Evaluation of the dog population in two Italian shelters in Central Italy (Marche region) as potential blood donors

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ABSTRACT

Background: In recent years, the field of transfusion medicine for dogs has advanced significantly, becoming a crucial aspect of veterinary clinical practice. Nowadays, blood still remains a fundamental biological source and the welfare and health status of eligible species-specific blood donors are essential for veterinary transfusion medicine.

Aim: This study focused on evaluating two shelters in Central Italy (Marche region), located in Tolentino (TS) and in Camerino (CS), in order to assess the potential of the shelter dogs as canine blood donors.

Methods: We evaluated a total of 45 dogs from these shelters based on physical (age and size), clinical, behavioural, and blood analysis criteria described in the Italian Ministerial Guideline for Veterinary Transfusion Medicine (2016).

Results: At the TS shelter, out of 206 resident dogs, 125 met the donation criteria (60.68%), with 28 (13.59%) selected for the study due to the impossibility to collect the samplings or other exclusion causes. In the CS shelter, of the 149 dogs, 17 (11.41%) were identified as potential blood donors and included in the study. Among these, seven dogs (25%) from TS and five dogs (29.41%) from CS were found to have DEA1 negative blood group. High percentages (TS = 25.24%, CS = 40.27%) of dogs were excluded for seniority and 29.53% in CS for behavioural issues. Notable findings included reduced erythrocyte mean corpuscular volume [mean TS = 63.93 fl; CS = 64.00 fl] and Reticulocyte Hemoglobin [mean TS = 22.39 pg; CS = 21.38 pg]. Additionally, in both shelters' dogs showed a modest increase in eosinophils levels [mean TS = 1.59 K/μl; CS = 1.02 K/μl].

Conclusion: Shelter dogs can fulfill the blood donation requirements set by the Italian Ministerial Guideline on Veterinary Transfusion Medicine. They are generally in good health and present a low risk of transmitting parasitic diseases; however, many are ineligible for donation due to behavioural pathologies or temperament issues and seniority.

Keywords: Dog, Transfusion medicine, Guideline, Blood donor, Shelter.

Introduction

Transfusion medicine in veterinary medicine is an expanding field, offering new opportunities for development, research, and applications. Its global adoption in recent years has led various countries to establish diverse regulatory frameworks. For instance, the American College of Veterinary Internal Medicine (ACVIM) in the United States issued the consensus statement in 2005 (Wardrop *et al*., 2005), then updated in 2016 (Wardrop *et al*., 2016), addressing the testing for infectious diseases in canine and feline blood donors. These laboratory tests, including blood type identification, crossmatching tests, and pathogen screening are essential to increase the safety of blood donation. They help monitor the donor's health, reduce post-transfusion adverse reactions in recipients, and minimize the risk of vector-borne diseases (Reine, 2004; Wardrop *et al*, 2023).

In Italy, the 2016 Ministerial Guideline on Veterinary Transfusion Medicine sets out detailed criteria for selecting donor animals (dogs, cats, and horses). It covers eligibility, exclusion standards, required blood tests, and procedures for collecting and storing blood. For example, donor dogs should weigh over 25 kg, be aged between 2 and 8 years, be docile, clinically healthy, regularly vaccinated, and undergo regular prophylaxis against heartworm, ectoparasite, and endoparasite.

Blood donor animals must be healthy and constantly monitored. This approach, not only supports preventive medicine, but also allows for ongoing health monitoring of the animals and tracking of epidemiological infectious disease trends (Morganti *et al*., 2022). This is particularly pertinent in shelter environments, where managing individual health and controlling infectious diseases can be more challenging, from the perspective of preventive medicine (Mutinelli *et al*., 2013).

Given this context, our study aimed to explore the feasibility of using shelter dogs in central Italy as blood donors. Historically, this has never been pursued due to unknown clinical and transfusion histories of shelter

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dogs and potential ethical concerns (Yagi and Bean, 2016). Currently, specific blood donation programs for dog shelters are absent, yet they could be viable candidates for transfusion medicine practices, provided their physical well-being is maintained and ethical considerations are respected (DeLuca *et al*, 2006; Mutinelli *et al*., 2013). Additionally, canine blood donation could foster awareness of transfusion practices and encourage blood donation among dog owners and, by extension, in humans (Ashall *et al*, 2017; Wilder and Humm, 2019).

In 2012, the Marche region implemented a surveillance plan for Leishmaniasis control, as it is an endemic area for this protozoan infection (Surveillance Plan, 2012). Blood samples for this study were collected as part of this regional plan ("Regional law"), with authorization from the respective shelter managers.

