pubvet
MEDICINA VETERINÁRIA E ZOOTECNIA
ISSN 1982-1263
https://doi.org/10.31533/pubvet.v14n3a534.1-9

Associated use of infrared thermography and ozone therapy for diagnosis and treatment of an inflammatory process in an equine – Case report

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Abstract. This study aims to was a clinical case report relating the use of infrared thermography (IR) associated to the ozone therapy as complementary tools to diagnose and treat a non-infectious inflammatory process in the locomotor system of an athlete-horse of the Amazon. The heart rate (HR), respiratory rate (RR), and rectal temperature (RT) were measured both rest and after walking. Radiographic evaluation, complete hemogram and creatine phosphokinase dosage (CPK) were primarily conducted. The IR examination was additionally undertaken, and the thermograms were analyzed using Flir Tools. Ozone therapy was performed via intramuscular in the scapular area. All comparisons were done using ANOVA and Tukey test (5%). The animal presented HR, RR, and RT all within normal ranges. When the animal was made to walk it demonstrated pain, and HR (48 beats.min^{-1}) and RR (60 breaths.min^{-1}). The creatine phosphokinase dosage was 79 UL^{-1} and the IR showed that the thoracic region had a surface temperature of up to 39.1ºC, indicating of an inflammatory process. After the ozone therapy was a reduction in the white color pattern from 39.1ºC to 37.2ºC. The infrared thermography is an efficient technique that can be used for the diagnosis of inflammation, and ozone therapy is an innovative treatment.
Keywords: Horse, claudication, veterinary medicine, thermogram

Uso associado da termografia por infravermelho e ozonioterapia para diagnóstico e tratamento de um processo inflamatório em equino: relato de caso

Resumo. O objetivo neste estudo foi relatar um caso clínico relacionando o uso da termografia infravermelha (RI) associada à ozonioterapia como ferramentas complementares para diagnosticar e tratar um processo inflamatório não infeccioso no sistema locomotor de um cavalo-atleta da Amazônia. A frequência cardíaca (FC), a frequência respiratória (RR) e a temperatura retal (TR) foram medidas tanto em repouso quanto após a caminhada. A avaliação radiográfica, a dosagem completa do hemograma e da creatina fosfoquinase (CPK) foram realizadas. O exame de IR foi realizado adicionalmente e os termogramas foram analisados usando o *Flir Tools*. A ozonioterapia foi realizada por via intramuscular na área escapular. Todas as comparações foram

realizadas utilizando ANOVA e teste de Tukey (5%). O animal apresentou FC, RR e TR, todos dentro dos limites da normalidade. Quando o animal foi levado a andar, demonstrou dor e FC (48 batimentos.min-1) e RR (60 respirações.min-1). A dosagem de creatina fosfoquinase foi de 79 UL-1 e o IR mostrou que a região torácica apresentava temperatura superficial de até 39,1ºC, indicando processo inflamatório. Após a ozonioterapia houve redução do padrão de cor branca de 39,1ºC para 37,2ºC. A termografia por infravermelho é uma técnica eficiente que pode ser usada para o diagnóstico de inflamação e a terapia com ozônio é um tratamento inovador.

Palavras chave: cavalo, claudicação, medicina veterinária, termograma

Uso asociado de la termografía infrarroja y la terapia con ozono para el diagnóstico y tratamiento de un proceso inflamatorio en caballos: Reporte de un caso

