Schweiz. Arch. Tierheilk.
B. Riond, E. Schuler, E. Rogg, R. Hofmann-Lehmann, H. Lutz, Band 153 Heft 8, August 2011, 369–374
DOI 10.1024/0036-7281/a000223

Prevalence of dog erythrocyte antigen 1.1 in dogs in Switzerland evaluated with the gel column technique

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Summary

Canine blood typing has become an established and essential laboratory test due to the rising demand for safe and efficient blood transfusions. The most immunogenic and clinically important blood type is DEA 1.1. Little is known about DEA 1.1 frequencies or special characteristics among different canine breeds. 304 dogs were tested for DEA 1.1. DEA 1.1-typing was performed using a commercial gel column technique (ID-Gel Test Canine DEA 1.1, DiaMed, Cressier, Switzerland). Fifty-three percent of all tested dogs reacted positive for DEA 1.1, whereas 49 % of the mixed breeds tested DEA 1.1-positive. All Bernese mountain dogs (n = 22) and Rottweilers (n = 9) tested positive for DEA 1.1, while all Boxers (n = 8), Flat-Coated Retrievers (n = 9), and Border Collies (6) tested negative for DEA 1.1. The prevalence of DEA 1.1 in dogs in Switzerland was found to be comparable to that reported from other countries. The tested breeds were found to differ considerably in the frequency of DEA 1.1. This knowledge is useful for selection of blood donors. However, DEA 1.1 blood typing of donor and recipient prior to transfusion and cross matching in sensitized dogs is unavoidable.

Keywords**:** dog, blood group, DEA 1.1 prevalence, gel test, transfusion medicine, immunohematology

Häufigkeit des Blutgruppenantigens DEA 1.1 bei Hunden in der Schweiz bestimmt mit der Geltechnik

Aufgrund der steigenden Nachfrage nach sicheren und effizienten Bluttransfusionen ist die Blutgruppenbestimmung beim Hund mittlerweile ein etablierter und unentbehrlicher Labortest geworden. DEA 1.1 ist das immunogenste und somit klinisch bedeutsamste Blutgruppenantigen beim Hund. Über DEA 1.1-Häufigkeiten oder rassespezifische Besonderheiten ist wenig bekannt. Im Rahmen dieser Studie wurden 304 Schweizer Hunde für DEA 1.1 getestet. Die DEA 1.1-Typisierung erfolgte mit der Geltechnik (ID-Gel Test Canine DEA 1.1, DiaMed, Cressier, Schweiz). Dreiundfünfzig Prozent aller Hunde sowie aller reinrassigen Hunde testeten positiv für DEA 1.1; 49 % der Mischlinge waren ebenfalls DEA 1.1 positiv. Alle Berner Sennenhunde (n = 22) und Rottweiler (n = 9) waren DEA 1.1 positiv, während alle Boxer (n = 8), Flat-Coated Retriever (n = 9) und Border Collies (6) DEA 1.1 negativ waren. Die DEA 1.1- Häufigkeit in der Schweiz ist ähnlich wie in anderen Ländern. Jedoch wurden erhebliche Unterschiede in der DEA 1.1- Häufigkeit zwischen einzelnen Rassen gefunden. Kenntnis der DEA 1.1 Häufigkeit innerhalb einer Rasse kann von Nutzen sein für die effiziente Auswahl von Blutspendern. Jedoch sind eine DEA 1.1 Blutgruppenbestimmung von Spender und Empfänger vor der Transfusion und die Durchführung einer Kreuzprobe vor der zweiten Transfusion unbedingt erforderlich.

Schlüsselwörter: Hund, Blutgruppe, DEA 1.1, Häufigkeit, Geltechnik, Transfusions- medizin, Immunhämatologie

