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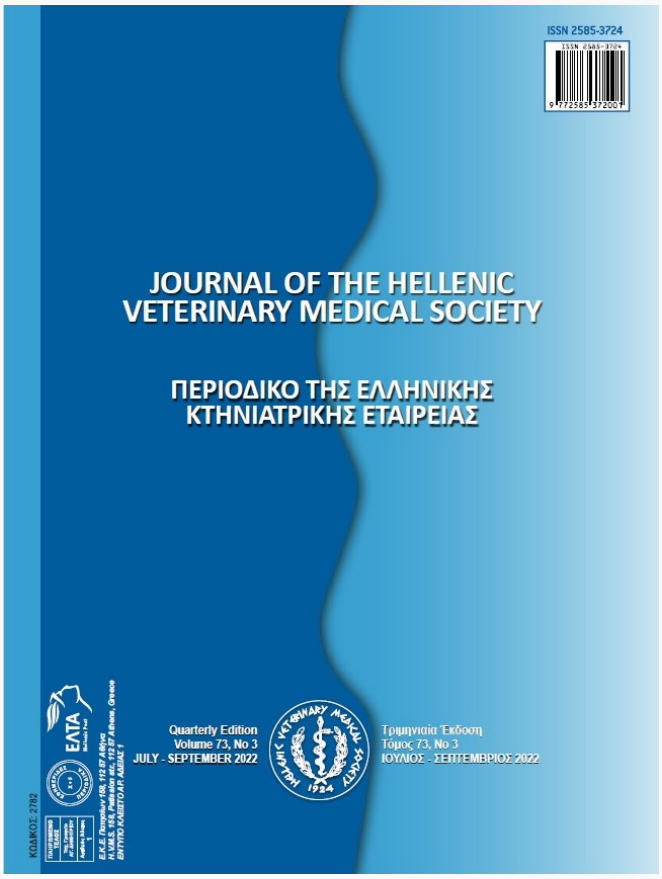

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Blood transfusions in dogs and cats from Portugal: a retrospective study

L. R. Dias[a], H. Vilhena[a,b,c,d], N. Carolino[a,e], A. Freire[f], M. Moura[g], R. Aleixo[g], A. C. Silvestre-Ferreira[c,d,g], S. C. Duarte[a,i,*]

[a]*Vasco da Gama Research Centre (CIVG)/ Department of Veterinary Sciences, Escola Universitária Vasco da Gama (EUVG), Coimbra, Portugal*

[b] *Baixo Vouga Veterinary Hospital, Segadães, Águeda*

[c]*The Animal and Veterinary Research Center (CECAV), Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*

[d]*Laboratório Associado para Ciência Animal e Veterinária (AL4AnimalS), Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*

[e] *Centre for Interdisciplinary Research in Animal Health (CIISA), Universidade de Lisboa.Faculdade de Medicina Veterinária, Lisboa, Portugal*

[f]*Universitary Veterinary Hospital of Coimbra, Coimbra, Portugal*

[g]*Foz Canis Figueira da FozVeterinary Hospital, Figueira da Foz, Portugal*

[h]*Department of Veterinary Sciences, Universidade de Trás-os-Montes e Alto Douro, Vila Real, Portugal*

[i]*LAQV-REQUIMTE, Faculdade de Farmácia da Universidade de Coimbra - Pólo III, Coimbra, Portugal*

ABSTRACT: This retrospective study aimed to characterize blood transfusions of whole blood and erythrocyte concentrates in companion animals performed in Portugal. In total, 116 animals were analyzed, of which 59 dogs and 57 cats. Pre-transfusion blood typing was not performed on most animals (63.8%). In the animals in which pre-transfusion blood typing was performed, most cats (96.2%) were type A and most dogs (75.0%) were DEA1 positive. After the transfusions, most of the animals survived (77.2% cats and 64.4% dogs). No transfusion reactions were recorded.

