ in 11 samples, 2+ in 12 samples, 3+ in seven samples. The strength of the suspected anti-DEA 7 antibodies was not specifically determined by titration.

The DEA 1, 4, 7 phenotypes and presence of suspected naturally occurring anti-DEA 7 antibodies did not vary by gender (male versus female), origin (Northern versus Southern Italy), coat colour (black, grey, fawn and brindle) or eye colour (brown, yellow, blue), nor between 10 breeders and 25 private owners. We found no association amongst presence of different DEAs and amongst DEAs and presence of suspected anti-DEA 7 antibodies ($P >$ 0.01).

However, DEA 1 and DEA 7 phenotypes differed from those reported previously in canine populations and other canine breeds ($P < 0.01$). Corso dogs had a higher prevalence of DEA 1-negative dogs than other canine populations, with the exception of Greyhounds, Italian canine blood donors and the canine population from an American study (Hale et al., 2008). Similarly, Corso dogs had a higher prevalence of DEA 7-negative dogs than the other canine populations, with the exception of Brazilian blood donors and Spanish greyhounds. Finally, Corso dogs had a higher prevalence of combined DEA 1- and 7-negative dogs (i.e. DEA 4-positive only) than the other canine populations, with the exception of Greyhounds (Table 2).

When a previously tested population of Italian canine blood donors (Spada et al., 2015a) were considered as donors for a Corso dog blood recipient, the risk of DEA 1 sensitisation when using DEA 1 untyped blood was 29%, and the risk of an acute HTR after a second untyped DEA 1-incompatible blood transfusion was 8%. The potential for a DTR between DEA 7-negative Corso dogs with suspected naturally occurring anti-DEA 7 antibodies receiving untyped DEA 7-positive blood from Italian dog blood donors was 11%. Conversely, when Corso dogs were considered as blood donors for the Italian canine population, the risk of DEA 1 sensitisation when using DEA 1 untyped blood was 17%, and the risk of an acute HTR after a second DEA 1-incompatible blood transfusion was 3%.

Discussion

The prevalence of DEAs and suspected naturally occurring antibodies in this population of Corso dogs has clinical implications. From the perspective of a blood bank program, it is highly advantageous that most dogs are DEA 1-negative, as DEA 1-blood type can be used to transfuse both DEA 1-negative and DEA 1-positive recipients.

The low prevalence of the DEA 7 blood type in Corso dogs is also a favourable characteristic for blood donors. In fact, in contrast to other canine blood types e.g., DEA 1, Dal, and the recently discovered Kai 1 and Kai 2 (Giger et al., 1995; Blais et al., 2007; Euler et al., 2016) for which there are no naturally occurring antibodies, naturally occurring anti-DEA 7 antibodies exist. Weak, naturally occurring anti–DEA 7 antibodies have been identified in up to 50% of all DEA 7–negative dogs that have never received transfusions (Giger et al., 1995; Hale and Wefelmann, 2006; Blais et al., 2009; Spada et al., 2016b) and although they might result in increased clearance of DEA 7-positive transfused RBCs (Smith, 1991; Hale, 1995; 2012;), neither *in vitro* haemolysis, HTRs nor neonatal haemolytic reactions have ever been reported (Blais et al., 2009). Additionally, there is a literature gap on the significance of naturally occurring anti-DEA 7 antibodies and studies documenting clinical transfusion reactions due to DEA 7 incompatibilities are dated and were performed on dogs previously sensitised by blood transfusion and not caused by naturally occurring anti-DEA 7 antibodies (Swisher and Young, 1961). However, it would not be possible clinically to detect a slow transfusion reaction, such as that caused by low titres of weak, non-haemolytic, naturally occurring anti-DEA 7 antibodies in a dog with ongoing haemolytic anaemia. There are no reports of a documented TR due to DEA 7 incompatibility, but conversely there are no studies that document that DEA 7-positive RBCs transfused into a DEA 7-negative recipient with naturally occurring anti-DEA 7 antibodies have a normal lifespan.

Suspected naturally occurring anti-DEA 7 antibodies were found in approximately one-third (32%) of Corso dogs tested in this study, a similar finding to recently published data in the general population where they were detected in 38% of dogs (Spada et al., 2016b). Only weak agglutination reactions were detected with suspected naturally occurring anti-DEA 7 antibodies (1+ in 11 samples, 2+ in 12 samples, 3+ in seven samples). Low agglutination titres are rarely associated with detectable clinical signs in the blood recipient. However study provides no information on the strength of the anti-DEA 7 alloantibodies, as this was not specifically determined by titration.

