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Serum total antioxidant status in dogs: Reference intervals and influence of multiple biological and analytical factors

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Abstract

Background: Total antioxidant status (TAS) is one of the most widely used oxidative stress biomarkers, but the lack of canine RI and the influence of analytical factors hinder its application in clinical practice.

Objectives: The aims of this study were to establish canine assay-specific RI for TAS and evaluate the sources of biological variation and the association between TAS and multiple hematologic and biochemical variables.

Methods: Blood samples from 190 clinically healthy dogs were collected, encompassing pet dogs (82), police dogs (56), and shelter dogs (52). After hematologic and biochemical analysis, serum TAS was determined by means of a commercial 2,2′-azinobis (3-ethylbenzthiazolin-6-sulfonic acid) (ABTS) test. The American Society for Veterinary Clinical Pathology guidelines were followed to establish the RI, employing nonparametric methods. Univariate analysis and multivariate analysis were conducted to assess the influence of biological and analytical variables, yielding a final regression model.

Results: The final reference population comprised 143 dogs, for which the RI was established (1.41–2.27 mmol/L). Partitioning was applied to the three study groups. The regression model revealed that police dogs had significantly higher TAS values than pet dogs. Furthermore, significant associations between four biochemical variables (albumin, globulins, cholesterol, and aspartate aminotransferase) and serum TAS were found.

Conclusions: This is the first study to establish RI for serum TAS in a large and heterogeneous canine population and provide data on its relationship with analytical variables. These findings could potentially improve the interpretation of TAS in clinical environments.

KEYWORDS
ABTS, canine, normal, oxidative stress, quality control, range

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1 | BACKGROUND

The concept of "oxidative stress" (OS) has undergone redefinition over the years. Currently, it is described as an imbalance between oxidants, originating from internal biochemical reactions or external agents, and the organism's antioxidant defenses, with a bias toward the oxidants. This imbalance arises due to an excess of free radicals and reactive species, specifically reactive oxygen species (ROS) and reactive nitrogen species (RNS). While some levels of oxidants are necessary for physiologic redox signaling, OS can also lead to molecular damage, with extensive evidence supporting its involvement in the development and progression of numerous diseases.[1–3]

Considering the multitude of compounds and pathways involved, assessing OS can be a challenging task. It can be estimated by measuring products of oxidative damage to biomolecules, or the organism's antioxidant capacity.[3–5] Given that measuring different antioxidant molecules independently is neither practical nor representative, several indexes that reflect the overall antioxidant capacity of a given sample have been developed. One of the most commonly used methods is the 2,2′-azinobis (3-ethylbenzthiazolin-6-sulfonic acid) (ABTS) test, also known as the total antioxidant status (TAS) assay.[6–9] This assay is based on the reaction between ABTS, a peroxidase (metmyoglobin) and hydrogen peroxide, which results in a color change of the sample that is determined spectrophotometrically at 660 nm. The suppression of color change is proportional to the concentration of antioxidants in the sample.

TAS assay has demonstrated clinical utility in dogs. It has been established as clinically valuable for evaluating antioxidant impairment in canine leishmaniosis,[10,11] ehrlichiosis,[12] babesiosis,[13] heart failure,[14,15] inflammatory bowel disease,[16] chronic kidney disease,[17] cystic endometrial hyperplasia,[18] and after anesthesia and surgical procedures.[19–21] TAS in dogs has also been found to be influenced by other factors such as exercise, psychogenic stress, or being housed in shelters.[22–27]

Despite these findings, the application of TAS in veterinary clinical practice remains challenging, and it is currently being used mostly in research. This could be related to the difficulties in its interpretation that arise from the different versions of the TAS assay, the absence of well-established RI, and the variety of individual and environmental factors that influence its result.[5,7,9,28–30]

Two studies have proposed RI for TAS in dogs.[9,28] Nevertheless, these studies sampled relatively small populations, lacking representation of the heterogeneity within this species. Furthermore, discrepancies in their results, when compared with other publications, complicate their interpretation. To the best of our knowledge, no prior studies have established RI for canine serum TAS estimated by the ABTS assay that complies with the latest recommendations of the American Society for Veterinary Clinical Pathology (ASVCP),[31] and no prior studies have explored the influence of multiple sources of biological variation and analytical variables on its result.