Introduction

Based on the last international workshop on standardization of canine blood groups in 1974 (Vriesendorp et al., 1976) , the dog erythrocyte antigen (DEA) system is the current used nomenclature for canine blood groups in the United States and Europe. The following canine blood group systems have been identified: DEA 1, DEA 3, DEA 4, DEA 5, DEA 6, DEA 7, and DEA 8. With the exception of the DEA 1 system, all canine blood group systems are bi-allelic; thus a dog either has a particular blood type or not. In the DEA 1 system, three allelic blood types have been identified: DEA 1.1 (also known as A1) and DEA 1.2 (A2). Two decades ago, a third subtype A3 has been described in German shepherd dogs in Australia (Symons and Bell, 1991), but is not yet approved by the international standardization committee. A dog shows only one phenotype meaning that a dog can be DEA 1.1-positive or -negative, and only a DEA 1.1-negative dog can be DEA 1.2-positive or DEA 1.2-negative. DEA 1.1 is dominant over the other types in the DEA 1 system (Hale, 1995; Hohenhaus, 2004). Typing sera is commercially available in the United States only for DEA 1.1,1.2, 3, 4, 5, 7 (Hale, 1995; Hohenhaus, 2004; Andrew and Penedo, 2010). DEA 1.1, DEA 1.2 and A3 are described as subtypes of a linear series meaning that isoimmune antisera produced to one of the antigens can result in cross-reactivity with the other antigens in the series (Andrew and Penedo, 2010). The DEA 1 system is the clinically most important blood group system in dogs. The clinical importance of DEA 1.2, DEA 3, 5 and 7 and A3 have not been documented (Kessler et al., 2010). Although naturally occurring antibodies to the DEA 1 system have not been reported, production of alloantibodies can be induced in the dog as a result of mismatched blood transfusions. DEA 1.1-negative dogs may become sensitized when receiving type DEA 1.1-positive blood and may develop acute haemolytic transfusion reactions when a second blood transfusion with DEA 1.1 blood is carried out (Callan et al., 1995; Giger et al., 1995; Hohenhaus, 2004; Giger, 2009). DEA typing for the dog varies, depending on the laboratory used, and the availability of antisera. Most of the laboratories only type for DEA 1.1 because of its high antigenicity; others type for more antigens. To assure safe and effective blood transfusions in the dog, and to prevent sensitization, blood donor and recipient should undergo DEA 1.1 blood typing. Additionally, crossmatching is indicated if earlier incompatible blood transfusions cannot be excluded (Howard et al., 1992; Giger et al., 1995; Giger et al., 2005).

Very limited surveys on the frequency of DEA 1.1 have been reported (Giger et al., 1995; Novais et al., 1999; van der Merwe et al., 2002; Hale et al., 2008) or on its distribution among canine breeds (van der Merwe et al., 2002; Hale et al., 2008), which leaves open the possibility of geographic and breed-associated differences. Several blood typing techniques for DEA 1.1 have been described including typing cards (DMS RapidVet-H, DMS Laboratories Inc., Flemington, USA) (Kohn et al., 1998; Giger et al., 2005; Seth et al., 2008), cartridge kits (Quick Test DEA 1.1, Alvedia, Lyon, France) (Seth et al., 2008), tube agglutination test (Animal Blood Resources International, Stockbridge, USA), and a gel column test (ID-Gel Test Canine DEA 1.1, DiaMed AG, Cressier sur Morat, Switzerland) (Giger et al., 2005; Seth et al., 2008). The ID-Gel test, originally developed for use in human medicine (Lapierre et al., 1990), is a highly sensible column agglutination assay in which red blood cells agglutinated by antibodies to DEA 1.1 antigen, become trapped and remain on top of the gel after a centrifugation step, while the free red blood cells pass through the column and form a pellet at the bottom of the tube (Giger et al., 2005; Seth et al., 2008). The ID-test is suitable for use in large clinical laboratories (Giger et al., 2005), whereas the typing cards and the cartridge kits a intended for in practice use as point-of-care tests. The purpose of the present study was to determine the prevalence of DEA 1.1 in the Swiss dog population and to gain information about the distribution of DEA 1.1 among individual canine breeds.

Animals, Material and Methods

Sample collection

EDTA blood samples from 304 dogs were typed for DEA 1.1. The samples had been sent to the Clinical laboratory by the Clinic for Small Animals, University of Zurich, from patients and blood donors for haematological and blood typing analysis over a period of two years (2006–2008). In total, 269 purebred dogs of 39 different breeds (Tab. 1) and 35 mixed-breed dogs were analysed. The blood samples were stored at 4 °C for < 3 days until analysis.