Sixty-seven percent of all dogs tested were positive for DEA 4 only. The definition of the ‘universal’ canine donor (referring to DEA 1- and 7-negative dogs, i.e., DEA 4-positive only) may not be accepted by all clinicians, due to the recent discovery of new blood types Dal (Blais et al., 2007), Kai 1 and Kai 2 (Euler et al., 2016) which could be implicated in TRs. DEA 1 and DEA 7 are the blood types that pose most problems in canine blood transfusion for the reasons cited above; therefore, identification of dogs negative for these blood types is certainly advantageous when screening for inclusion in blood donor programs. In the Corso population the prevalence of dogs with DEA 1- and DEA 7- negative and DEA 4-positive phenotype, was greater than in other canine populations previously tested (Hale et al., 2008; Sinnott Esteve et al., 2011; Spada et al., 2015a, 2015b, 2016c), with the exception of Greyhounds (Iazbik et al., 2010).

The prevalence of different DEAs in Corso dogs has a variety of clinical implications depending on whether dogs are blood donors or recipients. When used as blood donors the higher prevalence of DEA 1-negative animals reduces the risk of sensitisation and acute HTRs in the DEA 1-negative recipient, as demonstrated by the lower rate of calculated potential risk of sensitisation and HTRs when Corso dogs were used as blood donors in untyped DEA 1 transfusions (17% and 3%, respectively) in comparison with Italian blood donors (29% and 8%, respectively). However, the actual risk of sensitisation and transfusion reaction could be different from the calculated risk as development of alloimmunisation and subsequent reactions is influenced by a number of factors, such as quantitative differences in antigen expression, volume of blood administered, frequency of transfusion, and the recipient's immune status.

We did not determine Dal, DEA 3, 5 and the new Kai 1 and Kai 2 blood types (Blais et al., 2007; Euler et al., 2016); DEA 3 and DEA 5 blood types have prevalences of <25% in the general canine population (Swisher et al., 1962; Hale, 1995; Hale et al., 2008; Iazbik et al., 2010; Kessler et al., 2010; Euler et al., 2016;) and naturally occurring antibodies to these blood types exist in a low percentage of the canine population (Hale and Wefelmann, 2006). However, it was not possible to investigate these blood types as reagents for DEA 3 and DEA 5, and for Dal and Kai blood types are not commercially available.

For DEA 4 and DEA 7 blood typing, we used polyclonal antiserum derived from sensitised dogs, and this could have influenced the consistency of the results, particularly for DEA 7 blood types in which reactions were not as strong as for DEA 4. Polyclonal antiserum are heterogeneous and not optimal reagents for use in serologic testing because they can vary in concentration, serologic properties, and epitope recognition and can contain other antibodies of unwanted specificity. The ideal serum for serologic testing is a concentrated suspension of highly specific, well-characterised, uniformly reactive, immunoglobulin molecules such as monoclonal antibody which contain antibodies of a single specificity (Brechter, 2005). However only DEA 1 monoclonal antibodies are commercially available and, to author's knowledge, all studies that have been performed on DEA 7 rely on the use of polyclonal DEA 7 antiserum produced after sensitisation of a DEA 7-negative dog with DEA-7 positive RBC. In this study using polyclonal antiserum, positive agglutination reactions against DEA 4 (all 4+ agglutination) were much stronger than those against DEA 7

(1+ and 2+ agglutination). This suggests that the strength of reaction varies according to the titre and affinity of polyclonal antibodies to the different RBC antigens rather than there being a defined specificity and strength of reagent.

To identify naturally occurring anti-DEA 7 antibodies, plasma samples were cross-matched against only one DEA 7-positive RBC sample (and only one DEA 7-negative RBC sample); it is, therefore, possible that samples that were suspected to contain anti-DEA 7 alloantibodies could have contained other antibodies, such as naturally occurring anti-DEA 3 or anti-DEA 5 antibodies which cross-reacted with the samples. For this reason, in the study we refer to 'suspected' naturally occurring anti-DEA 7 antibodies.

The fact that all dogs in the study population originated in Italy and there might have been some interbreeding could have biased the results of prevalence of DEAs. However, most dogs were geographically distant and from 10 different breeders and from 25 private owners each having only one dog, so the level of interbreeding should be low. In fact, no statistically significant association was found between prevalence of DEAs and origin of the dogs. Furthermore, only 20 active blood donors from one blood bank were included in the survey, which should limit bias due to inclusion of many blood donors from blood banks where DEA 1-negative dogs are preferred.

Conclusions

Corso dogs appear to be an ideal breed to include in canine donor programs, as they have significantly higher DEA 1 and DEA 7-negative prevalence than most canine populations previously surveyed. Additional studies are needed to clarify whether the high prevalence of suspected naturally occurring anti-DEA7 antibodies in this breed could increase their risk of DTRs when they are blood recipients.

Conflict of interest statement

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

Acknowledgments

This study was supported by Piano di Sostegno alla Ricerca 2015-2017, Linea 2, University of Milan, Milan, Italy.

