pubvet
MEDICINA VETERINÁRIA E ZOOTECNIA
ISSN 1982-1263
https://doi.org/10.31533/pubvet.v16n10a1238.1-7

Analysis of hematological and biochemical variables in searchand rescue dogs

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Abstract. Exercise can induce a series of physiological and laboratory changes depending on some factors such as type of exercise, intensity, frequency, and level of training of the dog. This study aimed to evaluate changes in blood count, leukogram, and biochemical parameters before and after each search performed by the animals. Samples were collected before starting the first search (T0), right after the first search (T1), after the lunch break before starting the second search (T2) and right after the second search (T3). The study was based on verifying changes between these intervals, to also assess possible muscle injuries resulting from efforts made during exercise, with the hypothesis of changes in the blood count due to splenic contraction and gas exchange, we sought to verify changes. Six dogs were studied, four of the Bloodhound breed, a male Belgian Shepherd and a female Labrador Retriever that were submitted to search work. Clinical signs of exhaustion or exercise intolerance were not observed in the dogs during the study. In the course of the studies, no alterations were identified that deviate from the normal physiological pattern.

Keywords: Rescue, sniffer dogs, exercise physiology

Análise de variáveis hematológicas e bioquímicas em cães de busca e resgate

Resumo. O exercício pode induzir a uma série de mudanças fisiológicas e laboratoriais a depender de alguns fatores como tipo de exercício, intensidade, frequência e nívelde treinamento do cão. O presente estudo teve como objeto avaliar alterações no hemograma, leucograma e parâmetros bioquímicos antes e depois de cada busca realizada pelos animais. Foram coletadas amostras antes de iniciar a primeira busca (T0), logo após a primeira busca (T1), depois do período de descanso para almoço antes de iniciar a segunda busca (T2) e logo após a segunda busca (T3). O estudo se baseou em verificar alterações entre esses intervalos, para avaliar também, possíveis lesões musculares decorrentes dos esforços realizados durante o exercício, com a hipótese de alterações no hemograma por contração esplênica e trocas gasosas, buscou-se verificar alterações. Foram estudados seis cães sendo quatro da raça Bloodhound, um Pastor Belga macho e um Labrador Retriever fêmea que

foram submetidos a trabalho de busca. Sinais clínicos de exaustão ou intolerância ao exercício não foram observados nos cães durante o estudo. Ao decorrer dos estudos, não foram identificadas alterações que fogem do padrãofisiológico de normalidade.

Palavras-chave: Resgate, cães farejadores, fisiologia do exercício

Análisis de variables hematológicas y bioquímicas en perros de búsqueda y rescate

Resumen. El ejercicio puede inducir una serie de cambios fisiológicos y de laboratorio dependiendo de factores como el tipo de ejercicio, la intensidad, la frecuencia y el nivel de entrenamiento del perro. El presente estudio tuvo como objetivo evaluar los cambios en el hemograma, el leucograma y los parámetros bioquímicos antes y después de cada búsqueda realizada por los animales. Las muestras se recolectaron antes de comenzar la primera búsqueda (T0), justo después de la primera búsqueda (T1), después del período de descanso para almorzar antes de comenzar la segunda búsqueda (T2) y justo después de la segunda búsqueda (T3). El estudio se basó en verificar cambios entre estos intervalos, para evaluar también posibles lesiones musculares resultantes de los esfuerzos realizados durante el ejercicio, con la hipótesis de cambios en el hemograma debido a la contracción esplénica y al intercambio gaseoso, se buscó verificar los cambios. Se estudiaron seis perros, cuatro de la raza Bloodhound, un pastor belga macho y una labrador Retriever hembra que fueron sometidos a labores de búsqueda. No se observaron signos clínicos de agotamiento o intolerancia al ejercicio en los perros durante el estudio. Durante el transcurso de los estudios no se identificaron alteraciones fuera del patrón fisiológico normal.

Palabras clave: Rescate, perros rastreadores, fisiología del ejercicio

Introduction

The dog has been considered a fundamental part of families all over the world, it is extremely difficult to see a family environment that does not have a pet, especially dogs. In antiquity, these animals had a work function due to their functional ability and attribute of keen senses, in addition to their easy socialization with humans, being fundamental for hunting, herding and protection activities (Bradshaw, 2012).

