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Reference Intervals (RIs) in veterinary medicine

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ABSTRACT
Reference Intervals (RIs) are necessary in veterinary clinical pathology to provide a data base in order to compare results obtained from healthy versus diseased animals. Data are obtained from laboratory tests and, depending on whether they have a Gaussian or non-Gaussian distribution, are processed through statistical tests to define the RIs. This process begins with a healthy reference population made up of individuals who have been initially chosen based on inclusion or exclusion criteria. It is frequently challenging to have a large number of healthy individuals on which to establish de novo RIs, especially in wild and exotic animals. However, the use of reference intervals in daily clinical practice remains a fundamental instrument for therapeutic and diagnostic decisions, but it must always be accompanied by clinical findings that can confirm the hypothesis.

Section: RESEARCH PAPER

Keywords: reference intervals; clinical pathology; veterinary medicine; laboratory data; statistical test

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1. INTRODUCTION

Reference Intervals (RIs) in veterinary medicine have a fundamental role in the everyday medical practices and application. This topic could be strictly associated to the field of clinical pathology, but, at the same time they are implicitly used by everyone that processes and interprets laboratory data.

RIs of laboratory tests are calculated from a group or population of healthy adult animals (hence they are called population-based reference intervals). In fact, without a RI it would be very difficult to determine if the results of a laboratory test are altered or normal; other terms that could be used for RI include normal, normal values and reference range [1].

The terms "range" and "interval" have rather different meanings and should not be used interchangeably. A "range" refers to the difference between two values, the highest and lowest observations, and is a single whole number, whereas a "interval" only includes the values between two reference limits and the reference limits themselves. On the other hand, the term "normal value" is also discouraged, since it is difficult to define the concept of "normality", often many variations may appear "abnormal" due to age, physiology, diet, environment or other non-pathological factors [1], [2].

2. POPULATION-BASED REFERENCE INTERVALS

Firstly, these intervals are used to help identify abnormalities in a sick patient, compared to known values from healthy patients. It is important for a RI to represent a large, heterogeneous and appropriate demographic population in order to undertake a precise comparison [2].

In fact, reference values are used to describe the dispersion of variables in healthy individuals, comprising 95 % of the healthy population. In addition, they describe fluctuations observed in healthy populations or individuals that make the definition of health and health status. For this, it remains a fundamental tool in order to make clinical decisions for the patients [3].

This emphasises the first significant drawback of the reference intervals, which is that they differ when the composition of the group of healthy animals used to construct them varies. The most significant influencing factors are race, age, sex, environment, diet, and lifestyle choices; as a result, each laboratory should establish its own reference ranges based on the population of animals that visit the facility. However, this is frequently impractical, with the exception of large facilities that receive a large volume of samples [4].

3. METHODS OF DETERMINATION

Generally, a RI of an analyte for a given population could be determined in three ways: calculated de novo, transferred from a previous RI when a method or instrument is changed or validated from a previously established or transferred RI [3].

The ASVCP (American Society of Veterinary Clinical Pathology) published the reference interval guidelines in order to determine de novo RI in veterinary species, based on the CLSI (Clinical Laboratory and Standards Institutes) recommendations of 2008 [5], [6].

De novo determination of RIs is the most used and frequent procedure in veterinary medicine; an "a priori" method is advised for determining inclusion or exclusion criteria for the starting population, whereas a "a posteriori" approach is utilised when pre-existing data have not yet been mined to generate reference values. The reference individuals chosen form the reference population, are described as "an undefined number of individuals that represent the demographic for which the reference intervals will be used" [5].

The samples must be carried out from animals of a particular species, using a mixture of breeds, ages and gender. The animals of the population must be adults, clinically and serological testing healthy, since the age is a physiological pre-analytical variable that could induce different results in clinical pathology tests. For instance, hematocrit and protein concentration are lower and lymphocyte count is higher in younger animals compared to adults. The impact of age has been observed in a variety of species, including dogs, cats, horses, calves, and donkeys, thus this selection criterion needs to be handled with extreme caution.