Therefore, the aims of our study were to establish assay-specific RI for canine serum TAS, to assess the influence of individual and environmental factors on its result, as well as to evaluate its relationship with multiple hematological and biochemical variables. Given the growing body of evidence linking TAS with various pathologic conditions in dogs, providing data on these aspects is of considerable significance.

2 | MATERIALS AND METHODS

2.1 | Reference population, inclusion, and exclusion criteria

The study design aimed to follow the ASVCP's Quality Assurance and Laboratory Standards Committee (QALS) guidelines for the determination of de novo RI in veterinary species. Given that a minimum of 120 animals is necessary to enable the use of nonparametric statistical methods with 90% CI,[31] an initial population of 190 clinically healthy dogs was selected. The study involved animals situated in the Community of Madrid (Spain), categorized into three distinct groups: 82 privately owned pet dogs, 56 working police dogs, and 52 dogs residing in a rescue shelter. Samples from these dogs were submitted to the Clinical Pathology Service of the Veterinary Teaching Hospital of the Complutense University of Madrid as part of a routine health check, encompassing a hematologic and biochemical profile, along with the determination of antibodies against *Leishmania infantum* and *Ehrlichia canis*. The surplus serum volume was used to perform the TAS assay. The inclusion criteria for the participation in this study comprised clinically healthy dogs of any sex, age, breed, and size, without apparent physical or laboratory evidence of disease. Animals with a history of pre-existing diseases, recent medication use, or pathologic findings in laboratory analyses were excluded from the study.

2.2 | Blood sampling and pre-analytical factors

Venous blood samples were drawn during the routine health check and were transferred into 0.5 mL tubes containing K_3 EDTA for hematology analysis and into 5 mL plain tubes that were centrifuged after clotting (1200 g, 10 min) to obtain serum for biochemistry analysis and antibodies determination. Both the hematology and biochemistry analyses were carried out within 3 h of sample collection in all cases. The remaining serum samples were aliquoted and stored at −80°C for the determination of TAS, complying with previously reported stability data.[32] In accordance with a previous study revealing the interference due to hemolysis, icterus, and lipemia on the assay, specimens showing such alterations on visual examination were discarded.[33]

2.3 | Laboratory analyses

The hematology profile was performed using an automated hematology analyzer (URIT 2900Vet Plus TS, URIT Global Diagnostics

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Supplier, China) and included RBC, hemoglobin concentration (HB), HCT, MCV, MCH, MCHC, WBC, platelet count (PLT), and MPV. Blood smears were prepared and stained with May-Grünwald-Giemsa for evaluation and for manual differential leukocyte count of neutrophils (NEU), lymphocytes (LYM), monocytes (MONO), eosinophils (EOS), and basophils (BASO). The biochemistry profile was conducted on an automated biochemistry analyzer (TC220, Jiangxi Tecom Science Corporation, China) and included 17 variables: urea (UREA), creatinine (CRE), total protein (TP), albumin (ALB), globulin (GLOB), albumin/globulins ratio (A/G), ALT, AST, GGT, alkaline phosphatase (ALKP), LDH, calcium (Ca), inorganic phosphate (P), calcium/phosphate ratio (Ca/P), total cholesterol (CHOL), uric acid (UA), and CK. In addition, serum cortisol was measured as a potential indicator of psychogenic stress and was determined by a competitive ELISA, previously validated by the laboratory.[25,34,35] The determination of antibodies against *Leishmania infantum* and *Ehrlichia canis* was performed by immunofluorescence antibody test (IFAT). Serum TAS determination was conducted using the TAS-liquid stable colorimetric kit (Fortress Diagnostics Limited, UK), based on the ABTS assay. The method had been previously validated for canine serum samples, and results are expressed in mmol Trolox Equivalent/L.[8] The method is linear up to 2.7 mmol/L, and the imprecision is 4.04%.[33] Control samples were included in all batches, which yielded results within the control range (1.56–2.12 mmol/L).