Resumen. Objetivo de este estudio fue informar un caso clínico relacionado con el uso de la termografía infrarroja (IR) asociada con la ozonoterapia, como herramientas complementarias para el diagnóstico y el tratamiento de un proceso inflamatorio no infeccioso en el sistema locomotor de un caballo atleta del Amazonas. La frecuencia cardíaca (FC), la frecuencia respiratoria (RR) y la temperatura rectal (RT) se midieron tanto en reposo como después de caminar. Se realizó principalmente una evaluación radiográfica, un recuento sanguíneo completo y una medición de creatinina fosfoquinasa (CPK). El examen IR se realizó adicionalmente y los términos se analizaron usando *Flir Tools.* La ozonoterapia se realizó por vía intramuscular en el área escapular. Todas las comparaciones se realizaron con ANOVA y la prueba de Tukey (5%). El animal presentava HR, RR y TR, dentro de los límites normales. Cuando el animal fue llevado a caminar, mostró dolor, FC (48 latidos.min-1) y RR (60 respiraciones.min-1). El dosaje de creatina fosfoquinasa fue de 79 UL-1 e IR mostró que la región torácica tenia una temperatura superficial de hasta 39.1ºC, registrando un proceso inflamatorio. Después de la ozonoterapia, el patrón de color blanco disminuyó de 39.1ºC a 37.2ºC. La termografia por infrarrojo es una técnica eficiente que puede usarse para el diagnóstico de inflamación y la terapia de ozono es un tratamiento innovador.

Palabras clave: Caballo, cojera, medicina veterinaria, termograma

Introduction

Equines play an important role in sporting activities and high-performance horses are often involved in top level competitions or races. Thus, their musculoskeletal system is regularly subjected to mechanical forces that act in a continuous manner (Wilson & Weller, 2011). The performance of equines in sport-related activities is directly related to the integrity of their musculature, principally with respect to locomotion. Furthermore, the forces applied to their structures, due to intense physical activity, represent one of the main causes for inactivity of athlete-horses (Branly et al., 2018), leading to a decrease in animal welfare (Visser et al., 2014) and substantial economic losses.

In horses, muscular lesions can give rise to inflammatory processes, which are one of the causes for claudication (Lewis et al., 2017). Claudication indicates the occurrence of structural or functional perturbation of one or more members that is observable when the animal is in movement as well as when at rest (Thomassian, 2005). Inflammatory processes stimulate physiological mechanisms that increase blood flow and local temperature and can be diagnosed by alterations on the skin surface (Hildebrandt et al., 2010). In this context, infrared thermography has potential to be a precise and non-invasive technique that can aid in diagnosis of pathologies that have different degrees of negative effects on tissues through variations in surface temperature (TS) and thermal gradients (Menegassi et al., 2015; Sykes et al., 2012). The analysis of the color spectrum in a thermogram can be used to indicate

inflammatory processes when a pattern of elevated temperatures is detected (Silva et al., 2018; Soroko & Howell, 2018).

Clinically, when a lesion is detected, different therapeutic techniques can be applied to the animal. The choice of a particular therapeutic strategy depends, among other aspects, on the clinical condition of the patient, facility of adoption, and the prognostic for effective treatment. Ozone therapy is a form of treatment that possesses anti-inflammatory, analgesic, and antiseptic properties and can reduce platelet aggregation and consequently local inflammation and pain (Bernal et al., 2013; Kovach et al., 2017; Orakdogen et al., 2016; Silva et al., 2018; Somay et al., 2017). Currently, in veterinary medicine, ozone therapy is being used in the treatment of inflammatory processes, tumors, and trauma (Schwartz & Sánchez, 2012; Zobel et al., 2013), and is a promising and innovative technique that has received much attention in the treatment of diverse species of animals (Đuričić et al., 2016; Teixeira et al., 2013; Tsuzuki et al., 2015). Taking this into consideration, the objective of this study was to present a clinical case report relating the use of infrared thermography associated to the ozone therapy as complementary tools to diagnose and treat a non-infectious inflammatory process involving the locomotor system of an adult horse.

Materials and methods

Study Site

This study was conducted in Santarém, State of Pará, Brazil (02º 26' 35" S and 54º 42' 30" W), located in a tropical rainy climate and classified as Am3 according to the Köppen system (Köppen & Geiger, 1928). Annual rainfall varies between 2,000 to 2,500 mm with total rainfall below 60 mm during the month with the least rainfall. The maximum air temperature varies between 30.5 and 32.0°C and the minimum varies between 21.0 and 22.5° C (Köppen & Geiger, 1928).

Bioethics

All procedures undertaken were done in agreement with Brazilian legislation, and the publication of this procedure in the form of this article received the consent of the owner of the horse and the entire group that worked on this research.