The majority of age-related changes are primarily associated to the growth and differentiation that are unique to young animals, such as higher metabolism and grater tissue maturation. A study conducted on Dutch warmblood foals that were raised on pasture with free movement demonstrated that age has significant effects on several hematological values, with the exception of leucocytes (band-shaped, eosinophilic, basophilic and monocytes), platelets (PLT), creatinine, creatine phosphokinase (CPK), pCO_2 and sodium, potassium and calcium. Moreover, it appeared that raising conditions may impact other characteristics, although the clinical significance of these effects may be minimal [7].

Significant differences in values were also detected in newborn Martina Franca donkeys in the first three weeks of life. Compared to horse foals, Martina Franca donkey foals exhibited lower red blood cells (RBC) count, hematocrit (HCT) and hemoglobin (HGB) levels in their first few days of life. Additionally, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC) also resulted lower. These findings underline that RIs should be made to particular breeds species during the newborn stage [8].

Calves are another species in which hematological values have been observed to deviate from adult RIs. Both neonatal Holstein and Norwegian Red Breed calves present higher RBC and PLT counts during the first period of life. However, at the same time, MCV and MCHC values decrease [9], [10].

In clinical practice of small animals, it is important for clinicians to be aware of the temporal variations in biochemical and hematologic values for dog puppies during the first two months of life to prevent misinterpretation of findings [11]. During this period, haematological parameters change significantly, and a study conducted on 101 clinically healthy dog puppies has identified three different RIs, corresponding to 3 phases of 15 days, starting from 16th day after birth [12].

In contrast to calves, during the early stages of life, dogs tend to have lower RBC count, HCT, total proteins and creatinine levels; conversely, alkaline phosphatase (ALP), creatine kinase (CK) and MCV increase [11], [12].

In addition to the influence of age, it is important that the animals are non-treated with drugs that can affect test results, such as corticosteroids that increase neutrophil count and liver enzymes; because of their action, their use is an exclusion criterion for reference individuals in order to determine de novo RIs [5].

Moreover, if the animals are females, it is essential that they are not late pregnant or in early lactations; these physiologic states are uncontrollable pre-analytical variables, such as age. It is known from published studies that this state could affect clinical pathology tests.

For example, in Standardbred mares during peripartum (last month of gestation and first week after parturition), a reduction of HGB, HCT and WBC has been found, as well as in many other serum analytes; it is important to place the attention in these temporary haematological changes, which greatly differentiate the mares in perinatal from those not in lactation and not pregnant [13].

A similar study was carried out in Holstein dairy cows, covering the first month after calving; actually 39 analytes out of 52 tested were found significantly different due to lactation period. These differences due to the lactation phase can be statistically validated and biologically demonstrated, but they can also be affected by pre-analytic factors, such as herds, parity groups and management, which must be considered [14].

4. EXAMPLES

Breed is another crucial parameter to consider within the same species; numerous studies have been conducted to identify the main hematobiochemical differences between different breeds and standard RIs, with the majority of these studies focusing on dogs.

Greyhounds have been extensively studied in both veterinary transfusion medicine and clinical pathology. Their haematological and biochemical values are found to differ from those of the general canine population, indicating distinct physiological characteristics [15]. Numerous deviations have been observed in this breed, including higher HCT, MCV, MCHC, RBC counts and HGB concentrations. However, PLT count and total white blood cell (WBC) counts tend to be lower [15], [16].

There are hematological differences even among sighthounds of the same category, such as Whippet, Greyhound, Italian Greyhound, Sloughi, Saluki, Borzoi, Pharaoh Hound and Azawakh. In a study of 2013, it was documented that Whippets had the laboratory profile that matched Greyhounds the closest and, with the exception of Pharaoh Hounds, Italian Greyhounds demonstrated significantly higher alanine aminotransferase (ALT) activity than the other sighthound breeds [17].