2.4 | Statistical analysis

For the establishment of the RI, statistical analysis was conducted following the ASVCP and the Clinical and Laboratory Standards Institute (CLSI) guidelines, employing *Reference Value Advisor*, a set of macroinstructions for Microsoft Excel.[31,36,37] To assess the normality of data distribution, histograms were generated and the Anderson–Darling test was executed; *p*-value cut-off was corrected depending on the sample size ($p<.04$ for the whole population, $p<.067$ for pet dogs, $p<.076$ for police dogs, and $p<.075$ for shelter dogs).[38,39] Outliers were identified using Tukey's test and evaluated for potential removal, depending on pre-analytical, analytical possible sources of error, and biological criteria resulting in exclusion criteria. Reference limits with 90% CI were subsequently calculated through nonparametric methods, encompassing the central 95% of the reference values. Descriptive analysis was also provided. The partitioning into the three study groups (pet dogs, police dogs, and shelter dogs) was evaluated using the statistical recommendations of Lahti et al., as well as clinical considerations and data from the literature, as recommended.[31,40,41]

To evaluate the influence of the sources of biological variation, life conditions, and the analytical variables on TAS value, univariate and multivariate analyses were conducted. Firstly, univariate analysis was performed by nonparametric tests using SPSS Statistics (IBM, Spain). The Mann–Whitney *U* test was conducted to evaluate the differences between sexes while the Kruskal–Wallis test with Bonferroni's correction was performed to assess the differences between the three study groups and the differences between dog sizes (small, medium, and large). To examine the uniformity of groups in terms of dog sizes and sexes, the demographic data of the groups were subjected to a Chi-squared test. To evaluate the statistical correlation between age and TAS value, the Spearman's Rho test was used. This method was also employed to evaluate the correlation between hematological and biochemical variables, as well as the serum cortisol, with TAS value. In every case, a *p*-value <.05 was considered statistically significant.

Subsequently, a multivariate regression model was constructed using the statistical software STATA (StataCorp LLC, USA). TAS was defined as the dependent variable of the model, and the "reference dog" was defined as a male pet dog. The model evaluated the effect of all categorical variables (group, sex, age, size, and breed), along with the effect of those analytical (hematological and biochemical) variables that showed statistically significant association with TAS value in the univariate analysis.

3 | RESULTS

3.1 | Reference population

The study period comprised animal recruitment and sample collection between July 2021 and December 2022. From the initial set of samples of 190 dogs, 26 had to be excluded from the analysis. This exclusion was necessary either because of hematological or biochemical abnormalities detected, or due to clinical data not meeting the predefined exclusion criteria. In addition, 11 dogs were excluded due to positive results to *L. infantum* or *E. canis* serology, nine dogs were discarded due to hemolytic or lipemic serum specimens, and one dog had an insufficient serum sample to perform the TAS determination. Consequently, data from 143 dogs were subjected to statistical analysis.

The reference population ($n=143$) consisted of 57 pet dogs (40%), 43 police dogs (30%), and 44 shelter dogs (30%). Of these, 76 were males (53%) and 68 were females (47%), ranging in age from 6 months to 16 years [mean 3.93; SD 2.90]. Regarding size, 97 dogs were categorized as large (67%), 28 as medium-sized (20%), and 19 as small (13%). The population encompassed dogs of 31 breeds, including mixed-breed dogs (25% of the reference population), German Shepherd (14%), Belgian Malinois (14%), American Staffordshire Terrier (8%), Pitbull Terrier (7%), Labrador Retriever (5%), Spanish Greyhound (3%), Podenco (3%), and others (see supporting information).