Marche Region is a territory of Central Italy fronting on the Adriatic Sea and comprises five provinces; in the eastern part is bathed by the Adriatic Sea, the Umbrian-Marchigian section of the Apennines serves as its mountain backbone and it is mainly characterized by hills [Website Britannica.com].

This preliminary study is the first of its kind in the region, aiming to assess the suitability of shelter dogs as blood donors, considering their health status and the potential to contribute to transfusion medicine.

Materials and Methods

Blood samples were collected during the 2020–2021 years from two shelters in Central Italy-Marche Region: "Monti Azzurri" in Tolentino and "Colle Altino" in Camerino, located in the mountain area of the region. A manager and an official veterinarian are present in both shelters.

In these shelters, dogs are divided by fences, single or with more individuals, with indoor kennels and material for heating, bowls and a stool in front. At least twice a day food, water and cleaning by the operators are guaranteed.

As previously stated, this collection of blood samples was conducted as part of a regional initiative ("regional law"), aimed at evaluating the welfare status of dogs in shelters. The President of the Regional Council of Marche region has promulgated the Regional Law 20 April 2015, n. 18 concerning the amendments to the Regional Law 20 January 1997, n. 10 "norms in matter of pets and prevention of stray animals." The presence of a large fenced green area, and communicating with the boxes, for daily walks and socialization are institutionalized to guarantee animal welfare in the shelters; rules on health checks and proper management of the animals are also present.

Dog populations of shelters and recruitment
For the study, we used detailed shelter registers from both the Tolentino Shelter (TS) and the camerino shelter (CS). The TS register, provided by the shelter staff, included records from December 2020 and an update from December 2021. Meanwhile, the CS register, maintained by the animal manager, was complete and up to date as of October 2021. These registers contained extensive information about each dog, such as the animal's name, microchip number, estimated birth year, size (small, medium, and large), breed, sex, coat colour, date of entry into the shelter, and other relevant notes (e.g., if the dog was seized, previously owned, aggressive, and so on).

Dogs that did not meet the criteria set in the Ministerial Guideline on Veterinary Transfusion Medicine were excluded from both shelters as potential blood donors evaluated in this study (Table 1). Selected dogs were between 2 and 8 years old, weighed more than 25 kg, displayed docile behaviour and were regularly vaccinated against distemper, leptospirosis, hepatitis, parvovirosis, and rabies. Each dog was also registered with a microchip in the species registry, linked to a database and identified at the species registry. Furthermore, all dogs were regularly treated both for endoparasites and ectoparasites. Every year, all dogs are treated with pesticide repellents spot on (Frontline Tri-Act®-Boehringer Ingelheim) or collar (Seresto®-Bayer) to avoid punctures of phlebotomies and mosquitoes and the transmission of ectoparasites. Prophylaxis with ivermectin tablets (Cardotek 30®-Boehringer Ingelheim) against heartworm disease is carried out on all dogs in both shelters. Also, dogs are treated twice a year for gastrointestinal parasites with febantel, pyrantel and praziquantel (Drontal®-Vetoquinol).

During the shelters' dog evaluation, whenever a dog was chosen for examination after it was selected for the study, the facility's operators and the veterinary doctor provided the animal's health record, which included clinical and medical history. This was followed by a comprehensive physical evaluation, assessing various health indicators such as body condition score, skeletal and muscular development, sensory state, behavioural signs, weight, mucous membranes condition, and lifting of the skin to assess the state of hydration, and capillary refill time.

Each dog in both shelters was carefully evaluated through a behavioral visit by specialist veterinarians and experienced dog educators; a report on the behavioural evaluation is present for each dog in a personal data sheet.

Blood sampling and laboratory tests
For this study, in certain instances, dogs that met the criteria outlined in the Ministerial guideline were tested multiple times over the course of the study. Whole blood samples, each 5 ml, were drawn from the cephalic vein of dogs and immediately stored in two types of tubes: one containing K3-EDTA and one with granules and a clot activator. These samples were then processed at the Veterinary Teaching Hospital's Laboratory at the University of Camerino. The analysis included complete blood count (CBC), blood group typing, a biochemical profile and tests for hemoparasitosis. Electrophoresis

Table 1. Criteria and laboratory tests required by the Italian Ministerial Guideline on Veterinary Transfusion Medicine (2016) to evaluate a potential dog blood donor.