Recent studies on sighthounds have revealed that most RIs available in veterinary textbooks cannot be confirmed for Italian Greyhounds (Piccolo Levriero Italiano-PLI). Only a handful of these variants can be interpreted as peculiarities unique to this breed, perhaps as a result of its predisposition for racing. In fact, they present higher MCH, MCHC, cell haemoglobin concentration mean (CHCM), albumin, calcium and iron, while

large unstained cells (LUC) is lower. At the same time RIs for total proteins and cholesterol resulted wider and the morphology of RBC and reticulocytes different [18].

Standard RIs were also compared with populations of other dog breeds, underlining the presence of breed-specific variation in blood values. For example, Bernese Mountain dogs were found to require new RIs for 7 analytes (eosinophils, MCHC, ALP, γ-glutamyltransferase (GGT), total bilirubin, amylase, and cholesterol) [19], while in Shetland sheepdogs, cholesterol and glucose resulted to need specific RIs because of differences depending on breed peculiarities [20].

Concerning the French mastiff Dogue de Bordeaux (DDB), age was found to significantly affect RIs for HGB, HCT, MCHC, WBC, neutrophil, lymphocyte, and monocyte counts. Additionally, DDBs have higher RIs for HGB, HCT, MCV, MCHC and mean platelet volume (MPV), while lower RIs for reticulocytes counts, platelets by impedance (PLT-I) and optical count (PLT-O) and plateletcrit. For this reason, new RIs for RBC and PLT have been recommended to avoid pathological suspicions of polycythaemia and thrombocytopenia in this breed [21].

There are also breed-specific hematologic peculiarities observed in small dog breeds. For instance, hereditary poodle macrocytosis, a clinically silent condition, has been reported in some Miniature and Toy Poodles [22]. In Cairn and Norfolk Terriers, a condition known as hereditary macrothrombocytopenia has been identified, which is caused by mutations in the beta1-tubulin gene [23]. This condition is prevalent in the Cavalier King Charles Spaniel (CKCS) [24], that have been demonstrated to have a significantly lower whose platelet count than the general RI [25].

The hematologic profile of Miniature Dachshunds has been found to be like Greyhounds, with higher RBC count, packed cell volume (PCV) and hemoglobin concentration [26]. On the other hand, a 1995 Australian study reported that bile acids measurement may not be very useful in Maltese dogs, as they may have "artifactually" elevated serum bile acids due to unknown reacting substances [27].

The Japanese breeds, such the Akita or Shiba Inu and Hokkaido, are perhaps the ones with the most well-known hematological changes related to the breed in daily clinical practice. It has been observed in clinically healthy dogs that they have physiological microcytosis (red blood cells smaller than normal), resulting in lower MCV, MCH, MCHC, higher red cell distribution width (RDW) and significant anisocytosis (variation in red cell size) on smear exam [28].

By the way, veterinary transfusion centers and related databases can often be an excellent source for creating de novo RIs and studying breed differences for hematological and biochemical values. This is due to donor dogs being par excellence a healthy animal and must respect well-defined characteristics [29] and to the large number of samples processed. A recent study carried out through the blood donor database of the EMOVET-UNIPG blood bank and transfusion unit found significant differences in 5 hunting dog breeds (Ariégeois, Bleu de Gascogne, Bracco Italiano, Segugio Italiano, Briquet Griffon Vandeen) for 12 significant differences in hematologic and serum biochemical analytes; new RIs for HCT, MCH, MCHC, RDW, PLT, monocytes, eosinophils, albumin, urea, creatinine, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were supplied for at least one breed [30].

Several feline breeds have been evaluated for hematological and biochemical measurements in comparison to standard reference intervals. In most cases, the breed-specific data overlapped with the standard reference intervals, suggesting minimal impact on clinical interpretation [31]. However, certain breeds exhibited unique characteristics, such as the Birman, which has been found to have physiologically elevated levels of creatinine and serum symmetric dimethylarginine (SDMA) concentrations [32].

As mentioned above, in veterinary medicine it is often challenging to obtain the numbers suggested by guidelines, particularly in wild species or exotic animals. As a result, practitioners often have to make do with limited data sets where one has to make do with small numbers of samples that have been managed to collect.