The group of pet dogs ($n=56$) included 29 males (51%) and 27 females (49%), between 6 months and 10 years of age (mean 3.21; SD 2.81), were sized small (28% of the group), medium (37%), and large (35%). The group encompassed different breeds, mainly mixed-breed dogs (32%), Labrador Retriever (7%), Podenco (7%), Maltese (5%), and others (see supporting information). The group of police dogs ($n=43$) consisted of 18 males (42%) and 25 females (58%), ranging in age between 6 months and 9.5 years (mean 4.83;

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SD 2.37). All dogs in this group were large, including Belgian Malinois (47%), German Shepherd (44%), Labrador Retriever (5%), German Shorthaired Pointer (2%), and mixed-breed (2%). The group of shelter dogs ($n=44$) included 29 males (66%) and 15 females (34%), ranging from 6 months to 16 years of age (mean 3.98; SD 3.28). These dogs were mostly large (77%), followed by medium (16%) and small (7%) dogs. The group encompassed different breeds: mixed-breed dogs (39%), American Staffordshire Terrier (25%), Pitbull Terrier (21%), Spanish Greyhound (7%), and others (see supporting information).

The Chi-squared test was used to assess the uniformity of the three groups in terms of sex and size, which revealed that the groups were uniform regarding sexes ($p=.075$), but were not uniform in sizes ($p<.001$).

3.2 | Reference intervals

Descriptive statistics and the final RI for serum TAS obtained by using nonparametric methods in *Reference Value Advisor* are presented in Table 1. The RI of serum TAS determined in the reference population was 1.41 to 2.27 mmol/L (median 1.69; min 1.37; max 2.49). The frequency histogram of TAS results in the reference population is illustrated in Figure 1. The normality of the data was assessed using the Anderson–Darling method and revealed a non-Gaussian distribution ($p<.001$). Tukey's test did not identify any suspected outlier, and therefore the entire population was preserved ($n=143$).

Partitioning into the three groups (pet dogs, police dogs, and shelter dogs) was statistically evaluated following Lahti et al. recommendations for non-Gaussian distributions, which support partitioning into subclasses if more than 4.1% or less than 0.9% of a subclass falls outside the upper or lower limits of the entire population RI, and discourages partitioning if the proportion of the subclass which falls outside the combined RI is between 1.8% and 3.2%.[41] Considering these statistical recommendations, partitioning into the three groups could be applied to the TAS distribution (see supporting information). Nonparametric methods were utilized for group-specific RI, presented in Table 1, and no outliers were excluded for these subclasses. Clinical criteria and data from the literature were also taken into account to support partitioning, as recommended.[31,40,41]

3.3 | Univariate analysis

The Kruskal–Wallis test revealed statistically significant differences between groups in serum TAS ($p<.001$). Police dogs showed significantly higher TAS values than pet dogs ($p<.001$). Moreover, police dogs had higher TAS levels than shelter dogs ($p<.001$) and shelter dogs had higher values than pet dogs ($p=.037$) (Figure 2). The Kruskal–Wallis test also revealed statistically significant differences between sizes ($p<.001$), showing that large dogs had higher TAS values than medium ($p<.001$) and small dogs ($p=.006$). Differences between sexes were evaluated by the Mann–Whitney U test, which

TABLE 1 RI for serum total antioxidant status (TAS) in the canine population of the study.

TAS	Units	*n*	Mean	SD	Median	Min	Max	*p*-value	Distribution	Method	LRL of RI	URL of RI	CI 90% of LRL	CI 90% of URL
Reference population	mmol/L	143	1.76	0.23	1.69	1.37	2.49	0.000	NG	NP	1.41	2.27	1.37–1.47	2.14–2.49
Pet dogs	mmol/L	56	1.60	0.12	1.59	1.37	2.04	0.036	NG	NP	1.37	1.98	1.37–1.45	1.78–2.04
Police dogs	mmol/L	43	2.02	0.13	2.01	1.71	2.49	0.005	NG	NP	1.72	2.47	1.71–1.84	2.23–2.49
Shelter dogs	mmol/L	44	1.70	0.18	1.67	1.38	2.32	0.009	NG	NP	1.39	2.31	1.38–1.46	1.98–2.32

Note: The Anderson–Darling test was used for evaluating the distribution of the data; the *p*-value cut-off was corrected depending on the sample size ($p<.04$ for the whole population, $p<.067$ for pet dogs, $p<.076$ for police dogs, and $p<.075$ for shelter dogs).[38,39]

Abbreviations: LRL, lower reference limit; *n*, number of valid observations; NG, non-Gaussian; NP, nonparametric; URL, upper reference limit.