Keywords: Anemia; blood typing; blood compatibility; Portugal

Corresponding Author:
S.C. Duarte; Av. José R. Sousa Fernandes, Campus Universitário, Lordemão, 3020-210 Coimbra
E-mail address: s.cancela.duarte@gmail.com

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INTRODUCTION

In the dog, more than twelve blood groups are officially recognized, defined by the antigens expressed on the surface of the red blood cells (RBC). In the Dog Erythrocyte Antigen (DEA) system, the International Society for Animal Genetics recognizes seven blood types: DEA 1, 3, 4, 5, 6, 7 and 8 (Zaremba *et al*., 2019). DEA 1 is the blood type with greater antigenicity and can cause acute hemolytic reactions in DEA 1 negative dogs previously sensitized, involving reactions by induced alloantibodies (Ferreira *et al*., 2011; Odunayo*et al*., 2017). In dogs, there are natural alloantibodies (anti-DEA 3,5 and 7) that can cause destruction of transfused RBCs and lead to delayed transfusion reactions. In addition, Dal, Kai1 and Kai2 antigens are also recognized in the dog (Zaremba *et al*., 2019). The Dal antigen, described by Blais et al.(2007), was identified in Dalmatian, Doberman and Shih Tzu dogs, and can cause hemolytic reactions in negative animals in case of prior sensitization(Zaremba *et al*., 2019). Kai 1 and 2 antigens were identified in 2016, while no naturally occurring alloantibodies were identified (Euler *et al*., 2016). Most dogs (approximately 94% of the animals tested) are Kai 1 positive and Kai 2 negative. An animal cannot present Kai 1 and Kai 2 antigens simultaneously, however, they can be negative for both (Zaremba *et al*., 2019). In addition to these, based on incompatible results in crossmatching tests in previously transfused dogs, other canine blood group systems are suspected (Wardrop *et al*., 2016).

In the cat, the AB blood group system includes three blood types: A, B and AB. Type A cats, and especially type B cats, have natural alloantibodies that are formed about three weeks after birth (Davidow, 2013; Kisielewicz and Self, 2014). These alloantibodies are clinically significant, particularly in type B animals that are transfused with type A blood, causing acute and severe hemolytic and agglutination reactions. In type A cats, reactions can also occur if they receive type B blood, however these are delayed and clinically less relevant (Vieira *et al*., 2017). No naturally occurring alloantibodies exist for type AB cats (Davidow, 2013; Kisielewicz and Self, 2014). A new clinically relevant RBC antigen, *Mik*, has recently been described in cats. Cats may or may not express the *Mik* antigen. The absence of this antigen may be associated with the presence of natural anti-*Mik* alloantibodies, which can cause an acute hemolytic transfusion reaction after a compatible blood transfusion within the AB group (Priolo*et al*., 2017).

Transfusions of whole blood or blood components carry risks for animals, which can result in different types of reactions, such as immune-mediated or hemolytic, and acute or delayed (Ferreira *et al*., 2008). For this reason, blood transfusion should be associated with blood compatibility tests, such as blood typing and crossmatching to reduce the probability of occurrence of transfusion reactions (Vieira *et al*., 2017).

The commercial rapid blood typing tests to determine canine DEA 1 (positive or negative) and feline A, B and AB types are based in the techniques of immunochromatography, agglutination on gel-tube and card. For other dog and cat blood types, no commercial tests are available (Zaremba *et al*., 2019). Thus, determination of type DEA 1 (positive or negative) in dogs and types A, B or AB in cats does not prevent the occurrence of transfusion reactions due to incompatibilities of other blood groups, which may justify a reduction in the survival time of RBCs transfused. For this reason, in addition to blood typing, it is recommended to perform crossmatching before transfusions of whole blood or blood components (Zaremba *et al*., 2019).

Previous studies indicate that the ideal hematocrit, after a transfusion in dogs and cats, should be 10% higher than the initial value (Godinho-Cunha *et al*., 2011). For this purpose, several formulas given in the literature allow the estimation of the ideal volume of whole blood or its derivatives to be administered (Godinho-Cunha *et al*., 2011;Kisielewicz and Self, 2014).

Despite the fact that blood transfusions in veterinary medicine have become widespread in recent years, the available information related to their clinical practice in Portugal is scarce. Thus, the main objective of this study was to characterize blood transfusions of whole blood and erythrocyte concentrates in companion animals performed in Portugal, regarding the reason for transfusion, number of transfusions performed, origin and type of components transfused, prior execution of blood compatibility tests, and number of days of hospitalization.

MATERIALS AND METHODS

This study received the approval by the Scientific Council of Escola Universitária Vasco da Gama (minute nº43, of July 31st 2018), and respects the General Data Protection Regulation (EU) 2016/679 and other applicable legislation in force.

Study design and data collection

This retrospective study involved data collection

from six veterinary medical care centers (VMCC) in Portugal, in which all transfusions of whole blood and erythrocyte concentrates performed in dogs and cats between March 2014 and March 2019 were analyzed. All animals with no information regarding the transfused blood components or the hematocrit values were excluded. In animals submitted to repeated transfusions, only information regarding to the first blood transfusion was considered.