For example, for the 50th EAAM (European Association for Aquatic Mammals) Annual Symposium of 2023 that took place in Valencia in March, two posters were presented regarding the RIs of hematological and biochemical parameters in a particular species of dolphin, Risso's dolphin (*Grampus griseus*) [33], [34]. As for other exotic species, also in this case the bibliography available is very little numerous and the number of individuals involved in the studies is very small [35], [36].

Despite the small number of individuals (9 prepuberal females), the study on the RIs of hematological and biochemical parameters in Risso's dolphins presented at the 50th EAAM Annual Symposium in Valencia in March 2023 is noteworthy for the high number of samples under analysis (818 blood samples). This represents the first investigation on a large number of samples in this species and may serve as a preliminary study for future research on age and sex differences.

5. STATISTICAL METHODS

According to the guidelines, the nonparametric ranking approach with 90 % confidence intervals should be used to determine new Reference Intervals from a minimum of 120 Reference Individuals (CI) [6].

Based on the number of individuals, it is necessary to test if data follow a Gaussian distribution. Then, in order to determine reference limits and intervals, methods are used to remove outliers and select the central 95 % of reference values [1], [37].

When data do not follow a Gaussian distribution, a possible solution is applying a simple nonparametric method like percentile rank. This is called rank-percentile method, and it is used when there are more than 40 individuals not normally distributed [2]. Then, parametric (mean ± 2sd) or non-parametric (percentiles) methods can be used in order to establish reference limits [1].

In the reference interval guidelines of ASVCP there is a table that recommends procedures for establishing RIs based on reference sample size and distribution; for example, when reference samples are ≥ 20 and < 40, if the distribution is Gaussian, it could be used a parametric method, while, if non-Gaussian, a robust method [5].

This large number of required data are often impossible to achieve in veterinary clinical pathology, but at the same time it is stated that "the smaller the sample is, the higher is the degree of uncertainty in the estimation of reference limits" [5]. Consequently, for small samples, it is better to report them graphically in histograms or dot plot and subsequently use different methods in order to see which one could fit better [38].

This was tested in a study that randomly selected a small sample from a large sample group, showing that the RI estimated from small samples was highly variable and dissimilar from the

RI of the large whole sample. In conclusion, when there are small samples, the bias and not normality of distribution increase [38]. This is why reporting all values and forgoing calculations when there are less than 20 samples is preferable [39].

6. CONCLUSIONS

Laboratories should report the type of RIs used and how it was calculated, since incorrect interpretation of laboratory data can lead to clinical errors and is often underestimated. This is due to the fact that the reference intervals are "statistical artifacts" and the percentage of probability that a healthy animal taken at random from the population has an anomalous value is 5 % [4].

As a matter of fact, when multiple analytes are measured, the more analytes measured, the greater the risk of interpreting values as pathological when they are actually due to chance. On the other hand, just as a value falling within the reference limits does not mean that the presence of underlying pathologies can be excluded, meanwhile the mere fact that a value falls within the reference limits does not imply that the patient is healthy [4].

However, the importance of reference intervals remains fundamental in clinical practice and must always be accompanied by concrete clinical findings on the patient.

In conclusion, this paper highlights the importance of reference intervals (RIs) in clinical pathology. While RIs are used in other areas of veterinary medicine that involve measurements such as diagnostic imaging, with other units of measurements and values, the focus of this discussion has been on the importance of RIs in interpreting laboratory test results.

REFERENCES

[1] S. L. Stockham, M. A. Scott, Introductory concepts, in: Fundamentals of Veterinary Clinical Pathology 2nd ed., Ames Iowa, Blackwell Pub, 2008, pp 16-20, ISBN 9780813800769

[2] K. S. Latimer, J. R. Duncan, Generating and interpreting test results: test validity, quality control, reference values, and basic epidemiology, in: Duncan & Prasse's Veterinary Laboratory Medicine: Clinical Pathology, 5th ed. Chichester West Sussex UK, Wiley-Blackwell, 2011, pp. 369-373, ISBN 9780813820149.