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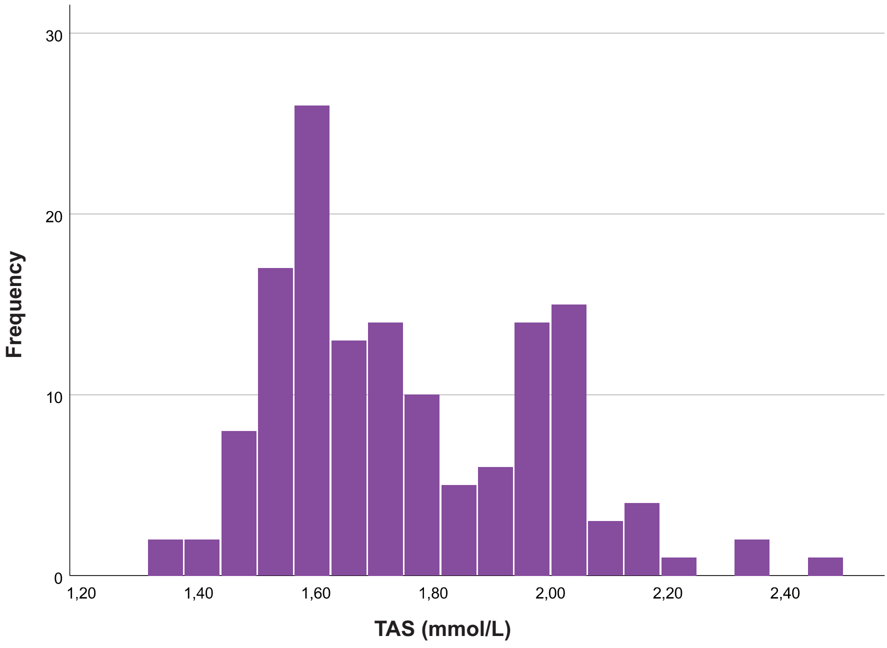

FIGURE 1 Histogram depicting the frequency distribution for serum total antioxidant status (TAS) value in the reference population (n = 143). According to the Anderson–Darling test, the histogram followed a non-Gaussian distribution (p < .001).