Anamnesis and clinical exam

An 8-year-old, athlete male American Quarter Horse, with a live weight of 420 kg and body condition score of 3 (1 to 5 scale) was examined at the Veterinary Clinic of the University Center of the Amazon (CLIVET-UNAMA). The main complaint concerned a limping and dissatisfaction with the functional aspects of locomotor abilities.

During anamnesis and the clinical exam, the animal stood, avoiding movement with plantar support (Figure 1A). When the animal entered into movement it showed an evident limping and a reduced functional capability of right forelimb during locomotion. According the owner this clinical symptom had begun four months before. Prior to the onset of the limping problem the animal participated in equestrian competitions that demand intense physical effort. During the clinical evaluation the heart rate was measured (HR; beats.min^{-1}) using a veterinary stethoscope, the respiratory rate (RR; breaths.min^{-1}) was visually monitored by counting respiratory movements, and rectal temperature (TR; ºC) was assessed using a clinical veterinary thermometer with a scale up to 44º C (Hodgson et al., 2013). Radiographic exams of the right forelimb were taken focusing the region between the knee and the hoof (Figure 1B, 1C), and blood collection was done through puncture of the jugular using vacuum tubes, with and without anti-coagulant, for the hemogram and the creatine phosphokinase (CPK) dose. Laboratory evaluation of total CPK was performed using a direct enzymatic method according to the International Federation of Clinical Chemists (Tai et al., 2017).

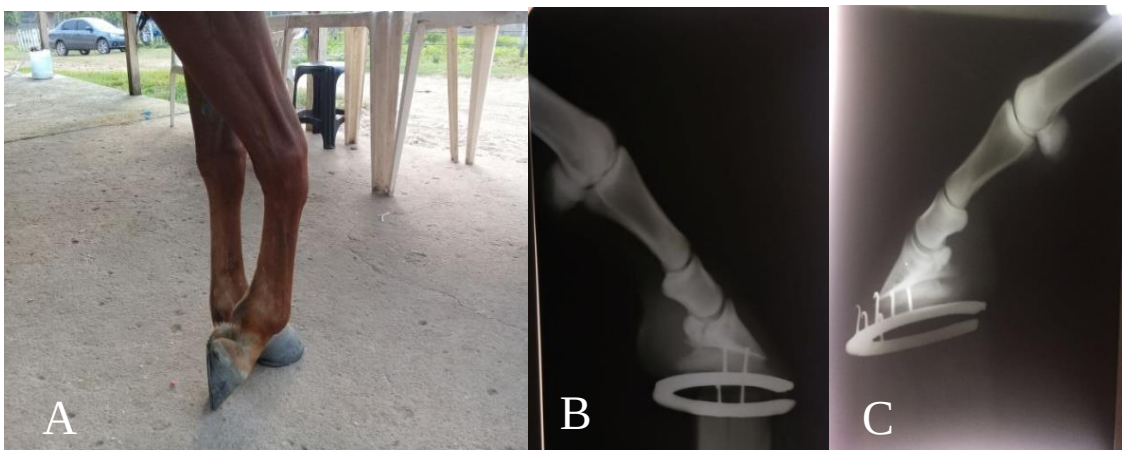

Figure 1. Images of the right forelimb. **(A)** Visual photography of the horse presenting difficulty standing on the right forelimb. **(B)** Radiographic image of the right forelimb (lateral-lateral view). **(C)** Radiographic image of the right forelimb (half-lateral view).

Thermographic imaging

After the physical exam, infrared thermographic images were used to aid in the diagnosis. The dynamic thermographic accompanying method was used before and after ozone therapy, and the variable monitored was surface temperature (TS, ºC). Thermographic scanning covered from the thoracic (scapular) region to the distal part of the right forelimb. The thermograms were generated using an infrared thermographic imager (FLIR T650sc, Wilsonville, OR, EUA). The thermographic imager had a 25 mm fixed lens, a temperature scale of -40 to 150º C, thermal sensibility of 50mK (<0.05 ºC at an ambient temperature of 30º C), a spectral scale with a range of 0.7 to 100µm, capable of receiving a response from imaged targets between 0.7 and 3.0 µm, and an optical resolution of 640x480 pixels. The emissivity index adopted for the analyses was 0.98 (Kahwage et al., 2017). The thermograms were subsequently analyzed using the program Flir Tools, 6.3v (FLIR Systems, Inc., Wilsonville, OR, USA). For the quantification of the TS of the evaluated regions the Rainbow HC palette was used with the following thermal patterns in decreasing order: white, red, yellow, and green. In each image four evaluation points were used for each analyzed color pattern (Figure 2).