Dogs for the search and rescue service can be of various breeds, and it is important to highlight that smell must be a point taken into account when selecting a dog, in addition to observing its physical state, keen senses, taste for playing and hunting (Costa, 2016). In addition, rigorous physical training is necessary. In tropical locations, dogs destined for military service undergo training to adapt to the demands of events, always taking into account the age of the animals before submitting them to exercise. When the animals are active, the physical demand becomes increasingly high, making it necessary to use all the body's systems in harmony for the success of the mission (Diverio et al., 2016; Rovira et al., 2008). Historically, to obtain animals that meet all the requirements described above, it was necessary to have a wide selection of efficient animals in both physical and olfactory patterns when it comes to search and rescue missions.

The most used breeds for searching and rescuing people are Bloodhound, German Shepherd and Labrador Retriever (Figure 1), precisely because they fit into important considerations such as aptitude for smell. In addition to the super-efficient sense of smell, dogs have enhanced hearing, which in risky situations is extremely important since, for the victim, every second is extremely important for survival (Layton, 2008).

To assess whether the animals are exerting too much effort or being subjected to overtraining, analyzes of blood concentrations of enzymes that make it possible to assess the athletic profile of the animals are necessary (Diverio et al., 2016; Rovira et al., 2008).

The object of this study was to analyze values in the biochemical and hematological samples of dogs submitted to the search test, in order to assist in the work performance of the trained animals. To analyze hematological (erythrocytes, platelets and leukocytes) and biochemical alterations including Lactate,

Glucose, Creatinine, Urea, Creatine kinase (CK) and Aspartate Aminotransferase (AST) in dogs submitted to scent training.

Figure 1. Sniffer dogs participating in the study of the Bloodhound (**A**), Labrador Retriever (**B**) and Belgian Shepherd Malinois (**C**) breeds.

Material and methods

Animals and experimental groups

In total, six dogs were used, four BloodHounds (three females and one male) between one and a half to three years old, weighing from 32 to 43 kg, a six-year-old male Belgian Shepherd weighing 28 kg and a Labrador Retriever, female of one year and a half weighing 27 kg. The study was carried out in December 2018 during the GBR Certification Exam - Sul Paulista, Franca, São Paulo, Brazil. The animals were maintained on Special Dog Adult Gold food (flavor: chicken/sweet potato) and water *ad libitum*.

It should be noted that the present study was submitted to the Ethics Committee in the Use of Animals of the University of Franca.

Training program

Initially, all animals had regular training, performed three times a week, twice physical and once functional (training with a helper simulating a real search for missing persons). All animals took part in the man trailing course of the GBR-Brazil Rescue Search Group (Figure 2) and all were able to practice this exercise.

Figure 2. Logo of the Search and Rescue Group GBR – Brazil.

Data collects

Blood samples from all animals were collected by external jugular venipuncture. The first collection was before the beginning of scent and search training (T0). Subsequently, blood samples were obtained at the end of each search trail, with an average of 25 min each (Track 1 = T0 – T1 longest route), there was a lunch break for the tutors for about an hour, after which the second search began. (Track 2 = T2 – T3 shortest route), according to routes intended for the animal.

The samples were stored in the K2 EDTA, SST and NaF Na² EDTA Collection Tube (2 ml, 4 ml and 0.5 ml) and refrigerated until processing.

Laboratory tests

The dosages of the respective exams were carried out in the laboratories of the Veterinary Hospital of the University of Franca and in the JL Laboratório Veterinário.

The hemogram determined the hematocrit, counting of the number of red blood cells, calculation of the hematimetric indexes, mean corpuscular volume (MCV) and total and differential leukocyte count, by means of flow cytometry and electrical impedance using the Poch-100iv Diff device (Sysmex) .

The biochemical quantification of AST, urea, creatinine and glucose were performed using the spectrophotometry technique (RA 500 XT, Technicom). The Ck enzyme was quantified by spectrophotometry performed in the Labquest Analyzer (Labtest) device.

Lactate measurement was performed using specific test strips with the aid of a portable lactimeter, by the colorimetric method, by the ChemWell T machine.

Statistical analysis

The statistical software SIGMA PLOT 11.0 was used and the data were submitted to one-way analysis of variance, followed by Tukey's post-hoc test. Differences were considered significant for $p < 0.05$. The comparison was performed between the times, comparing T0 to the rest of the times.

Results and discussion

The average results by evaluation time are shown in Table 1.