The medical records of each animal were consulted, and data were recorded in two groups: a) Characterization of the study population - species, gender, reproductive status, age, weight and breed; and b) Characterization of transfusions - number of transfusions performed, blood origin (blood bank or VMCC donors), reason for transfusion, prior blood typing, transfused blood components, pre- and post-transfusion hematocrit values, days of hospitalization, death or euthanasia during the hospitalization period after transfusion.

The reason for the transfusion was systematized according to Klaser and col. (2005) and Paltrinieri and col (2016) in hemolytic anemia, hemorrhagic anemia, non-regenerative anemia, and anemia of undetermined etiology. Pre-transfusion hematocrit values were determined immediately before transfusions, while post-transfusion values were determined up to 24 hours after transfusion. Normal range for hematocrit in dogs was considered as 37-55% and in cats as 24-45%. In the dog, anemias were classified as mild for hematocrit values between 30 and 37%, moderate for values between 20 and 29%, severe if between 13 and 19%, and very severe if <13% (Bellier and Cordonnier, 2010). In cats, anemias were classified as mild for hematocrit values between 20 and 26%, moderate between 14 and 19%, severe between 10 and 13%, and very severe if <10% (Tasker, 2012).

Statistical analysis

The statistical analysis was performed separately for each species, although according to an identical methodology. Blood parameters (initial hematocrit, post transfusion hematocrit and difference between the two evaluations) and all variables / factors collected on the animals were subjected to several preliminary analyses with PROCMEANS and PROCFREQ from the SAS™ program to characterize the corresponding records through their descriptive characteristics and frequencies.

The PROCCORR of the SAS™ program was used to estimate Pearson's correlation coefficients between the different quantitative parameters evaluated, to quantify the intensity and direction of the linear relationship between these same parameters.

Subsequently, the values of the final hematocrit and the difference between the values of the final and initial hematocrit were submitted to covariance analysis with the PROCGLM of the SAS™ program to verify the main factors that influenced this blood parameter.

RESULTS

Characterization of the study population

During the study period, 116 animals submitted for transfusion of whole blood or erythrocyte concentrates were selected, of which 59 were dogs and 57 cats. In both species, transfusion was more frequent in males. The characterization of the analyzed population is shown in Table 1.

Fifty-two European shorthair cats (91.2%) were studied, and the remaining purebred animals studied were two Persian cats (3.5%), two Siamese cats (3.5%) and one Norwegian Forest cat (1.8 %).

In dogs, the distribution of purebred animals was as follows: seven Labrador Retrievers (11.8%), three Yorkshire Terriers (5.1%), three Poodles (5.1%), three Pitbull (5.1%), two Pinschers (3.4%), two Siberian Husky (3.4%), two Samoyeds (3.4%), and two Golden Retrievers (3.4%). An exemplar (1.7%) of each of the following breeds was also included: Sharpei, English Cocker Spaniel, Castro Laboreiro Dog, Brazilian Fila Dog, Rottweiler, Pekingese, Chihuahua, Epagneul Breton, Lion of Rhodesia, Dogue de Bordeaux, French Bulldog, Swiss Cattleman, Maltese Bichon, Dalmatian, English Setter, German Shepherd Dog and Boxer. The remaining animals (n = 18; 30.5%) were mixed breed dogs.

Characterization of transfusions

Of a total of 116 transfusions performed in dogs and cats, in 74 (63,8%) cases included in the study, no blood typing tests were performed prior to the transfusion. In cats, 31 out of 57 (54.4%) animals were not typed before blood transfusion; and in blood typed animals (n=26), no type B cat was identified. In 43 out of 59 (72.9%) dogs, no blood typing before transfusion was performed. Of the total transfusions (n=116), 63(54.3%) corresponded to whole blood transfusions and 53 (45.7%) to erythrocyte concentrates transfusions (Table 2).

Table 1. Characterization of the studied population.