Weight	>25 kg
Age	2–8 years
Regularly vaccinated for:	Distemper, leptospirosis, hepatitis, parvovirosis and rabies
Behaviour	Docile
Regular prophylaxis	Heartworm disease
Identification	Species registry with a microchip registered linked to a database
Blood group	DEA 1.1[a] (only at the first blood sample)
Hematological parameters (at every blood donation)	n° RBC, Hgb, MCV, MCH, MCHC, RDW (RBC morphology) n° WBC, neutrophils, eosinophils, basophils, lymphocytes, monocytes; WBC morphology n° PLT (PLT morphology) macroscopic research of *Babesia* spp. in the buffy coat*
Serological (at every blood donation)	*Leishmania infantum*[a], *Ehrlichia canis*[a], *Babesia canis*[b*], *Anaplasma phagocytophilum*[a], *Dirofilaria immitis*[a**].

*In alternative or with IFAT-PCR for Babesia canis; **If the treatment is certified, it can be omitted; a: It's possible to use rapid tests instead of IFAT or PCR; b: IFAT or PCR. RBC: red blood cell; Hgb: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration; RDW: red distribution width; WBC: white blood cells; PLT: platelets.

was employed in ambiguous cases of Leishmaniasis diagnosis. Occasionally, challenging field conditions or the dogs' restlessness post-venipuncture hindered the collection of adequate blood quantities for all tests stipulated in the Ministerial Guideline on Veterinary Transfusion Medicine. In such instances, only a subset of the panels could be conducted. For each blood sample, an aliquot of serum was frozen for further study.

Blood group typing was carried out on blood with EDTA by rapid immunochromatographic test (Lab Test BT DEA1®-Alvedia®). CBC evaluation were performed on samples with K3 EDTA anticoagulant with the Procyte® IDEXX analyzer and a blood smear test was carried out. Evaluated parameters included: red blood cell (RBC); hematocrit (HCT); hemoglobin (Hb); mean corpuscular volume (MCV); mean corpuscular Hb (MCH); MCH concentration (MCHC); red distribution width (RDW); % reticulocytes; reticulocyte Hb (Retic-Hb); Leukocytes; % segmented neutrophils; % lymphocytes; % monocytes; % eosinophils; % basophils; absolute value of segmented neutrophils, lymphocytes, monocytes, eosinophils, and basophils; platelets (PLT); PLT distribution width (PDW); mean PLT volume (MPV); Plateletcrit. Serum was obtained using the Rotina 46R of Hettich Zentrifugen® centrifuge and then analyzed using the machine BT3500 Vet of Biotecnica Instruments®. The biochemical profile took into account the following parameters: Calcium (Ca; Arsenazio III); Blood urea nitrogen (ultra violet-U.V. method); Gamma-glutamide transferase (GGT; IFCC-International Federation of Clinical Chemistry method); Alkaline phosphatase (ALP; DEA-diethanolamine method); Glucose (Glu; Trinder method); Creatinine (Jaffè method); glutamic-oxalacetic transaminase (GOT; IFCC method); Alanine amino-transferase (GPT; IFCC method); cholesterol (Trinder method); Triglycerides (Trinder method); albumin (Alb; VBC-Bromocresol Green method); Phosphorus (U.V.-Ultra Violet method); Direct bilirubin (Sulfanilic method); Total Bilirubin (sulfanilic method); indirect bilirubin; total proteins (TP; biuret method); Albumin/globulin ratio; corrected calcium; Calcium/phosphorus ratio; globulins.

The separated serum samples were employed for hemoparasitoses testing, as required by the Ministerial Guideline on Veterinary Transfusion Medicine. The SNAP® Test 4Dx® Plus (IDEXX) and the SNAP® Leishmania Tests were used for the control of Ehrlichiosis, Anaplasmosis, Borreliosis and Heartworm Disease, and Leishmaniasis. For the latter, dogs had already been tested with indirect immunofluorence according to the Marche region surveillance plan for the control of Leishmaniasis. In the case of Babesiosis and Brucellosis, testing was sporadically carried out on a random selection of the specimens. The smear test was used to exclude the presence of *Babesia canis*, while Canine Brucellosis Antibody Test Kit D-TEC® CB (Zoetis) for *Brucella canis*.

Stool examination by flotation test was performed twice a year on random samples.

Data processing

Microsoft® Excel® program for Microsoft 365 MSO (Version 2201 Build 16.0.14827.20180) was used to make the statistical part. The functions used were: sum, mean, median, standard deviation, and percentage.

Ethical approval
Not needed for this study.