References

Blais, M.C., Berman, L., Oakley, D., Giger, U., 2007. Canine Dal blood type: A red cell antigen lacking in some Dalmatians. Journal of Veterinary Internal Medicine 21, 281–286.

Blais, M.C., Rozanski, E.A., Hale, A.S., Shaw, S.P., Cotter, S.M., 2009. Lack of evidence of pregnancy-induced alloantibodies in dogs. Journal of Veterinary Internal Medicine 23, 462–465.

Brechter, M.E., 2005. AABB Technical Manual, 15th Ed. AABB, Bethesda, Maryland.

Ergul Ekiz, E., Arslan, M., Ozcan, M., Gultekin, G.I., Gulay, O.Y., Kirmizibayrak, T., Giger, U., 2011. Frequency of dog erythrocyte antigen 1.1 in 4 breeds native to different areas in Turkey. Veterinary Clinical Pathology 40, 518–523.

Euler, C.C., Lee, J.H., Kim, H.Y., Raj, K., Mizukami, R., Giger, U., 2016. Survey of two new (Kai 1 and Kai 2) and other blood groups in dogs of North America. Journal of Veterinary Internal Medicine 30, 1642–1647.

Giger, U., Gelens, C.J., Callan, M.B., Oakley, D.A., 1995. An acute hemolytic transfusion reaction caused by dog erythrocyte antigen 1.1 incompatibility in a previously sensitized dog. Journal of the American Veterinary Medical Association 206, 1358–1362.

Hale, A.S., 2012. Canine blood groups and blood typing, in: Day, M., Kohn, B. (Eds.), BSAVA Manual of Canine and Feline Haematology and Transfusion Medicine. BSAVA, Glouchester, UK, pp. 280–283.

Hale, A.S., 1995. Canine Blood Groups and their Importance in Veterinary Transfusion Medicine. Veterinary Clinics of North America: Small Animal Practice 25, 1323–1332.

Hale, A.S., Wefelmann, J., 2006. Incidence of canine serum antibody to known dog erythrocyte antigens in potential donor population, In: Research Abstract Program of the 24th Annual ACVIM Forum Louisville, KY, May 31 - June 3, 2006. Journal of Veterinary Internal Medicine, pp. 768–769.

Hale, A.S., Werfelmann, J., Lemmons, M., Smiler, B., Gerlach, J., 2008. An evaluation of 9570 dogs by breed and dog erythrocyte antigen typing, in: Research Abstract Program of the 26th Annual ACVIM Forum San Antonio, TX, June 4 – June 7, 2008 Index. p. 740.

Harmening, D.M., 2012. Part II. Blood Groups and Serological Testing. 12. Other Technologies and Automation, in: Harmening, D.M. (Ed.), Modern Blood Banking & Transfusion Practices. F.A. Davis Company, Philadelphia, PA, pp. 273–288.

Iazbik, M.C., O'Donnell, M., Marin, L., Zaldivar, S., Hudson, D., Couto, C.G., 2010. Prevalence of dog erythrocyte antigens in retired racing Greyhounds. Veterinary Clinical Pathology 39, 433–435.

Kessler, R.J., Reese, J., Chang, D., Seth, M., Hale, A.S., Giger, U., 2010. Dog erythrocyte antigens 1.1, 1.2, 3, 4, 7, and Dal blood typing and cross-matching by gel column technique. Veterinary Clinical Pathology 39, 306–16.

Melzer, K.J., Wardrop, K.J., Hale, A.S., Wong, V.M., 2003. A hemolytic transfusion reaction due to DEA 4 alloantibodies in a dog. Journal of Veterinary Internal Medicine 17, 931–3.

Novais, A.A., Santana, A.E., Vicentin, L.A., 1999. Prevalence of DEA 1 canine blood group system in dogs (*Canis familiari*s, Linnaeus, 1758) reared in Brazil. Brazilian Journal of Veterinary Research and Animal Science 36, 23–27.

Shulman, I.A., Downes, K.A., Sazama, K., Maffei, L.M., 2001. Pretransfusion compatibility testing for red blood cell administration. Current Opinion in Hematology 8, 397–404.

Sinnott Esteve, V., de Almeida Lacerda, L., Lasta, C.S., Pedralli, V., Gonzalez, F.H.D., 2011. Frequencies of DEA blood types in a purebred canine blood donor population in Porto Alegre, RS, Brazil. Pesquisa Veterinária Brasileira 31, 178–181.

Smith, C.A., 1991. Transfusion medicine: the challenge of practical use. Journal of American Veterinary Medical Association 198, 747–752.

Spada, E., Gavazza, A., Ferro, E., Lubas, G., Mangili, V., Antognoni, M.T., Miglio, A., Proverbio, D., 2015a. Prevalence of Dog Erythrocyte Antigens 4 and 7 in Italian canine blood donors using gel agglutination technique, In: 25th ECVIM-CA Congress, September 10th-12th, Lisbon, Portugal. p. 188.