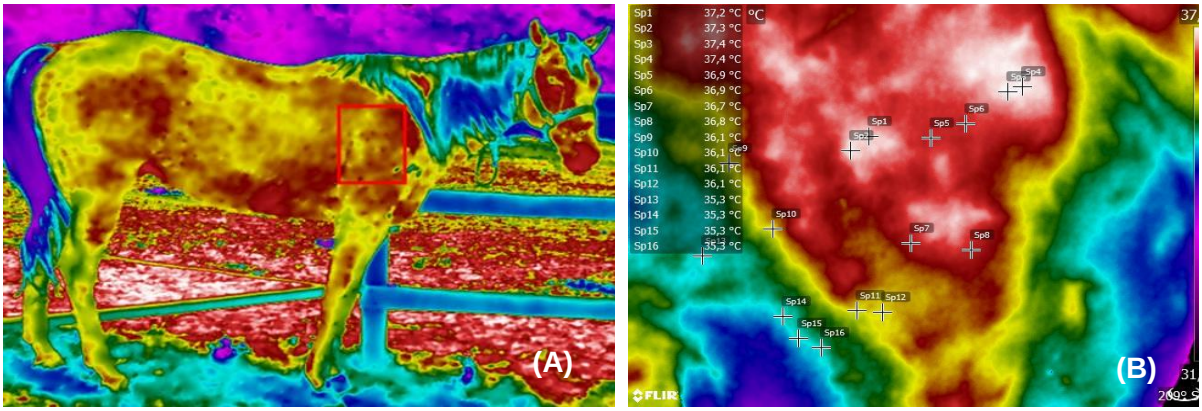

Figure 2. (A) Infrared thermogram of the right antimer of the athlete-horse. **(B)** Detailed image showing different thermographic patterns evaluated in the affected anatomic region.

Ozone therapy

Ozone therapy was conducted with the animal standing, and five consecutive sessions for application were done over three-day intervals for a total of 15 days of treatment. The ozone therapy was administered by deep intramuscular injections in the scapular region. An ozonizer (Philozon, O3R, São Paulo, SP, Brazil) with an oxygen concentrator at 92% (10 L min^{-1}), maximum ozone generation of 15 g of O3 at 8 min L^{-1} of O_2 with a static mixer injection system/diffuser, bypass and one-inch venturi injector, was used. In each application, 120 mL of the mixture was injected.

Statistical Analysis

The data were previously submitted to descriptive analysis. Analysis of variance (ANOVA) was conducted, followed by mean comparisons using the Tukey test in order to identify decreasing thermal patterns (white, red, yellow, and green) in responses of the evaluated regions. The statistical software used was BioEstat, version 5.3 (Ayres, 2007) and the probability level used was 5%.

Results

During anamnesis and clinical evaluation, the animal was nervous while standing and presented claudication in the right forelimb when submitted to physical exertion of differing intensities, from a simple walk to running.

In the present study, during the palpations conducted during the clinical exam and the handling of the joints of the affected member, including the shoulder, the animal manifested no sign of discomfort. The animal presented pallid mucous membranes, normal capillary refill time and cutaneous turgor, and cardiac and respiratory rates of 42 beats/min^{-1} and 44 breaths/min^{-1}, respectively. The internal temperature of 37.2º C was within normal range, and there were no lesions on the skin that could be related to previous traumatism. When the animal was made to walk along a straight line for about 100 m it demonstrated pain, and an increase in the cardiac (48 beats/min^{-1}) and respiratory rates (60 breaths/min^{-1}). The radiographic exam was negative for skeletal alterations, thus eliminating the possibility of a bone fracture or micro fissures.