Results

Dogs' populations
In 2021, the TS accommodated 206 dogs and CS hosted 149 dogs. Table 2 illustrates the demographic composition of these populations, focusing on the distribution and percentage of sex, age, and size across both shelters.

Notably, both shelters exhibited a higher percentage of male dogs [TS = 64.08%; CS = 61.07%] compared to females [TS = 35.92%; CS = 38.93%]. A significant majority (approximately 80%) of the dogs were of medium-large size (weighing over 25 kg). The sum of the percentages of medium-large breed dogs in TS was 83.49% while in CS 77.18%.

While specific breeds were not identifiable, there was a notable prevalence of mixed breeds. These mixed breeds predominantly displayed characteristics akin to Maremmano shepherds and hound breeds.

Figure 1 presents two flow diagrams that describe the criteria for the progressive exclusion of dogs, as outlined in the Ministerial Guideline on Veterinary Transfusion Medicine.

Each diagram begins with the total population of the respective shelter, starting with the total consistency of the populations, categorized by sex. On the left side of the diagram, selection criteria (age, size, and pathologies) are delineated. Some dogs considered suitable for collection in the preliminary phase, once physically contained outside or inside the box, proved not to be able to sustain too close contact with the operators, not maintaining their well-being and tranquillity, which is why they did not return to the chosen population. Concerning CS, most of the dogs with behavioural pathologies resulted to be phobic, unpredictable or unruly. The pathologies found in the preliminary phase of the dogs' clinical history were leishmaniasis, heart disease, paraplegia, and hypothyroidism.

The selection criteria are used to identify and exclude unsuitable candidates, which are shown on the right side of the diagram. The process concludes with the final counts: the total number of dogs included in and excluded in the study, along with the count of specimens that were effectively tested.

Out of the TS population, one hundred and twenty-five dogs (60.68%) were potential candidates for this study based on the Ministerial guideline on Veterinary Transfusion Medicine criteria, yet only twenty-eight underwent blood testing due to the COVID-19 pandemic situation and, later, the continuous changing of the population.

In the case of CS, all one hundred and forty-nine dogs in the population were evaluated based on the anamnestic and/or clinical records, with only seventeen selected for the study because they met the criteria to become possible blood donors. Most of the excluded dogs exhibited behavioural pathologies or temperament issues, making them ineligible for donation, as opposed to clinical and organic diseases.

Table 3 provides a comprehensive overview of the selected populations and their respective characteristics, encompassing both TS and CS.
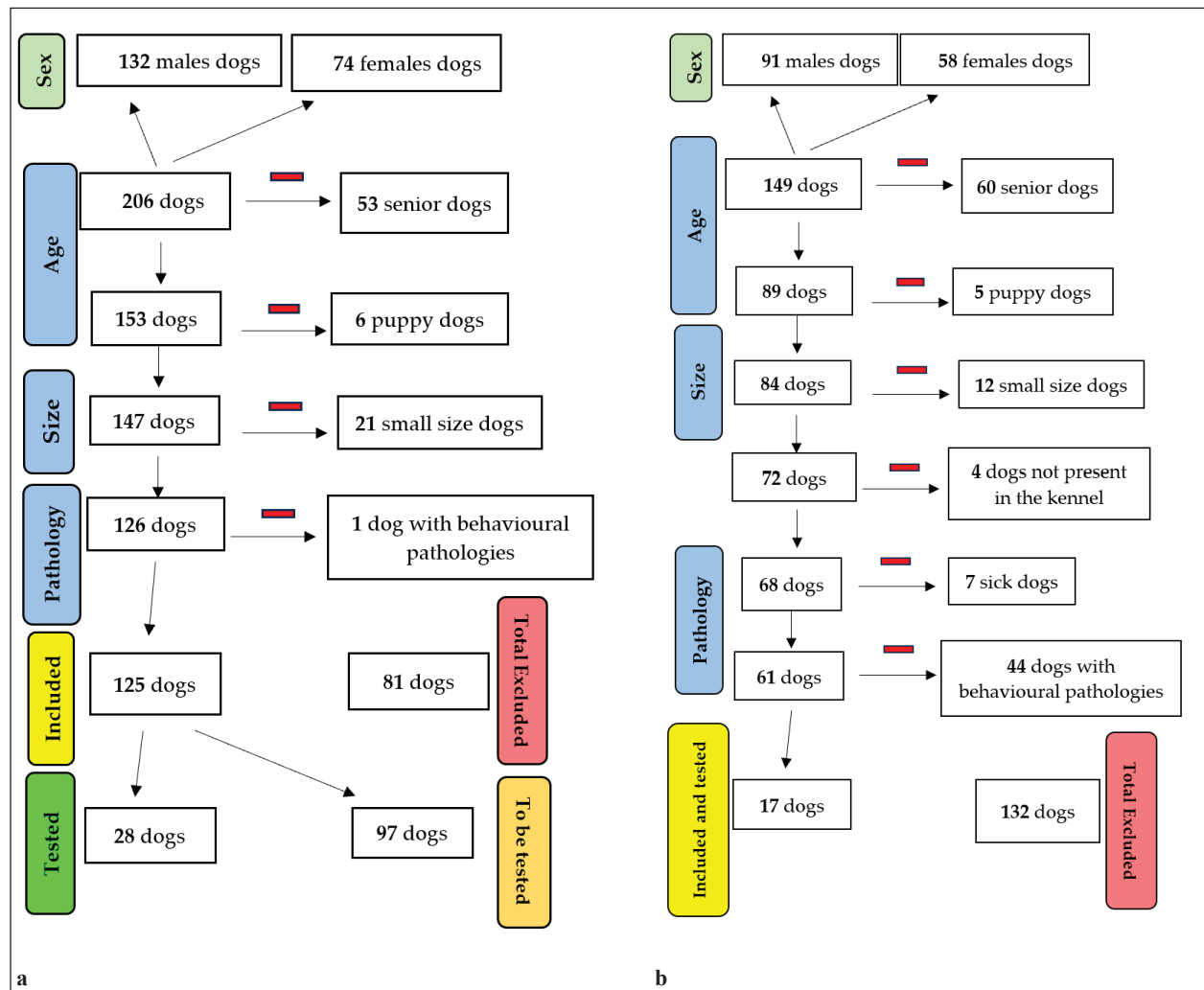
Blood type typing and laboratory tests
The percentage of the dogs that resulted to be DEA1 positive was higher [TS = 75%; CS = 70.59%] than DEA1 negative [TS = 25%; CS = 29.41%].