Table 1. Mean and standard deviation of hematological and biochemical parameters of search and rescue dogs at different times of exercise

Parameters	Time 0	Time 1	Time 2	Time 3
Red blood cells - HE(x10^6)	7,32±0,76	7,23 ± 0,58	6,91±0,67	7,07±0,40
Hemoglobin - HB(g/dL)	16,05±1,61	15,97±1,38	15,08±1,01	15,48±0,67
Hematocrit -VG (%)	46,80±4,32	46,88±2,84	44,17±2,13	45,68±1,29
VCM (page)	64,38±2,15	64,46±2,15	64,57±2,00	64,33±1,99
HCM (page)	22,08±0,68	29,37±17,81	22,02±0,61	21,80±0,74
CHCM (%)	34,26±0,72	32,08±5,27	34,11±0,77	33,90±0,74
RDW (%)	11,11±1,16	14,70±8,90	10,83±1,00	10,65±0,85
Platelets (µl)	270.500±66.443,21	273.500±68.242,95	234.800±52.959,42	225.000±40.521,60 [C]
Leukocytes (µl)	11.450,00±2.412,26	11.816,67±2.513,49	13.416,67±3.079,23 [B]	13.716,67±2.685,82 [C]
Rods (µl)	-	-	-	-
Segmented (µl)	7726,66±1449,53	7829,33±2349,83	9538,83±336,13	9568,00±2485,75
Lymphocytes (µl)	3700,50±1268,40	2774,50±864,53	2263,33±783,71 [B]	2860,83±813,48
Monocytes (µl)	-	316,83±148,35	322,83±337,56	187,50±163,47
Eosinophils (µl)	22,83±55,93	875,66±497,53 [A]	1332,83±877,75 [B]	1027,66±574,12 [C]
Basophils (µl)	-	20,33±49,80	103,16±161,39	47,00±72,85
Glucose (mg/dL)	86,33±5,71	92,71±13,30	101,36±5,42 [B]	94,98±0,79
Lactate (mg/dL)	12,33±3,66	13,83±4,95	12,33±4,96	8,83±2,71
Creatinine (mg/dL)	1,03±0,13	1,01±0,11	1,00±0,14	0,98±0,10
Urea (mg/dL)	28,41±6,65	28,48±7,00	28,48±5,53	27,81±5,39
CK (UI/L)	159,90±110,74	176,41±133,91	160,13±95,53	171,08±104,88
AST/TGO (UI/L)	44,10±13,66	46,68±10,60	43,38±8,74	42,91±8,72

[A]Statistical significant difference from T0xT1; [B]Statistical difference from T0xT2; [C]Statistical difference from assessment T0xT3 ($P < 0.05$).

The six dogs studied performed the searches without showing fatigue, exercise intolerance and dehydration, in Figure 03 shows images of the animals during the GBR-Brasil 2018 certification test. Changes linked to exercise in the blood count parameters in sporting dogs vary according to with the type of exercise performed (Rovira et al., 2008). The hematological results of red and white blood cells remained within the reference intervals established by Thrall et al. (2015), which was the parameter used for the present study, thus, showing no significant changes, only within the physiological standards, except for the value of HCM (mean capsular hemoglobin) which is calculated from the hemoglobin

concentration and the blood count. erythrocytes, as it is considered redundant to other measurements, is not useful (Kerr, 2003; Thrall, 2015).

Confirming the absence of dehydration in this study, relative polycythemia was not evidenced, taking into account that the presence of acute polycythemia may be the result of dynamic splenic contraction in response to increased oxygen demands and metabolic stress (Ilkiw et al., 1989; Snow et al., 1988) and with a slight decrease in hydration caused by the loss of steam for thermoregulation (Rovira et al., 2008). In addition, the present study did not observe an increase in urea, since urea nitrogen is filtered by the glomeruli, the amount reabsorbed will vary according to the flow rate in the tubules, therefore, the lower the flow, the more nitrogen urea is reabsorbed, therefore, in dehydration, its disproportionate increase in relation to creatinine can be observed (Meuten, 2015), corroborating the findings of the present work of absence of dehydration.

Figure 3. GBR Certification Exam – Brazil: Bloodhound sniffer dogs (**A**) and Labrador Retriver (**B**).

Regarding possible muscle damage, characterized as a frequent consequence of intense exercise in humans and other animals (Kuipers, 1994), the quantitative assessment of muscle damage is of interest in exercise physiology and sports medicine to compare training programs and understand the exercise-induced muscle pathology (Chanoit et al., 2001). Creatine kinase (CK) is a highly specific enzyme for muscle injuries, being found mainly in the cytosol of skeletal and cardiac muscle cells, but also in the kidneys, brain, diaphragm, gastrointestinal tract, uterus and bladder (Valberg, 1996). Its peak serum concentration occurs between four to six hours post-injury and values can return to normal within 24 to 96 hours (Thomassian et al., 2007). This fact was not evidenced in the present study.