[3] A. Geffré, K. Friedrichs, K. Harr, D. Concordet, C. Trumel, J.P. Braun, Reference values: a review, Vet. Clin. Pathol. 38(3) (2009), pp. 288-298.
DOI: 10.1111/j.1939-165X.2009.00179.x

[4] S. Paltrinieri, W. Bertazzolo, A. Giordano, Come fare e intepretare correttamente un esame: errori preanalitici e interpretativi - How to do and interpret correctly examination: analytical and interpretative errors, in: Patologia clinica del cane e del gatto: approccio pratico alla diagnostica di laboratorio - Clinical pathology of the dog and cat: practical approach to laboratory diagnostics, Italia, ed. Elsevier Health Sciences Italy, 2015, pp. 18.20, ISBN 8821431592 [In Italian]

[5] K. R. Friedrichs, K. E. Harr, K. P. Freeman, B. Szladovits, R. M. Walton, K. F Barnhart, J. Blanco-Chavez; American Society for Veterinary Clinical Pathology, ASVCP reference interval guidelines: determination of de novo reference intervals in veterinary species and other related topics, Vet. Clin. Pathol. 41(4) (2012), pp. 441-453.
DOI: 10.1111/vcp.12006

[6] Clinical and Laboratory Standards Institute (CLSI) document EP28-A3c, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline-Third Edition. Wayne, PA, 2008, ISBN 978-1-56238-682-5.

[7] H. Brommer, M. M. Sloet van Oldruitenborgh-Oosterbaan, B. Kessels, Haematological and blood biochemical characteristics of Dutch warmblood foals managed under three different rearing conditions from birth to 5 months of age, Vet Q. 23(2) (2001), pp. 92-95.
DOI: 10.1080/01652176.2001.9695090

[8] M. C. Veronesi, A. Gloria, S. Panzani, M. P. Sfirro, A. Carluccio, A. Contri, Blood analysis in newborn donkeys: hematology, biochemistry, and blood gases analysis, Theriogenology 82(2) (2014), pp. 294-303.
DOI: 10.1016/j.theriogenology.2014.04.004

[9] H. C. Brun-Hansen, A. H. Kampen, A. Lund, Hematologic values in calves during the first 6 months of life, Vet. Clin. Pathol. 35(2) (2006), pp. 182-187.
DOI: 10.1111/j.1939-165X.2006.tb00111.x

[10] N. Panousis, N. Siachos, G. Kitkas, E. Kalaitzakis, M. Kritsepi-Konstantinou, G. E. Valergakis, Hematology reference intervals for neonatal Holstein calves, Res Vet Sci. 118 (2018), pp. 1-10.
DOI: 10.1016/j.rvsc.2018.01.002

[11] E. Rosset, B. Rannou, G. Casseleux, K. Chalvet-Monfray, S. Buff, Age-related changes in biochemical and hematologic variables in Borzoi and Beagle puppies from birth to 8 weeks, Vet. Clin. Pathol. 41(2) (2012), pp. 272-282.
DOI: 10.1111/j.1939-165X.2012.00415.x

[12] R. Rørtveit, B. K. Saevik, A. V. Eggertsdóttir, E. Skancke, F. Lingaas, S. I. Thoresen, J. H. Jansen, Age-related changes in hematologic and serum biochemical variables in dogs aged 16-60 days, Vet. Clin. Pathol. 44(1) (2015), pp. 47-57.
DOI: 10.1111/vcp.12220

[13] J. Mariella, A. Pirrone, F. Gentilini, C. Castagnetti, Hematologic and biochemical profiles in Standardbred mares during peripartum, Theriogenology 81(4) (2014), pp. 526-534.
DOI: 10.1016/j.theriogenology.2013.11.001

[14] P. Moretti, S. Paltrinieri, E. Trevisi, M. Probo, A. Ferrari, A. Minuti, A. Giordano, Reference intervals for hematological and biochemical parameters, acute phase proteins and markers of oxidation in Holstein dairy cows around 3 and 30 days after calving, Res. Vet. Sci. 114 (2017), pp. 322-331.
DOI: 10.1016/j.rvsc.2017.06.012