Table 4 reports the CBC parameters evaluated in TS and CS and only the biochemical values whose mean did not fall within the reference intervals for the parameter. A urine test was not considered because, in a situation like the shelter, it is quite impossible to collect the urine individually with a non-invasive method.

The SNAP tests conducted in both the TS and CS groups yielded negative results for *Ehrlichia canis*, *Borrelia burgordferi*, *Dirofilaria immitis*, *Anaplasma phagocytophilum,* and *Leishmania infantum*. A singular dog, exhibiting clinical conditions of Leishmaniasis, underwent serum electrophoresis, but the results were normal and did not show hypergammaglobulinemia. All dogs subjected to indirect immunofluorescence serological tests (IFATs) as part of Leishmania infantum (Surveillance plan) resulted in negative. Simultaneously, random tests for Babesiosis and Brucellosis resulted in a negative for this parasitosis. All the fecal flotation tests showed negative results.

Discussion

This study aims to delineate the health status and characteristics of shelter dogs as eligible blood donors. Upon evaluation, the majority of excluded dogs had behavioural pathologies, rending them not eligible for

Table 2. Description of total shelter populations and their main characteristics.

		TS	CS
Sex	Females	74 (35.92%)	58 (38.93%)
	Males	132 (64.08%)	91 (61.07%)
Age	>8 years	52 (25.24%)	60 (40.27%)
	6–8 years	81 (39.32%)	33 (22.15%)
	2–5 years	67 (32.52%)	51 (34.23%)
	<2 years	6 (2.92%)	5 (3.35%)
Size	Small (< 20 kg)	25 (12.14%)	34 (22.82%)
	Medium (20–35 kg)	131 (63.59%)	70 (46.98%)
	Large (>35 kg)	41 (19.90%)	45 (30.20%)
	Unknown	9 (4.37%)	-
Total		**206**	**149**

TS: Tolentino shelter; CS: Camerino shelter.