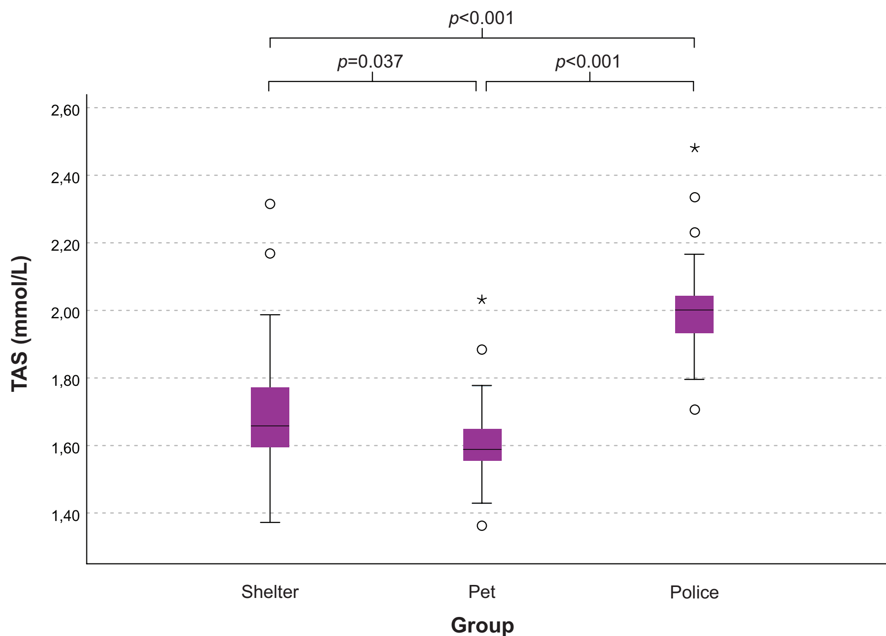

FIGURE 2 Box plots showing median (line within box), 25th and 75th percentiles (box), and minimum and maximum values (whiskers) of TAS results in the three groups of study (pet, police, and shelter dogs). Brackets show the associated p-values, revealing the statistically significant results found. Data points lying between 1.5 (○) and 3 times (*) the interquartile range above the third quartile or below the first quartile are represented in the figure.

revealed a significantly higher TAS value in females compared to males (p = .023). The Spearman's Rho test revealed a significant positive correlation coefficient (R) between TAS value and age (p < .001, R = .304). This test was also employed to evaluate correlations between TAS and all the hematological and biochemical variables tested. Regarding hematology, it revealed a significant positive correlation between TAS and erythroid variables: RBC (p = .001; R = .267), HB (p = .005; R = .232), and HCT (p = .004; R = .240). It also revealed significant correlations with several biochemical variables: TP (p < .001; R = .476), ALB (p = .010; R = .220), GLOB (p < .001;

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$R = .433$), A/G ($p = .004$, $R = -.242$), AST ($p = .010$; $R = .217$), and CHOL ($p = .013$; $R = .209$). In addition, serum cortisol also showed a significant negative correlation with TAS ($p = .004$; $R = -.243$).

3.4 | Multivariate regression model

Given that the study groups were not uniform in terms of both dog sizes and breeds, a multivariate regression model was conducted to assess the influence of the ensemble of biological factors, along with the analytical variables that exhibited significant correlation with TAS value in the univariate analysis. Consequently, the initial model included RBC, HB, ALB, GLOB, AST, CHOL, and cortisol, as well as the categorical and biological factors (group, sex, size, and age). TP and A/G variables were not included in the model given that they were already represented by ALB and GLOB parameters.

The model, presented in Table 2, revealed an interaction between group and sex. Police dogs, both males and females, had significantly higher ($p < .001$) TAS values than the reference dog (pet male dog). Regarding shelter dogs, only the females showed higher TAS values than the reference dogs ($p = .001$). In addition, the following analytical variables showed a significant positive association with TAS value: ALB ($p < .001$), GLOB ($p < .001$), AST ($p = .025$), and CHOL ($p < .001$). Conversely, RBC, HB, HCT, and serum cortisol were excluded from the model as they did not result in statistically significant associations with TAS ($p > .050$).

4 | DISCUSSION

4.1 | RI for the reference population

The RI of serum TAS established in this study was 1.41 to 2.27 mmol/L (median 1.69; min 1.37; max 2.49). The reference population did not follow a Gaussian distribution, in accordance with Tomsič et al. who also obtained a non-Gaussian distribution for canine plasma TAS.[9] While Gaussian distributions tend to appear in tightly regulated biochemical analytes (e.g., glucose and electrolytes), many other measurands do not fit such distributions.[42] The latter could be the case of an individual's antioxidant capacity, which is highly influenced by the multiple systems governing oxidative status.[3]

In line with our findings, four studies reported canine serum or plasma TAS values in control dogs within our established RI,[18–20,43] which is also similar to the reported RI for human serum by the manufacturer (1.3–2.3 mmol/L) (TAS-*liquid stable kit*, Fortress Diagnostics Limited, UK). In contrast, other studies have found lower[12,13,24,27,44] and higher[11,21,45] mean TAS values in dogs. This discrepancy could be attributed to the different commercial kits and assay variations for TAS, the sample type (plasma or serum), and the demographic differences of the control populations. The two studies that proposed RI for TAS found slightly lower values but were conducted using smaller (30 and 19 dogs) and more homogeneous populations than the present one.[9,28] Hence, this study stands as the first to establish assay-specific RI for TAS in a large population of dogs in natural conditions, encompassing diverse biological and environmental backgrounds.