The results for the hemogram indicated anemia (red blood cells: 4.5 x10^{6} cells.µL^{-1}) macrocytic (mean corpuscular volume, MCV: 66.7 fL), normochromic (mean corpuscular hemoglobin concentration, MCHC: 34.3 g.dL^{-1}), and a reduction in hemoglobin (10.3 g.dL^{-1}) and low hematocrits (30%) (Table 1). The biochemical exam showed that CPK was 79 U.L^{-1}, a level that is within normal range (145-380 U.L^{-1}).

Table 1. Results obtained in the current study and reference values for hematological parameters in horses.

Variable	Result	Reference Values*
Red blood cells (x10^{6} cells. µL^{-1})	4.5	6.8-12.9
Mean corpuscular volume-MCV (fL)	66.7	37.0-58.5
Mean corpuscular hemoglobin concentration-MCHC (g.dL^{-1})	34.3	31.0-38.6
Hemoglobin (g.dL^{-1})	10.3	11.0-19.0
Hematocrit (%)	30.0	32.0-53.0
Creatine phosphokinase-CPK (U.L^{-1})	79	145-380

* Jain & Jain (1993).

In the evaluation of the thermograms, in the thoracic region, at the insertion point of the scapula with the cervical trapezius muscle, the animal presented an elevated temperature, with a TS of up to 39.1º C (white color pattern) (Figure 3.A). After ozone therapy through deep intramuscular injection in the scapular region there was a reduction in the white color pattern from 39.1º C to 37.2º C (Figure 3.B).

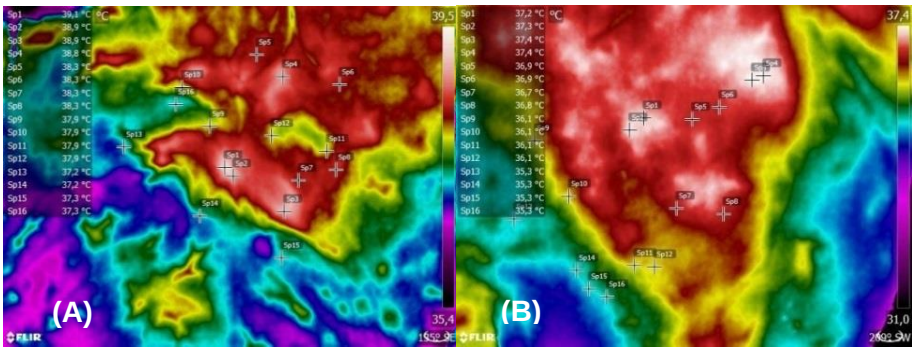

Figure 3. Infrared thermograms of the thoracic region of a horse. **(A)** Thermographic image indicating an inflammatory process characterized by an increase in surface temperature. **(B)** Thermographic image of the same anatomic region after ozone therapy. Images parametrized for Rainbow HC palette.

There was a reduction in TS for all the thermographic patterns that were analyzed in the affected region after the end of ozone therapy treatment (Tables 2).

Table 2. Surface temperatures (Mean±standard deviation) registered by infrared thermography in the thoracic region of an athlete-horse before and after ozone therapy.

Thermographic Pattern	Mean Temperature (ºC)	
	Before Ozone therapy	After Ozone therapy
White	38.9±0.1[a]	37.3±0.0[b]
Red	38.3±0.0[a]	36.8±0.0[b]
Yellow	37.9±0.0[a]	36.1±0.0[b]
Blue	37.2±0.0[a]	35.3±0.0[b]

[a,b]Different letters in the same line indicate statistical difference (P<0.05).

Discussion

In horses, claudication can be due to diverse etiologies, including the routine of exercise (Thomassian, 2005; Hodgson et al., 2013). Furthermore, in athlete-horses, claudication is the most common sign of pain in the locomotive structure (Wagner, 2010).