Laboratory testing

DEA 1.1 testing and interpretation of the results were performed using a commercial gel column technique (ID-Gel Test Canine DEA 1.1, DiaMed AG, Cressier sur Morat, Switzerland) according to the manufacturer's instructions and as described previously (Giger et al., 2005; Seth et al., 2008).

Statistical Analysis

All results were compiled in a table-calculation program (Analyse-It for Microsoft Excel, v. 2.09, Analyse-it-Software Ltd., Leeds, UK; http://www.analyse-it.com). A Fisher's exact test was performed to assess differences in DEA 1.1 prevalences among different breeds. Values of $p < 0.05$ were considered significant.

Table1: Canine samples analyzed for DEA 1.1.

Breed	Sample size	DEA 1.1- positive	DEA 1.1- negative	Prevalence*	Fisher's exact test +
Airedale	4	0	4	–	
Akito Inu	2	2	0	–	
Australian Shepard	4	1	3	–	
Borsoi	2	2	0	–	
Beagle	9	3	6	33%	$p < 0.05$
Bearded Collie	2	0	2	–	
Berger des Pyrenées	2	0	2	–	
Bernese mountain dog	22	22	0	100%	
St. Bernhard	2	2	0	–	
Bobtail	2	0	2	–	
Border Collie	6	0	6	0%	$P < 0.05$
Boxer	8	0	8	0%	$P < 0.05$
Cairn Terrier	3	1	2	–	
Cocker Spaniel	8	4	4	50%	n. s.
Dachshund	7	5	2	71%	n. s.
Dalmation	4	3	1	–	
German Shepherd	12	3	9	25%	$P < 0.05$
Gordon Setter	5	5	0	–	
Great Dane	3	2	1	–	
Doberman	6	2	4	33%	$P < 0.05$
German Hunt terrier	2	0	2	–	
Flat-coated retriever	9	0	9	0%	$P < 0.05$
French Bulldog	3	0	3	–	
Golden Retriever	13	10	3	77%	n. s.
Greyhound	2	0	2	–	
Hovawart	3	0	3	–	
Jack Russell Terrier	12	6	6	50%	$P < 0.05$
Canadian Shepherd	5	3	2	–	
Labrador Retriever	20	15	5	75%	n. s.
Leonberger	4	4	0	–	
Maltese	2	1	1	–	
Mixed breed	35	17	18	49%	$P < 0.05$
Newfoundland dog	4	3	1	–	
Poodle	6	2	4	33%	$P < 0.05$
Pinscher	2	1	1	–	
Rhodesian Ridgeback	2	1	1	–	
Rottweiler	9	9	0	100%	
Tervueren	7	1	6	14%	$P < 0.05$
Tibetan Terrier	4	4	0	–	
Yorkshire Terrier	13	7	6	54%	$P < 0.05$

** given for breeds ≥ 6 samples*
+ DEA 1.1 prevalence of Rottweilers and Bernese Mountain dogs compared with given prevalence of breeds ≥ 6 samples.

Results

Thirty-six of 211 tested samples showed anaemia, with hematocrit values below 40 % (reference range: 42–55 %). In fact, one dog had a PCV of 15 %. In all cases, reading and interpretation of the anaemic samples was performed without difficulty.

None of the 304 blood samples tested showed positive reactions in the negative control; thus, no auto-agglutination was suspected and all samples were included in the study. Fifty-three percent of the dogs (160/304) tested positive for DEA 1.1. In 112/160 positive samples, the intensity of the agglutination reactions was recorded: 2 + was found in six samples, 3 + in 70 samples, and 4 + in 36 samples. Forty-seven percent (144/304) did not react in the gel test and were therefore considered DEA 1.1-negative. Of the purebred dogs, fifty-three percent (143/269) tested positive for DEA 1.1. The prevalence in mixed breeds was slightly lower (49 %; 17/35). The results of DEA 1.1 typing in 39 different canine breeds are shown in Table 1. Canine breeds with sample volumes < 2 are not listed. Notable differences among the breeds were observed. When comparing Bernese mountain dogs and Rottweilers showing the highest prevalence with breeds were ≥ 6 dogs were analyzed, significant differences were found in 11 canine breeds (Tab. 1). Major differences in DEA 1.1 prevalence were observed between the overall average and the average within a single breed, as well as between the overall average and the average across the 39 pure breeds tested. The percentage of DEA 1.1-positive dogs ranged from 0 % until 100 % among individual breeds (Tab. 1). Interestingly, some canine breeds reacted in a uniform manner: All Bernese Mountain dogs (22) and Rottweilers (9) tested positive for DEA 1.1, whereas all Boxers (8), Flat-Coated Retrievers (9), and Border Collies (6) were negative.