4.2 | Influence of biological and environmental factors, and partitioning into study groups

Regarding biological sources of variation, the age and size of the dogs did not show a significant influence on TAS value, similar to the findings of Tomsič et al.[9] It has been suggested that OS plays an important role in aging and cellular senescence. However, the relationship between age and oxidative status has not been proven in all animal models, reflecting that this relationship might be nonlinear and depends on multiple individual factors such as genotype, cellular metabolism, and mitochondrial function, among others.[46] Moreover,

TABLE 2 Output of the final multivariate regression model for factors associated with serum total antioxidant status (TAS) value in the canine reference population.

TAS	Coefficient	SE	*t*-value	*p* value	95% CI	
Intercept (male pet dog)						
Pet—Female	.0020077	0.0351355	0.06	.955	−0.0675192	0.0715346
Police—Male	**.3105697**	0.0387118	8.02	**.000**	0.2339661	0.3871734
Police—Female	**.3752127**	0.0356242	10.53	**.000**	0.3047189	0.4457065
Shelter—Male	-.0015589	0.0334385	−0.05	.963	−0.0677276	0.0646098
Shelter—Female	**.1439048**	0.0410946	3.50	**.001**	0.0625859	0.2252236
ALB	**.1592191**	0.0433416	3.67	**.000**	0.0734538	0.2449844
AST	**.0021527**	0.000948	2.27	**.025**	0.0002767	0.0040288
CHOL	**.0008303**	0.0002146	3.87	**.000**	0.0004056	0.001255
GLOB	**.0938373**	0.0257297	3.65	**.000**	0.0429228	0.1447517
Constant	.5322658	0.1828979	2.91	.004	0.1703439	0.8941877

Note: Statistically significant associations ($p < .050$) are displayed in bold.

Abbreviations: ALB, albumin; CHOL, cholesterol; GLOB, globulins; SE, standard error.

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the lack of influence of age and size on TAS could be explained by the demographic characteristics of this study. The canine aging process is a complex phenomenon that displays remarkable differences depending on both the dog's size and breed. It has been suggested that studies performed in heterogeneous populations of dogs may not detect the specific associations between these factors and OS biomarkers,[47–50] which could be the case in the present study. The breed could not be statistically evaluated as an independent factor due to its heterogeneity in the reference population, which occasionally comprised only one animal of certain breeds.

Sex was not found to have a significant independent effect on TAS value in the reference population of this study, in accordance with previous studies.[9] The model only revealed a significantly higher TAS value in female shelter dogs compared to the reference dog (pet male dog). However, the shelter dogs group encompassed a limited number of females (15) and included two distant values (2.32 and 2.18 mmol/L) which could have affected statistical results. Other studies have found significant fluctuations of ROS levels across the estrus cycle in the bitch,[51] although this aspect could not be evaluated in the present study due to the absence of data on the animals' reproductive status.

The group (pet/police/shelter) showed a notable effect on the TAS model, revealing a significantly higher TAS value in police dogs, both males and females. This difference corresponds to a median TAS value of 2.01 mmol/L (SD 0.13) in police dogs and a median TAS value of 1.59 mmol/L (SD 0.12) in pet dogs. Several clinical considerations could explain this finding. It has been documented that the genetic component influences up to 50.9% of the individual antioxidant capacity.[52] Notably, the group of police dogs was more homogeneous in that aspect, consisting mostly of German Shepherd and Belgian Malinois dogs. These dogs are usually acquired from specific breeders that select the animals based on their attitude toward work, and therefore some of them could belong to common litters. Additionally, environmental factors may have also played a role. Police dogs included in this group undergo rigorous physical training, which has been shown to enhance antioxidant defenses.[53–56] Specifically, the police dogs included in this study belonged to the apprehension and defense units, which followed even more strict physical training than those specialized in explosives or drug detection. Nutritional data were not recorded in this study, which may have also influenced TAS differences.[7] However, studies in dogs have demonstrated that the antioxidant capacity is not directly related to food intake and it is more affected by the individual factor.[57]