Fig. 1. Flow diagrams of the dogs populations and the progressive exclusion of dogs, as outlined in the Italian Ministerial Guideline on Veterinary Transfusion Medicine. At the beginning the total population of the respective shelter, and the total consistency of the populations categorized by sex. On the left side selection criteria (age, size, pathologies) are delineated. At the end the dogs included in the study and the blood tested ones. (**a**) Flow chart of the exclusion criteria for Tolentino shelter. (b) Flow chart of the exclusion criteria for CS.

donation, as opposed to presenting clinical and organic diseases (Dufour *et al*., 2005; Descamps *et al*, 2023). In the selected populations, approximately 70% of dogs tested positive for DEA1, indicating they could therefore donate blood exclusively to DEA1+ animals. This result aligns with previous studies on the prevalence of blood groups in both mongrels and purebreds' dogs in Italy (Carli *et al*., 2017; Medina Valentin *et al*., 2017), Europe (Paleckaitis *et al*., 2018), and worldwide (Mangiaterra *et al*., 2021), where the percentage of dogs DEA1+ where higher than DEA1-. On the contrary, studies on greyhounds, frequently employed as blood donors in clinical practice due to their favourable physical and behavioural characteristics, showed a significantly lower percentage of DEA1-positive dogs (Iazbik *et al*., 2010; Mesa-Sanchez *et al*., 2014).

Table 3. Description of the 45 selected dogs for the study from the shelter populations and their main characteristics.

		TS	CS
Sex	Females	10 (35.71%)	8 (47.06%)
	Males	18 (64.29%)	9 (52.94%)
Age	6–8 years	9 (32.14%)	9 (52.94%)
	2–5 years	19 (67.86%)	8 (47.06%)
Size	Medium	22 (78.57%)	12 (70.60%)
	Large	6 (21.43%)	5 (29.40%)
Total		**28**	**17**

TS: Tolentino shelter; CS: Camerino shelter.

Table 4. Mean and median of the hematological and biochemical parameters evaluated of the selected dogs for the study from the shelter populations.

	Shelters	RBC [M/µL]	HCT [%]	Hb [g/dL]	MCV [fL]	MCH [pg]	MCHC [g/dL]	RDW [%]	Retic [K/µL]
Mean ± s.d.	TS	7.52 ± 0.84	48.04 ± 5.57	16.97 ± 1.93	63.93 ± 2.66	22.58 ± 0.82	35.35 ± 0.93	18.04 ± 1.70	57.91 ± 30.67
	CS	7.26 ± 0.92	46.75 ± 6.29	16.42 ± 2.23	64.00 ± 2.40	22.48 ± 0.87	35.13 ± 0.89	17.68 ± 1.85	60.35 ± 33.45
Median	TS	7.57	48.70	17.20	64.20	22.60	35.30	18.40	52.30
	CS	7.26	47.40	16.90	63.50	22.20	34.80	18.40	60.0
RI		5.65—8.87	37.30—61.70	13.10—20.50	61.60—73.50	21.2—25.9	32.0—37.9	13.6—21.7	10—110
% <RI	TS	—	2.70	5.41	18.92	5.41	—	–	–
	CS	5.88	11.76	11.76	—	—	—	–	–
% >RI	TS	8.11	—	2.70	—	—	2.70	–	2.70
	CS	5.88	—	—	—	—	—	–	5.88

	Shelters	Retic—Hb [pg]	Leukocyte [K/µL]	Segmented Neutrophil [K/µL]	Lymphocyte [K/µL]	Monocyte [K/µL]	Eosinophil [K/µL]	Basophil [K/µL]	PLT [K/µL]
Mean ± s.d.	TS	22.39 ± 1.18	12.79 ± 3.38	6.83 ± 2.02	3.42 ± 1.02	0.89 ± 0.27	**1.59 ± 1.39**	0.05±0.07	270.68±96.56
	CS	**21.38 ± 1.63**	12.83 ± 3.50	7.92 ± 2.12	16.42 ± 2.23	1.08 ± 0.41	1.02 ± 0.60	0.03±0.05	269.88±87.81
Median	TS	22.40	12.14	6.32	3.67	0.85	**1.25**	0.01	279.0
	CS	**21.60**	13.79	8.11	16.90	1.13	0.99	21.60	275.0
RI		22.30—29.60	5.05—16.76	2.95—11.64	1.05—5.10	0.16—1.12	0.06—1.23	0.00—0.10	148—484
% <RI	TS	47.95	—	—	—	—	—	—	16.22
	CS	76.47	—	—	—	—	—	—	5.88
% >RI	TS	—	13.51	5.41	—	21.62	51.35	21.62	—
	CS	—	11.76	5.88	5.41	52.94	41.18	—	—