Discussion

This is the first study that provides information about DEA 1.1 frequencies in the dog population in Switzerland. The prevalence of DEA 1.1 positive dogs in Switzerland is comparable to those determined with other methods in the United States (45 %) (Swisher and Young, 1961; Hale, 1995), (42 %) (Hale et al., 2008), Brazil (52 %) (Novais et al., 1999) , South Africa (47 %) (van der Merwe et al., 2002), and Germany (53 %) (Kohn et al., 1998). The slight differences observed in the DEA 1.1 prevalence in different studies may be due to the method used and to the particular dog populations and breeds under investigation. Differences among canine blood typing methods have been documented, and can be explained by the use of different DEA 1.1 reagents. Beside the gel test several canine blood typing methods are available using monoclonal DEA 1.1 antibodies. These include the card test, in which a monoclonal DEA 1.1 antibody different from the gel test is used. False-positive DEA 1.1 reactions were reported for this card test (Moritz et al., 1998) with subsequent incompatible transfusions or deleterious exclusion of potential blood donors. A tube agglutination test, which is based on a canine blood typing classification different from the DEA system, recognizes four blood types by monoclonal antibodies (Giger et al., 2005). The only canine blood typing method in which polyclonal antibodies are used is the Michigan State University test (Giger et al., 2005). The gel test is a reliable and rapid clinical laboratory method for DEA 1.1 typing (Giger et al., 2005). It can be adapted for an extended canine blood typing using polyclonal reagents for detection of DEA 1.1, 3, 4, and 7, and for cross matching (Kessler et al., 2010).

Geographical variations and differences among breeds have been already reported for feline blood types (Giger et al., 1991; Hubler et al., 1993; Giger, 2009). Therefore, it can be assumed that each country or geographic region has predominant dog breeds differing from those of other countries. More precisely, the average DEA 1.1 prevalence in a country can be influenced by indigenous breeds with high or low DEA 1.1 prevalence. This influence became evident in a Croatian study, where the authors reported a DEA 1.1 prevalence of 84 % (Gracner et al., 2004). However, those authors analysed only three Croatian dog breeds showing a high prevalence of DEA 1.1.

In accordance with the findings of other groups, we found considerable differences in DEA 1.1 frequency among different canine breeds. Despite the large number of dogs tested in this study, some breeds were underrepresented. Reasonable numbers of animals (≥ 6/breed) were tested in 16 of 39 breeds, and DEA 1.1 prevalence was calculated (Tab.1). In agreement with a previous report (van der Merwe et al., 2002), some breeds showed very low DEA 1.1 frequencies, whereas others had high frequencies of up to 100 %. All Bernese Mountain dogs and Rottweilers were found to be DEA 1.1-positive, whereas all Flat-coated Retrievers, Boxers, and Border Collies tested DEA 1.1-negative. Today many canine breeds are characterized by reduced genetic diversity resulting from a small number of founders. It has been shown that selection pressure within a breed leads to loss of genetic variation (Irion et al., 2003). The homogeneity of DEA 1.1 in some breeds in our study can be explained by this phenomenon.

Knowledge of DEA 1.1 frequencies within and between canine breeds is helpful in canine transfusion medicine as screening for blood donors in blood banks will be facilitated by the more efficient selection of blood donors. Based on the result of this study in Switzerland it can be suggested that it is more likely to identify DEA 1.1 negative blood donors among Flat-coated Retrievers, Boxers, and Border Collies, whereas Bernese Mountain dogs and Rottweilers may probably not be suitable as universal blood donors. Nevertheless, knowing the prevalence of a blood type in a breed is no substitute for DEA 1.1 typing of donor and recipient prior to transfusion, as well as cross matching in sensitized dogs.