Spada, E., Proverbio, D., Baggiani, L., Canzi, I., Perego, R., 2016a. Activity, specificity, and titer of naturally occurring canine anti–DEA 7 antibodies. Journal of Veterinary Diagnostic Investigation 28, 705–708.

Spada, E., Proverbio, D., Viñals Flórez, L.M., Del Rosario Perlado Chamizo, M., Perego, R., Bagnagatti De Giorgi, G., Baggiani, L., 2015b. Prevalence of dog erythrocyte antigens 1, 4, and 7 in galgos (Spanish Greyhounds). Journal of Veterinary Diagnostic Investigation 27, 558–561.

Spada, E., Proverbio, D., Vinals Florez, L.M., Del Rosario Perlado Chamizo, M., Serra Gómez De La Serna, B., Perego, R., Baggiani, L., 2016b. Prevalence of naturally occurring antibodies against dog erythrocyte antigen 7 in a population of dog erythrocyte antigen 7–negative dogs from Spain and Italy. American Journal of Veterinary Research 77, 877–881.

Spada, E., Proverbio, D., Viñals Flórez, L.M., Serra Gómez De La Serna, B., Del Rosario Perlado Chamizo, M., Baggiani, L., Perego, R., 2016c. Prevalence of dog erythrocyte antigens 1, 4, and 7 in Podenco Ibicenco (Ibizan Hounds) from Ibiza Island. Veterinary Medicine International 2016, 1048257.
.
Swisher, S.N., Young, L.E., 1961. The blood grouping systems of dogs. Physiological Reviews 41, 495–520.

Swisher, S.N., Young, L.E., Trabold, N., 1962. In vitro and in vivo studies of the behavior of canine erythrocyte-isoantibody systems. Annals New York Academy of Sciences 97, 15–25.

Vriesendorp, H.M., Albert, E.D., Templeton, J.W., Belotsky, S., Taylor, B., Blumenstock, D.A., Bull, R.W., Cannon, F.D., Epstein, R.B., Ferrebee, J.W., et al., 1976. A joint report of the Second International Workshop on Canine Immunogenetics. Transplantation Proceedings 8, 289–314.

Table 1.

Prevalence and strength of agglutination DEA 1, 4, and 7-positive samples in a population of 100 Italian Corso dogs.

DEA	% (95% CI)	Strength of agglutination (%)			
		1+	2+	3+	4+
1-positive	29 (19.4-41.6)	N/A	N/A	N/A	N/A
4-positive	100 (81.3-121.6)	0	0	0	100
7-positive	5 (1.6-11.6)	4	5	0	0

DEA, dog erythrocyte antigen; CI, confidence intervals; N/A, not applicable.

Table 2

Comparison of the results of prevalence of blood type DEA 1, 4 and 7 in 100 purebred Italian Corso dogs and DEAs prevalence in other canine populations tested worldwide.

Canine population Investigated	Number of dogs blood typed	DEA 1-positive		DEA 4-positive		DEA 7-positive		DEA 4-positive, DEA 1 and DEA 7-negative	
		%	*P*	%	*P*	%	*P*	%	*P*
Corso dogs (this study)	100	29	N/A	100	N/A	5	N/A	67	N/A
Italian blood donors (Spada et al., 2015a) (different breeds)	84	41	0.1029	100	N/A	33	<0.0001[a]	38	0.0001[a]
Spanish greyhounds (Spada et al., 2015b)	205 (150 for DEA 4 and DEA 7)	55	0.0003[a]	100	N/A	8	0.5662	47	0.0067[a]
Ibizan hounds (Spada et al., 2016c)	92	75	<0.0001[a]	99	1.000	25	0.0002[a]	17	< 0.0001[a]
Greyhounds (Iazbik et al., 2010)	206 (113 for DEA 4)	13	0.0092[a]	100	N/A	29	<0.0001[a]	52	0.0437
Brazilian blood donors (Sinnott Esteve et al., 2011) (different breeds)	100	61	<0.0001[a]	100	N/A	17	0.0129	14	<0.0001[a]
US canine population (Hale et al., 2008) (different breeds)	9570	42	0.0762	98	0.4773	20	0.0028[a]	29	<0.0001[a]

DEA, dog erythrocyte antigen; N/A, not applicable

[a] Statistically significant (P<0.01)

*Highlights (for review)

Highlights

- Information on prevalence of dog erythrocyte antigens (DEAs) is important in selection of blood donors.
- Corso dogs fulfil common requirements for blood donors: large size, good-tempered, easy to handle during blood collection.
- There is a high prevalence of DEA 1 and DEA 7-negative dogs in the Corso breed.
- The presence of suspected naturally occurring anti-DEA 7 antibodies may be implicated in delayed transfusion reactions.