		Dogs (n=59)	**Cats** (n=57)
Gender	**Males**	**32 (54.3%)**	**37 (64.9%)**
	Intact	27 (45.8%)	15 (26.3%)
	Neutered	5 (8.5%)	22 (38.6%)
	Females	**27 (45.7%)**	**20 (35.1%)**
	Intact	15 (25.4%)	7 (12.3%)
	Neutered	12 (20.3%)	13 (22.8%)
Age (years)	Mean ± SD	7.0 ± 4.6	6.3 ± 4.6
	[Min-Max]	[0.2-14.0]	[0.3-19.0]
Weight (kg)	Mean ± SD	18.1± 13.1	3.8± 1.4
	[Min-Max]	[2.4-51.0]	[1.1-8.2]
Breed	Purebred	18 (30.5%)	5 (8.8%)
	Undetermined	41 (69.5%)	-
	European shorthair	-	52 (91.2%)

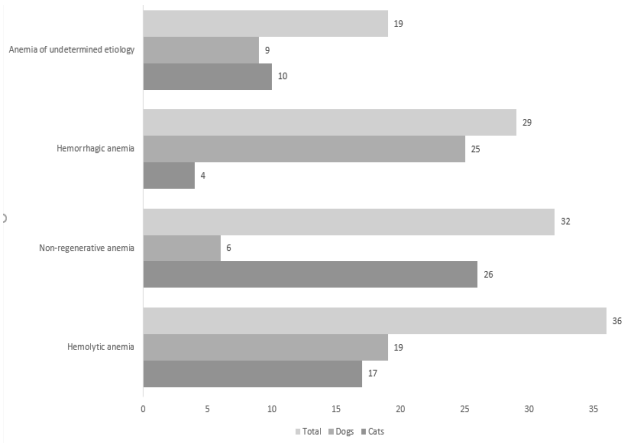
Max. maximum; Min. minimum; SD. Standard deviation

Table 2. Characterization of transfusions.

Total number of transfusions performed per animal	**Dogs**(n=59)	**Cats**(n=57)
n=1	51 (86.4%)	47 (82.5%)
n=2	7 (11.9%)	10 (17.5%)
n=3	1 (1.7%)	0 (0.0%)
Origin of blood and transfused components	**Dogs**(n=59)	**Cats**(n=57)
Blood bank	29 (49.2%)	39 (68.4%)
RBCs concentrates	19 (32.2%)	34 (59.6%)
Whole blood	10 (17.0%)	5 (8.8%)
VMCC donors	30 (50.8%)	18 (31.6%)
RBCs concentrates	0 (0.0%)	0 (0.0%)
Whole blood	30 (50.8%)	18 (31.6%)
Blood types	**Dogs**(n=59)	**Cats**(n=57)
Undetermined	**43 (72.9%)**	**31 (54.4%)**
Determined	**16 (27.1%)**	**26 (45.6%)**
A	n.a.	25 (96.2%)
B	n.a.	0 (0.0%)
AB	n.a.	1 (3.8%)
DEA 1 positive	12 (75.0%)	n.a.
DEA 1 negative	4 (25.0%)	n.a.
Classification of anemia	**Dogs**(n=59)	**Cats**(n=57)
Mild	4 (6.8%)	3 (5.3%)
Moderate	9 (15.3%)	16 (28.1%)
Severe	30 (50.8%)	15 (26.3%)
Very severe	16 (27.1%)	23 (40.3%)

(VMCC, Veterinary Medical Care Center; n.a., not applicable)

The main reason for transfusion in cats was non-regenerative anemia, while in dogs was hemorrhagic anemia (Graph 1). However, it was not possible to determine the reason for transfusion (anemia of undetermined etiology) in 19 (16.4%) of the cases (dogs and cats) analyzed. The two most frequent causes for non-regenerative anemia in cats were infection by feline leukemia virus (n=15; 12.9%) and chronic kidney disease (n=5; 4.3%). Hemorrhagic anemia in dogs was mainly associated with coagulopathies (n=11; 9.5%) and surgery (n=10; 8.6%).



Graph 1. Number (x axis) of animals distributed according to the reason of the transfusion (dogs and cats together depicted as light grey bars; dogs depicted as grey bars; cats depicted as dark grey bars).

The mean value of pre-transfusion hematocrit for cats was 11.8 ± 5.4% (SD) and, for dogs, 16.7 ± 6.7% (SD). Regarding post-transfusion hematocrit, the mean was 18.3 ± 5.6% (SD) in cats, and in dogs 25.7 ± 8.1% (SD). Among the pre- and post-transfusion hematocrits, there was a mean variation of 9.0% in dogs and 6.5% in cats. Cats that survived after blood transfusion increased the hematocrit by 6.6% in comparison with the pre-transfusion hematocrit, and those that died by 6.2%. In dogs, the increase in hematocrit among those who survived was 9.3% while in those who died it was 8.5%.