	Shelters	ALP [IU/L]	GGT[UI/L]	GOT [UI/L]	TP [g/dL]	Alb [g/dL]	Creatinine [mg/dL]
Mean ± s.d.	TS	120.44 ± 48.35	4.68 ± 2.77	45.15 ± 31.68	6.47 ± 0.59	**3.33 ± 0.32**	1.12 ± 0.19
	CS	**169.01 ± 105.41**	**7.47 ± 2.61**	40.63 ± 10.74	6.95 ± 0.63	3.13 ± 0.36	1.14 ± 0.21
Median	TS	113.0	4.26	39.21	6.43	**3.37**	1.08
	CS	148.81	**7.00**	37.38	7.06	3.08	1.09
RI		20.0—156.0	1.20——6.40	23.0——66.0	5.40——7.10	2.60——3.30	0.50——1.50
% <RI	TS	3.45	3.45	3.45	——	——	——
	CS	——		——	——	6.25	——
% >RI	TS	24.14	24.14	10.34	17.24	55.17	3.45
	CS	43.75	68.75	——	43.75	25.0	6.25

RI: Reference Intervals; TS: Tolentino shelter; CS: Camerino shelter. RBC: Red Blood Cell; HCT: haematocrit; Hb: Hemoglobin; MCV: Mean Corpuscular Volume; MCH: Mean Corpuscular Hemoglobin; MCHC: Mean Corpuscular Hemoglobin Concentration; RDW: red distribution width.
ALP: alkaline phosphatase; GGT: gamma–glutamide transferase; GOT: glutamic–oxalacetic transaminase; TP: total proteins; Alb: albumin. The parameters in bold are not within the reference intervals.

In terms of hematological findings, the majority of dogs had no significant alterations in RBC, Hct and Hb levels, which is a positive factor in relation to their potential suitability as donors. However, the average MCV values observed in both TS and CS, were approximately 64 fL, just above the minimum limit of the reference interval. Notably, MCV indicates the size of RBCs which is already determined at the medullary level. Consequently, any condition that slows erythrocyte maturation or Hb formation can generate smaller cells, called microcytes. Microcytosis is often associated with iron deficiency and can have different etiologies; when iron levels decrease, the body tends to produce smaller erythrocytes, ensuring that the limited Hb is concentrated within them, maintaining a concentration of Hb at saturation.

This trend may serve as an indicator of subclinical pathologies not yet manifested or non-optimal nutritional status (Harvey, 2012). Despite this, the parameters found closely align to the reference intervals. Considering the challenging management conditions in shelters, characterized by a high number of animals and consistent environmental factors, these results are commendable.

Indeed, iron deficiency anaemia is prevalent in dogs and cats, often stemming from factors such as malabsorption with impaired gastroenteric pH, chronic bleeding (bleeding ulcers, parasites especially in puppies) and gastroenteric tract neoplasms leading to continuous dripping. This deficiency cannot be compensated by the introduction of iron through the digestion and intestinal absorption processes. Consequently, these anaemias are chronic haemorrhagic conditions, and over time can become iron deficiency (Naigamwalla *et al*., 2012).

Retic-Hb coupled with reduced MCV emerges as a good indicator for detecting subclinical states of iron deficiency, allowing for early diagnosis, potentially as soon as 3–4 days into the pathological process (Steinberg *et al.*, 2005). The association between clinical syndromes and Retic-Hb lacks specificity, focusing solely on the insufficient Hb production level rather than the underlying mechanism (Fuchs *et al*., 2017). In both shelters, the mean of Retic-Hb is almost overlaps with the minimum value of the reference range; moreover, in CS, the average of Retic-Hb dogs is 21.38 pg, slightly below the lower limit of the RI set at 22.30 pg. Unfortunately, both MCV and Retic-Hb have limitations as relatively late indicators of iron deficiency, incapable of distinguishing an iron deficiency anaemia, stemming from continuous blood loss, from an inflammatory anaemia due to iron sequestration. It is worth acknowledging that reticulocyte haemoglobin concentration can be influenced by inflammatory conditions, impacting both anaemic and non-anaemic dogs (Meléndez-Lazo *et al*., 2015; Schaefer *et al*., 2015). In this study, the dogs considered did not present an anaemic condition.

Even if the faecal flotation tests resulted negative and the dogs were treated for gastrointestinal parasites, the high animal density in shelters, leading to increased environmental faecalization a potential stress-induced immunosuppression, often resulted in a higher prevalence of direct-life-cycle parasites in shelter dogs compared to family dogs, even when properly treated with endo and ectoparasite medications (Scaramozzino *et al*., 2018). Eosinophils were identified as the only leukocyte parameter that was significantly higher (Scaramozzino *et al*., 2018). Specifically, in CS, the average eosinophil count fell within the RI, while in TS it exceeded the upper limit. Elevated eosinophilic granulocytes may be indicative of allergic diseases and parasitosis, with recorded eosinophilia suggesting ongoing gastro-intestinal subclinical parasitosis (Raza *et al*., 2018).