When submitted to walking presented pain and increase in heart rate and respiratory. Clinical studies have shown that horses with condition of sharp pain present an activation of the autonomic nervous system which increases the heartbeats and respiration rate, besides increasing the levels of serum catecholamine, beta-endorphins, and cortisol (Driessen & Zarucco, 2007). Thus, the pain during the execution of the movements also explains the increase observed in these rates.

The radiographic exam was negative for skeletal alterations, thus eliminating the possibility of a bone fracture or micro fissures. A radiograph can diagnose fractures, bone dislocations, and modifications of subcutaneous tissues such as edema in soft tissues and calcification, but it is not able to identify inflammatory processes at the muscular level (Brukner & Khan, 2006).

Results that were most likely related to the inflammatory processes detected in the animal. Borges et al. (2007) affirm that inflammatory processes can sequester iron, which can lead to hypoferremia followed by a reduction in red blood cells and hematocrit, and consequently a reduction in the production of hemoglobin.

The CPK within normality discards the possibility of myositis. CPK is an enzyme that is directly related to acute lesions and/or intense muscular effort, with elevation in cases of myositis, muscular dystrophy and trauma after moderately intense exercise, surgery or convulsions (Aleman, 2008).

The increase in temperature in the thoracic region was indicative an inflammatory process, due to an increase in blood flow (Hildebrandt et al., 2010). Therefore, analyzing the results from anamnesis, the clinical evaluation, the hemogram, and the thermographic images, the final diagnosis of an inflammatory process in the muscle tissue was confirmed. The use of thermography in this case was concluded to be of great utility because the initial suspicion was claudication due to a lesion in the distal part of the right front leg. However, an inflammatory process was detected, using the infrared thermography, in the proximal part of the limb, in the thoracic region.

The ozone therapy treatment reduces the inflammation, probably by combating platelet aggregation and the immunological response (Orakdogen et al., 2016; Somay et al., 2017). Furthermore, there was an improvement in the clinical condition of the animal, with a reduction in symptomatology and pain after consecutive applications of ozone. Ozone therapy has been used in the treatment of inflammatory processes and has been shown to be effective for severe and chronic inflammatory processes (Raeissadat et al., 2018) and infections (Lu et al., 2018), besides being a technique that is easily applied and that has no collateral effects.

Conclusions

Infrared thermography is an efficient technique that can be used for increasing the diagnostic accuracy of inflammatory processes. Its use in veterinary medicine can favor the diagnosis, reveal the exact location of the inflammation source, and can greatly aid the monitoring the evolution of the treatment, thus contributing

to reestablish the animal welfare. On the other hand, ozone therapy is an innovative treatment, capable of reducing surface temperature and thus helping to eliminate inflammatory processes. Therefore, the use of ozone therapy in the treatment of inflammatory processes is a promising alternative for veterinary medicine clinics. When these techniques are used in combination there are innumerous benefits because thermography can aid in the detection of lesions that can subsequently be treated using ozone therapy. This reduces the time that the animal would be feeling pain, improving its quality of life and allowing for a quicker return to the participation in sporting activities.

Conflict of interest statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Acknowledgements

The authors thank the Brazilian Coordination for the Improvement of Higher Education Personnel (CAPES) for the post-doctorate scholarship for the first author. The authors also thank the Brazilian Agricultural Research Company-Embrapa for the opportunity to share the thermographic imager (Project MP5 PECUS/INFRA CNPq).

References

Aleman, M. (2008). A review of equine muscle disorders. *Neuromuscular Disorders*, *18*(4), 277–287.

Ayres, M. (2007). *BioEstat 5.0: aplicações estatísticas nas áreas das ciências biológicas e médicas*. Sociedade Civil Mamirauá.

Bernal, D. S., Dupláa, G. R., Tabasco, M. M. M., Palomares, M. G., & Sánchez, V. M. (2013). Tratamiento de la gonalgia por gonartrosis con ozono intrarticular. *Revista de La Sociedad Española Del Dolor*, *20*(3), 107–112.

Borges, Ai. S., Divers, T. J., Stokol, T., & Mohammed, O. H. (2007). Serum iron and plasma fibrinogen concentrations as indicators of systemic inflammatory diseases in horses. *Journal of Veterinary Internal Medicine*, *21*(3), 489–494.