DISCUSSION

In dogs, of the 16 animals that were blood typed prior to transfusion, 12 (75.0%) were DEA 1 positive and 4 (25.0%) DEA 1 negative type. These values differ from those presented by Ferreira and col.(2011) and Valentin and col.(2017), reporting prevalence of 56.9% and 61.2% for DEA 1 positive. However, in these studies the number of animals typed was much higher (274 and 7414 respectively), when compared to our study. Most dogs (72.9%, n = 43) were not blood typed prior to transfusion. This high percentage is associated with the fact that only the first transfusions were considered; and in dogs, the first transfusion, even without prior typing, is considered a safe method given the lack of natural alloantibodies for DEA 1 (Kisielewicz and Self, 2014).

The frequency of distribution of different blood types in cats is dependent on the geographical area and the animal´s breed. Even so, blood type A is described as the most prevalent globally, with percentages ranging from 73.3 to 100% (Kisielewicz and Self, 2014). The frequencies of the blood types of cats included in this study were similar to the few previous studies carried out in Portugal. In the North of the country, Silvestre-Ferreira and col. (2004) reported a blood type distribution as follows: 90.3% for type A cats, 3.8% for type B and 5.9% for type AB. In the Lisbon area, Marques and col. (2011) registered a distribution of 97.5% for type A, 2.1% for type B and 0.4% for type AB.

In the present study, cats belonging to type B were not identified. Type B is generally prevalent in cats of the Angora Turco, Devon rex, Cornish rex, British shorthair and Scottish fold breeds (Davidow, 2013). No cats of these breeds were included in this study, and the number of animals tested was low. Blood typing in cats is essential before any transfusion, as the risk of developing an acute hemolytic reaction is inevitable if a type B cat receives type A blood (Castellanos *et al*., 2004). In addition, in type A cats receiving type B blood, a late hemolytic reaction will occur, and the life span of the transfused erythrocytes will be shorter, which also compromises the success of the transfusion (Zaremba *et al*., 2019).

In 54.4% of cats undergoing transfusion, compatibility tests, whether blood typing or crossmatch, were not performed prior to transfusion. However, none of these animals had a post-transfusion hemolytic reaction, probably because type A blood was the most prevalent (96.2%), and therefore the risk of a post-transfusion reaction was low. Due to the retrospective nature of this study, we should consider the possibility that, in some cases, the blood typing was performed, however it was not recorded in the animal's clinical record.

The mean pre-transfusion hematocrit presented by cats was significantly lower (11.8%) than dogs (16.7%). Many cats with chronic anemia have a compensatory mechanism that shifts the oxyhemoglobin dissociation curve to the right, which favors the release of oxygen to the tissues, and consequently allows greater tolerance to lower percentages of hematocrit, so in several cases manifestation of clinical signs only occur with very low hematocrit (hematocrit<10%) (Spada *et al*., 2017; Barfield andAdamantos, 2011).

In this study, the mean post-transfusion hematocrit in cats was 18.3%, and in dogs was 25.7%. Among pre- and post-transfusion hematocrits, there was a variation of 9.0% in dogs and 6.5% in cats. Within

these, the cats that survived showed an increase in hematocrit of 6.6% and those that died of 6.2%. In dogs, the increase in those who survived was 9.3%, while in those who died was 8.5%. The desired final hematocrit value, according to Godinho-Cunha and col. (2011), is 10% higher than the initial hematocrit value. By the above, the goals were not achieved globally in cats, but were close in dogs. Such percentages in cats may be related with the reduced volume of RBC transfused in a unit of whole blood or RBC concentrates, due to the small size of donors in this species. In this study, it was not known whether the minimum transfusion volumes required were achieved.

CONCLUSIONS

According to the results obtained in this study, most dogs are DEA 1 positive, although the number of blood typed animals was small, and the vast majority of cats have blood type A. In a high percentage of animals, no compatibility tests prior to transfusion were performed. The mean of pre-transfusion hematocrit in cats was lower than in dogs, due to the greater adaptability of the former to anemia. In dogs, the increase in post-transfusion hematocrit was close to 10%, as recommended by the literature consulted, in contrast to cats, due to the reduced weight of donor cats in our country. No transfusion reactions were recorded.

It is essential to raise awareness among the Portuguese veterinary practitioners on the importance of performing blood compatibility testingprior to the blood transfusions, to prevent transfusion reactions and increase the success of the transfusion.

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CONFLICTS OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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