Aspartate Aminotransferase is an enzyme present in hepatocytes and muscle cells; therefore, it is not a hepatospecific enzyme. Precisely because it lacks specificity, it must be analyzed together with the CK to identify muscle damage. Still, doubt may arise due to the fact that the half-life of CK is shorter than that of AST, that is, in a situation of muscle injury, we can find both increased, or only increased AST and CK in their normal values (Kerr, 2003; Thrall, 2015). Mild increases in these enzymes after exercise are not associated with muscle cell injury, but with increased membrane permeability (Thomassian et al., 2007). In the present study, no alterations were found in the AST enzyme.

Rovira et al. (2008) reported CK (108. 0 ± 143.0) and AST (31.50±6.25 IU/L) values in search and rescue dogs, also CK (43.13 ± 36.9) and AST (23.0 ± 6.70 IU/L) in agility dogs. In the same study reported values of CK 218±163 and AST (60.0 ± 19) in sled dogs. In the present study, there was a greater increase in mean CK, but still within the reference range and AST remained constant. Therefore, it is assumed, according to the results obtained, that there was no muscle injury.

The study carried out by Belfort et al. (2018) evaluated the plasma activity of CK in two groups of dogs, one group of dogs submitted to walking sessions on an aquatic treadmill and the other to swimming. The dogs in the aquatic treadmill group showed CK values within the reference range, unlike the dogs in the swimming group, which showed a greater increase in CK, suggesting that these animals had a greater muscular effort. Unlike the present study, where there were no major changes in CK. The study carried out by Maia et al. (2019), showed CK levels in Mangalarga Machador horses athletes before and after 40 minutes of training, during 60 days, where there was a gradual increase in CK both pre- and post-training. There was a slight simultaneous increase between pre and post exercise, however,

there was a significant increase between the days. Lavender & Nosaka (2008) mentioned that, due to daily training, there may be an increase in CK. In this study, there was a small increase in CK between searches, but still within physiological values.

Thomassian et al. (2007) reported an increase in AST and CK in horses submitted to the standard progressive treadmill exercise test. At times, CK rose higher and AST remained constant. This can be explained, as mentioned by Mattosinho et al. (2017), AST has a long plasma half-life of approximately 8 days and its peak occurs around 24 hours after injury (Thomassian et al., 2007). In the present study, no alterations in CK and AST enzymes were found. Regarding the conditioning of dogs and muscle fatigue, which was not evidenced in this study, lactate is an important marker, as it allows diagnosing the effectiveness of training, since muscle exercise requires a constant supply of adenosine triphosphate (ATP) so that there is sufficient energy. for muscle contraction. As ATP stores are very low in muscle, the body has several mechanisms to obtain energy (Boffi, 2007). One of these mechanisms is through the anaerobic pathway of pyruvate produced by the glycolytic pathway of glycogen breakdown, where lactate will be produced that will serve as an instantaneous energy source for muscle contraction (Rose & Hodgson, 1994). However, when the intensity and duration of the exercise increases, the fibers are not able to use all the lactate produced. At this point, blood lactate concentrations rise rapidly, decreasing the pH of the muscles and causing fatigue, a fact that was not evidenced in the present study with search and rescue animals. This level of effort intensity is known as the lactate threshold (Boffi, 2007; Lindner et al., 2006). Lactate itself is not harmful to the body, but the hydrogens associated with it (Reed et al., 2009), which promote fatigue, due to events that make the muscle work slowly (Pösö & Puolanne, 2005).

In a study carried out by Costa et al. (2018), there was an increase in serum glucose proportional to the increase in lactate in Quarter Horses post-training, compared to pre-training, indicating the use of the anaerobic pathway for lactate metabolism and energy supply, justifying an increase in blood glucose. post-exercise (Corrêa et al., 2010), which differs from the results obtained in the present study, therefore, no important changes were identified that could suggest injury to the muscle tissue through physical effort, together with the physical examination that do not indicate fatigue and exercise intolerance.

Conclusions

In view of the results presented, no changes were identified that indicated muscle or physiological injuries, evidencing good physical preparation of these animals, requiring further studies with blood gas analysis to identify other possible physiological or pathological changes in search and rescue dogs.

Acknowledgments

We appreciate all the technical support, assistance and participation of the Grupo de Busca e Resgate Sul Paulista and the Universidade de Franca without you it would not be possible to carry out this project.

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Article History:

Received: August 31, 2022
Accepted: September 26, 2022
Available online: November 4, 2022