Branly, T., Contentin, R., Desancé, M., Jacquel, T., Bertoni, L., Jacquet, S., Mallein-Gerin, F., Denoix, J.-M., Audigié, F., & Demoor, M. (2018). Improvement of the chondrocyte-specific phenotype upon equine bone marrow mesenchymal stem cell differentiation: influence of culture time, transforming growth factors and type I collagen siRNAs on the differentiation index. *International Journal of Molecular Sciences*, *19*(2), 190.

Brukner, P., Khan, K. (2006). Clinical Sports Medicine, 3 ed. Australia: McGraw-Hill.

Driessen, B., & Zarucco, L. (2007). Pain: from diagnosis to effective treatment. *Clinical Techniques in Equine Practice*, *6*(2), 126–134.

Đuričić, D., Valpotić, H., Žura Žaja, I., & Samardžija, M. (2016). Comparison of intrauterine antibiotics versus ozone medical use in sheep with retained placenta and following obstetric assistance. *Reproduction in Domestic Animals*, *51*(4), 538–540.

Hildebrandt, C., Raschner, C., & Ammer, K. (2010). An overview of recent application of medical infrared thermography in sports medicine in Austria. *Sensors*, *10*(5), 4700–4715.

Hodgson, D. R., Mcgowan, C., Mckeever, K. (2013). The Athletic Horse: Principles and Practice of Equine Sports Medicine. 2nd ed. Philadelphia, PA: Saunders.

Jain, N. C., & Jain, A. H. (1993). *Essentials of Veterinary Hematology* (1st ed.). Wiley-Blackwell.

Kahwage, P. R., Esteves, S. N., Jacinto, M. A. C., Junior, W. B., Pezzopane, J. R. M., de Andrade Pantoja, M. H., Bosi, C., Miguel, M. C. V., Mahlmeister, K., & Garcia, A. R. (2017). High systemic and testicular thermolytic efficiency during heat tolerance test reflects better semen quality in rams of tropical breeds. *International Journal of Biometeorology*, *61*(10), 1819–1829.

Köppen, W., & Geiger, R. (1928). Klimate der Erde. Gotha: Verlag Justus Perthes. *Wall-Map 150cmx200cm*.

Kovach, I., Kravchenko, L., Khotimska, Y., Nazaryan, R., & Gargin, V. (2017). *Influence of ozone therapy on oral tissue in modeling of chronic recurrent aphthous stomatitis*. *264*, 115–119. https://doi.org/http://dx.doi.org/10.1556/1646.9.2017.32.

Lewis, S. S., Nicholson, A. M., Williams, Z. J., & Valberg, S. J. (2017). Clinical characteristics and muscle glycogen concentrations in warmblood horses with polysaccharide storage myopathy. *American Journal of Veterinary Research*, *78*(11), 1305–1312.

Lu, J., Li, M., Huang, J., Gao, L., Pan, Y., Fu, Z., Dou, J., & Xiang, Y. (2018). Effect of ozone on Staphylococcus aureus colonization in patients with atopic dermatitis. *Journal of Central South University*, *43*(2), 157–162.

Menegassi, S. R. O., Barcellos, J. O. J., Dias, E. A., Koetz, C., Pereira, G. R., Peripolli, V., McManus, C., Canozzi, M. E. A., & Lopes, F. G. (2015). Scrotal infrared digital thermography as a predictor of seasonal effects on sperm traits in Braford bulls. *International Journal of Biometeorology*, *59*(3), 357–364.

Orakdogen, M., Uslu, S., Emon, S. T., Somay, H., Meric, Z. C., & Hakan, T. (2016). The effect of ozone therapy on experimental vasospasm in the rat femoral artery. *Turk Neurosurg*, *26*(6), 860–865. https://doi.org/http://dx.doi.org/10.5137/1019-5149.JTN.14162-15.2.

Raeissadat, S. A., Rayegani, S. M., Forogh, B., Abadi, P. H., Moridnia, M., & Dehgolan, S. R. (2018). Intra-articular ozone or hyaluronic acid injection: which one is superior in patients with knee osteoarthritis? A 6-month randomized clinical trial. *Journal of Pain Research*, *11*, 111.