Fréquence de l'antigène de groupe sanguin DEA 1.1, déterminé par la méthode de filtration en gel, chez les chiens en Suisse

Au vu de la demande croissante de transfusions sûres et efficaces, la détermination des groupes sanguins chez le chien est devenue un test de laboratoire établi et indispensable. DEA 1.1 est l'antigène de groupe sanguin le plus immunogène et donc celui qui présente la plus grande importance clinique chez le chien. On connaît peu de choses sur la fréquence ou les spécificités raciales du DEA 1.1 . Dans le cadre de cette étude, 304 chiens suisses ont été testés à l'égard du DEA 1.1. La typisation a été réalisée par la méthode filtration en gel (ID-Gel Test Canine DEA 1.1 Diamed, Cressier, Suisse). 53 % de tous les chiens, ainsi que de tous les chiens de pures races ont été testés positifs. 49% des croisés étaient DEA 1.1 positifs, de même que tous les bouviers bernois (n = 22) et tous les rottweiler (n = 9). Tous les boxers (n = 8), les flat coated retriever (n = 9) et les border collies (n = 6) étaient DEA 1.1 négatifs. En Suisse, la fréquence du DEA 1.1 est similaire avec celles des autres pays, toutefois, des différences importantes sont constatées entre les diverses races. La connaissance de cette fréquence à l'intérieur d'une race peut être utile pour le choix optimum de donneurs. Toutefois, une détermination du groups sanguin DEA 1.1 du donneur ou du receveur avant la transfusion, de même que la réalisation d'un cross match avant la 2e transfusion sont absolument indispensables.

Frequenza dell'antigene del gruppo sanguigno DEA 1.1 nei cani in Svizzera, determinato con il metodo di gel

A causa della domanda crescente di trasfusioni sanguigne sicure ed efficienti, la determinazione del gruppo sanguigno nel cane è diventato un test di laboratorio usuale ed essenziale. Il DEA 1.1 è il più immunogeno ed il clinicamente più importante antigene dei gruppi sanguigni nel cane. Informazioni sulla frequenze del DEA 1.1 o di particolarità specifiche alla razza sono poco note. In questo studio, sono stati testati per il DEA 1.1, 304 cani svizzeri. La tipizzazione del DEA 1.1 è stata eseguita con il metodo di gel (ID-Gel Test Canine DEA 1.1, DiaMed, Cressier, Svizzera). Il 53% di tutti i cani, compresi quelli di razza sono risultati positivi al DEA 1.1 e il 49% di quelli incrociati sono risultati anche loro positivi al DEA 1.1. Tutti i Bovari bernesi (n = 22) e i Rottweiler (n = 9) sono risultati positivi al DEA 1.1, mentre tutti i Boxer (n = 8), i Flat-Coated Retriever (n = 9) e i Border Collie (6) sono risultati negativi al DEA 1.1. L'incidenza del DEA 1.1 in Svizzera è simile agli altri paesi. Tuttavia, sono state riscontrate differenze rilevanti nella frequenza del DEA 1.1 tra razze diverse. La conoscenza della frequenza del DEA 1.1 all'interno di una razza può essere utile per una efficiente selezione dei donatori di sangue. Tuttavia, una tipizzazione del DEA 1.1 del sangue del donatore e del ricevente prima della trasfusione e l'attuazione di una prova incrociata prima della seconda trasfusione è necessaria.

Conclusion

The present study determined the overall frequency of DEA 1.1 for dogs in Switzerland and reported DEA 1.1 frequencies within and among different European breeds. These data are valuable for veterinary transfusion medicine, especially for blood banks. Nevertheless, the establishment of blood type frequencies in the dog has to be extended in order to build up a solid database. Laboratory but also in-clinic DEA 1.1 typing methods are available for use prior to transfusing dogs.

Acknowledgments

We thank Y. Bosshart, U. Egger, E. Grässli, M. Huder, B. Lange, J. Wälchli, M. Maurer, and Y. Gahler for excellent laboratory assistance. The authors thank DiaMed AG, Cressier sur Morat, Switzerland for providing the tests and technical support. R.H.-L. is the recipient of a professorship from the Swiss National Science Foundation (PP00B-102866 and PPOOP3–119136).

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Received: 14 January 2010
Accepted: 26 November 2010