4.3 | Relationship between TAS and analytical variables

Although the univariate analysis suggested potential associations between several analytical variables and TAS value, some of them were discarded by the multivariate regression model. The latter was the case of serum cortisol, whose association with TAS value was excluded by the model. Previous studies have assessed the level of psychogenic stress in dogs by means of blood cortisol concentration and its association with different OS biomarkers, particularly in sheltered dogs. Nonetheless, their results have been conflicting, which has been attributed to the complexity of the assessment of psychogenic stress and the potential lack of specificity of blood cortisol for this matter.[22,23,58–62]

On the other hand, the model revealed significant associations between TAS value and the serum concentrations of ALB, GLOB, CHOL, and AST. The positive influence of protein fractions (ALB, GLOB) on TAS value is fully in line with the characteristics of the assay and with the biochemical properties of proteins. Plasma ALB is considered the major and predominant extracellular antioxidant of plasma[63–65] due to several functions. ALB binds multiple molecules, including free transition metals like iron and copper, limiting their availability to react in Fenton-type reactions that result in the formation of ROS, like hydroxyl radicals.[64,65] Its binding of other molecules, such as bilirubin, homocysteine, and lipids, also inhibits oxidation processes such as lipid peroxidation.[64,65] Additionally, ALB exerts reactive species trapping properties, mainly due to its thiol group of Cys34 residue, which is able to neutralize ROS and RNS such as hydrogen peroxide, peroxynitrite, superoxide anion, and hypochlorous acid.[64,66,67] In fact, it has been reported that ABTS assay measures mainly ALB, representing up to 53% of its total value.[29] The total GLOB variable encompasses multiple proteins that also contribute to the redox state by binding metals, such as the metal-binding proteins, including ceruloplasmin, ferritin, myoglobin, transferrin, lactoferrin, and metallothioneins.[63] GLOB also comprises other proteins whose thiol groups act as antioxidants, such as glutathione, thioredoxins, and other cysteine-containing proteins.[68]

The model also revealed a significant positive association between TAS and CHOL values, which could be explained by in vitro and in vivo mechanisms. A previous study found a significant interference of lipids in TAS assay.[33] Given that CHOL is transported in serum within lipoproteins,[42] samples with higher CHOL may have also contained higher levels of other lipids exerting chemical interference. Furthermore, several studies have demonstrated that high-density lipoproteins (HDL) exhibit antioxidant activities, mainly due to their apoprotein moiety and its associated enzymes (paraoxonase and PAF-acetyl hydrolase).[69,70] Given that dogs have predominantly HDL cholesterol, rather than low-density lipoproteins (LDL),[42] this could have also contributed to the finding.

Lastly, a positive influence of AST concentration on TAS was also found although it represented the lowest significance level of the model. Given that AST may increase due to in vitro hemolysis,[42] samples with minimal hemolysis may have exhibited slightly higher TAS values, in accordance with a previous interference study.[33]

5 | CONCLUSIONS

Although the TAS assay has demonstrated clinical value in several pathologic conditions in dogs, limited information on its serum RI

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has been published. This study stands as the first to establish RI for serum TAS in a large and heterogeneous population of dogs (1.41–2.27 mmol/L). Additionally, specific RIs were established for pet dogs (1.37–1.98 mmol/L), police dogs (1.72–2.47 mmol/L), and shelter dogs (1.39–2.31 mmol/L) in accordance with biological and clinical criteria. The model revealed police dogs exhibit significantly higher TAS values than pet dogs, along with significant associations between TAS and four biochemical variables (ALB, GLOB, CHOL, and AST), in line with the underlying OS biochemical processes. These findings hold substantial practical utility and could potentially contribute to enhancing the clinical interpretation and application of TAS assay in dogs.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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