[15] S. Zaldívar-López, L. M. Marín, M. C. Iazbik, N. Westendorf-Stingle, S. Hensley, C. G. Couto, Clinical pathology of Greyhounds and other sighthounds, Vet. Clin. Pathol. 40(4) (2011), pp. 414-425.
DOI: 10.1111/j.1939-165X.2011.00360.x

[16] C. Campora, K. P. Freeman, F. I. Lewis, G. Gibson, F. Sacchini, M. J. Sanchez-Vazquez, Determination of haematological reference intervals in healthy adult greyhounds, J. Small Anim. Pract. 52(6) (2011), pp. 301-309.
DOI: 10.1111/j.1748-5827.2011.01070.x

[17] I. Uhríková, A. Lačňáková, K. Tandlerová, V. Kuchařová, K. Řeháková, E. Jánová, J. Doubek, Haematological and biochemical variations among eight sighthound breeds, Aust. Vet. J. 91(11) (2013), pp. 452-459.
DOI: 10.1111/avj.12117

[18] P. Scarpa, B. Ruggerone, S. Gironi, T. Vitiello, S. Paltrinieri, Haematological and biochemical reference intervals in healthy racing and retired Italian Greyhounds, Acta Vet. Hung. 68(1) (2020), pp. 71-78.
DOI: 10.1556/004.2020.00006

[19] L. Nielsen, M. Kjelgaard-Hansen, A. L. Jensen, A. T. Kristensen, Breed-specific variation of hematologic and biochemical analytes in healthy adult Bernese Mountain dogs, Vet. Clin. Pathol. 39(1) (2010), pp. 20-28.
DOI: 10.1111/j.1939-165X.2009.00186.x

[20] B. Ruggerone, M. Giraldi, S. Paltrinieri, P. Scarpa, Hematologic and biochemical reference intervals in Shetland Sheepdogs, Vet. Clin. Pathol. 47(4) (2018), pp. 617-624.
DOI: 10.1111/vcp.12665

[21] R. Lavoué, A. Geffré, J. P. Braun, D. Peeters, F. Granat, N. Bourgès-Abella, C. Trumel, Breed-specific hematologic reference intervals in healthy adult Dogues de Bordeaux, Vet. Clin. Pathol. 43(3) (2014), pp. 352-361.
DOI: 10.1111/vcp.12151