Schwartz, A., & Sánchez, G. M. (2012). Ozone therapy and its scientific foundations. *Ozone Therapy Global Journal*, *2*(1), 199–232.

Silva, L. K. X., Sousa, J. S., Silva, A. O. A., Lourenço Junior, J. B., Faturi, C., Martorano, L. G., Franco, I. M., Pantoja, M. H. A., Barros, D. V, & Garcia, A. R. (2018). Testicular thermoregulation, scrotal surface temperature patterns and semen quality of water buffalo bulls reared in a tropical climate. *Andrologia*, *50*(2), e12836.

Somay, H., Emon, S. T., Uslu, S., Orakdogen, M., Meric, Z. C., Ince, U., & Hakan, T. (2017). The histological effects of ozone therapy on sciatic nerve crush injury in rats. *World Neurosurgery*, *105*, 702–708. https://doi.org/http://dx.doi.org/10.1016/j.wneu.2017.05.161.

Soroko, M., & Howell, K. (2018). Infrared thermography: current applications in equine medicine. *Journal of Equine Veterinary Science*, *60*, 90–96.

Sykes, D. J., Couvillion, J. S., Cromiak, A., Bowers, S., Schenck, E., Crenshaw, M., & Ryan, P. L. (2012). The use of digital infrared thermal imaging to detect estrus in gilts. *Theriogenology*, *78*(1), 147–152.

Tai, H., Cui, L., Guan, Y., Liu, M., Li, X., Shen, D., Li, D., Cui, B., Fang, J., & Ding, Q. (2017). Correlation of creatine kinase levels with clinical features and survival in amyotrophic lateral sclerosis. *Frontiers in Neurology*, *8*, 322.

Teixeira, L. R., Luna, S. P. L., Taffarel, M. O., Lima, A. F. M., Sousa, N. R., & Joaquim, J. G. F. (2013). Comparison of intrarectal ozone, ozone administered in acupoints and meloxicam for postoperative analgesia in bitches undergoing ovariohysterectomy. *The Veterinary Journal*, *197*(3), 794–799.

Thomassian, A. (2005). *Enfermidades dos cavalos*. Livraria Varela.

Tomašević-Čanović, M., Daković, A., Rottinghaus, G., Matijašević, S., & Đuričić, M. (2003). Surfactant modified zeolites—new efficient adsorbents for mycotoxins. *Microporous and Mesoporous Materials*, *61*(1), 173–180.

Tsuzuki, N., Endo, Y., Kikkawa, L., Korosue, K., Kaneko, Y., Kitauchi, A., Katamoto, H., Hidaka, Y., Hagio, M., & Torisu, S. (2015). Effects of ozonated autohemotherapy on the antioxidant capacity of Thoroughbred horses. *Journal of Veterinary Medical Science*, *77*(12), 1647–1650.

Visser, E. K., Neijenhuis, F., Graaf-Roelfsema, E., Wesselink, H. G. M., De Boer, J., van Wijhe-Kiezebrink, M. C., Engel, B., & van Reenen, C. G. (2014). Risk factors associated with health disorders in sport and leisure horses in the Netherlands. *Journal of Animal Science*, *92*(2), 844–855.

Wagner, A. E. (2010). Effects of stress on pain in horses and incorporating pain scales for equine practice. *Veterinary Clinics: Equine Practice*, *26*(3), 481–492.

Wilson, A., & Weller, R. (2011). The biomechanics of the equine limb and its effect on lameness. In *Diagnosis and Management of Lameness in the Horse* (pp. 270–281). Elsevier.

Zobel, G., Leslie, K., Weary, D. M., & von Keyserlingk, M. A. G. (2013). Gradual cessation of milking reduces milk leakage and motivation to be milked in dairy cows at dry-off. *Journal of Dairy Science*, *96*(8), 5064–5071. https://doi.org/http://dx.doi.org/10.3168/jds.2012-6501

Article History:
Received 29 January, 2020
Accepted 17 March, 2020
Available online 17 April, 2020