Regarding the biochemical profile, nearly all parameters, with rare exceptions, fell within the RIs. Notably, attention was drawn to the trend of GGT, an enzyme associated with different tissues' membranes (bile ducts, acini and pancreatic ducts, renal tubular cells and those of the breast epithelium). GGT serves as a marker for liver cholestasis, demonstrating lower sensitivity but higher specificity than ALP, whose mean in CS also exceeded the RI. GGT plasma concentration typically increases in conditions such as hepatobiliary diseases, hyperadrenocorticism, glucocorticoid, or anticonvulsant therapies (although the ALP increase is much more significant), cholangiohepatitis, diabetes mellitus, pancreatitis, liver neoplasms. While an increase in GGT plasma concentration is less sensitive than ALP in signalling hepatobiliary diseases, it is more specific, although cases of possible enzymatic induction by endogenous or exogenous and barbiturate corticosteroids should be excluded. It is mainly associated with increased bile production resulting from bile stasis and/or bile cell hypertrophy (Paltrinieri *et al*., 2017).

The average GGT in CS was above the RI, whereas in TS it remained within the RI. The causes of this increase in GGT may be attributed to nutritional imbalances and inflammatory processes of the gastroenteric apparatus, with consequent cholestasis phenomena.

Finally, all SNAP tests performed for hemoparasitosis and protozoan infection yielded negative results. This is highly encouraging as the Marche Region is an endemic territory for Leishmaniasis (Mendoza-Roldan *et al*., 2020). The prophylaxis and controls carried out by the shelters, such as IFAT for Leishmania, have proven effective in maintaining the health of the host dogs and limiting this infectious disease. In fact, in 2021, within the dog shelters in the province of Macerata, where TS and CS are located, the IFAT test identified four positive dogs (3.2%) with antibody titres equal to or greater than 1:160. The test also detected 12 dogs (9.7%) with a "dubious" infection, featuring antibody titres between 1:40 and 1:80. According to regulations,

these dogs are monitored over time with clinical and serological control every 8-16 weeks (Website VeSa Marche).

However, selecting healthy blood donor canines in a region where vector-borne illnesses are prevalent poses significant challenges. It is crucial to opt for a serological and biomolecular inquiry panel that best fits the donor's surroundings (Antognoni *et al*., 2022). The prevalence and distribution of many canine vector-borne disease are present and evolving all over Europe and are constantly monitored (Miró *et al*., 2012, 2022). The SNAP tests, accepted by the Ministerial Guideline on Veterinary Transfusion Medicine, resulted in negative for the hemoparasitoses. Despite being less sensitive than molecular and immunofluorescence tests for non-diseased animals, previous studies on rapid tests such as the SNAP® 4Dx® Plus Test Kit have demonstrated very high sensitivity and specificity (Chandrashekar *et al*., 2010; Stillman *et al.,* 2014; Liu *et al*., 2018). This suggests that in daily clinical practice, rapid tests for hemoparasitoses can be used due to their ease of use and the provision of reliable quick results (Proverbio *et al*., 2016). Moreover, the Surveillance Plan of Marche region ensures the confirmation of dogs' negativity for Leishmaniasis through the IFAT technique.

A limitation of the study was the inability to perform the Babesiosis test (examined only on the blood smear) and urine analyses for all dogs. In addition, in a One Health approach, in future, it will be necessary to use more specific tests for hemoparasitosis, such as PCR in favour of the epidemiological supervision of the shelter dogs and reducing the transmission risk of zoonosis.

Moreover, utilizing shelter dogs for blood donation presents various challenges due to the rapid and heterogeneous changes in their populations, coupled with the difficulty of obtaining a comprehensive clinical history.

Conclusion

This study demonstrates that both the chosen populations of TS and CS could be suitable candidates for blood donations. Nevertheless, a significant limitation was found to be the large percentage of behavioral pathologies among the dogs, hindering the execution of clinical checks and blood sampling. Additionally, the dogs' advanced age and the greater number of DEA1-positive dogs than DEA1-negative ones create obstacles. This could potentially enhance the adoption rate for older and larger dogs, often overlooked in adoption processes.

In conclusion, the feasibility of using shelters for transfusion medicine in relation to the progress of research in this field, especially in the monitoring of vector-borne diseases, remains to be assessed. At the same time, the shelters, already integral to the health landscape of the territory, can play a crucial role in advancing veterinary practices and contributing to the overall well-being of both animals and the community.

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