[22] O. W. Schalm, Erythrocyte macrocytosis in miniature and toy poodles, Canine Practice 3 (1976), pp. 55-57.
[23] M. E. Gelain, W. Bertazzolo, G. Tutino, E. Pogliani, F. Cian, M. K. Boudreaux, A novel point mutation in the β1-tubulin gene in asymptomatic macrothrombocytopenic Norfolk and Cairn Terriers, Vet. Clin. Pathol. 43(3) (2014), pp. 317-321. DOI: 10.1111/vcp.12168
[24] H. D. Pedersen, J. Häggstrom, L. H. Olsen, K. Christensen, A. Selin, M. L. Burmeister, H. Larsen, Idiopathic asymptomatic thrombocytopenia in Cavalier King Charles Spaniels is an autosomal recessive trait, J. Vet. Intern. Med. 16(2) (2002), pp. 169-173. DOI: 10.1111/j.1939-1676.2002.tb02350.x
[25] J. Kelley, L. C. Sharkey, P. W. Christopherson, A. Rendahl, Platelet count and plateletcrit in Cavalier King Charles Spaniels and Greyhounds using the Advia 120 and 2120, Vet. Clin. Pathol. 43(1) (2014), pp. 43-49. DOI: 10.1111/vcp.12116
[26] A. R. Torres, S. E. Cassle, M. Haymore, R. C. Hill, Hematologic differences between Dachshunds and mixed breed dogs, Vet. Clin. Pathol. 43(4) (2014), pp. 519-524. DOI: 10.1111/vcp.12178
[27] P. L. Tisdall, G. B. Hunt, G. Tsoukalas, R. Malik, Post-prandial serum bile acid concentrations and ammonia tolerance in Maltese dogs with and without hepatic vascular anomalies, Aust. Vet. J. 72(4) (1995), pp. 121-126. DOI: 10.1111/j.1751-0813.1995.tb15029.x
[28] O. Aniołek, A. Barc, A. Jarosińska, G. Zdzislaw, Evaluation of frequency and intensity of asymptomatic anisocytosis in the Japanese dog breeds Shiba, Akita, and Hokkaido, Acta Veterinaria Brno 86 (2017), pp. 385-391. DOI: 10.2754/avb201786040385
[29] Gazzetta Ufficiale della Repubblica Italiana, Linea guida relativa all'esercizio delle attività sanitarie riguardanti la medicina trasfusionale in campo veterinario - Guideline on the practice of transfusion medical health activities in the veterinary field, n.25, 1 February 2016. Online [Accessed 15 February 2024] https://www.fnovi.it/sites/default/files/LineeGuidaMedicinaTrasfus_0.pdf [In Italian]
[30] A. Miglio, A. Gavazza, D. Siepi, F. Bagaglia, A. Misia, M. T. Antognoni, Hematological and Biochemical Reference Intervals for 5 Adult Hunting Dog Breeds Using a Blood Donor Database, Animals 10(7) (2020), art. No. 1212. DOI: 10.3390/ani10071212
[31] S. Paltrinieri, F. Ibba, G. Rossi, Haematological and biochemical reference intervals of four feline breeds, J. Feline Med. Surg. 16(2) (2014), pp. 125-136. DOI: 10.1177/1098612X13499337
[32] S. Paltrinieri, M. Giraldi, A. Prolo, P. Scarpa, E. Piseddu, M. Beccati, B. Graziani, S. Bo, Serum symmetric dimethylarginine and creatinine in Birman cats compared with cats of other breeds, J. Feline Med. Surg. 20(10) (2018), pp. 905-912. DOI: 10.1177/1098612X17734066
[33] A. Gavazza, M. Quagliardi, G. Rossi, I. Jen, K. Mu, A. Roncarati, L. Galosi, B. Biancani. Hematological parameters in subadult female Risso's dolphins (*Grampus griseus*), Proc. of the EAAM 51st annual symposium, Oceanografic-Valencia, Spain, 7-11 March 2023, pp. 43.
[34] B. Biancani, M. Quagliardi, G. Rossi, I. Jen, K. Mu, A. Roncarati, L. Galosi, A. Gavazza. Biochemical parameters in subadult female Risso's dolphins (*Grampus griseus*), Proc. of the EAAM 51st annual symposium, Oceanografic-Valencia, Spain, 7-11 March 2023, pp. 41.
[35] P. Natchtigall, J. L. Pawloski, J. P. Schroeder, S. Sinclair, Successful maintenance and research with a formerly stranded Risso's dolphin (*Grampus griseus*), Aquatic Mammals 16(1) (1990), pp. 8-13.
[36] K. Shirai, T. Sataki, Haematological findings in captive dolphins and whales, Aust. Vet. J. 75(7) (1997), pp. 512-514. DOI: 10.1111/j.1751-0813.1997.tb14384.x
[37] P. S. Horn, L. Feng, Y. Li, A. J. Pesce, Effect of outliers and nonhealthy individuals on reference interval estimation, Clin. Chem. 47(12) (2001), pp. 2137-2145. DOI: 10.1093/clinchem/47.12.2137
[38] A. Geffré, J. P. Braun, C. Trumel, D. Concordet, Estimation of reference intervals from small samples: an example using canine plasma creatinine, Vet Clin Pathol. 38(4) (2009), pp. 477-484. DOI: 10.1111/j.1939-165X.2009.00155.x
[39] J. P. Braun, D. Concordet, A. Geffré, N. Bourges Abella, C. Trumel, Confidence intervals of reference limits in small reference sample groups, Vet. Clin. Pathol. 42(3) (2013), pp. 395-398. DOI: 10.1111/vcp.12065