

EVALUATION OF HEMOACUPUNCTURE IN THE TREATMENT OF DOGS WITH THROMBOCYTOPENIA

AVALIAÇÃO DA HEMOACUPUNTURA NO TRATAMENTO DE CÃES COM TROMBOCITOPENIA

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Resumo: O presente trabalho teve o objetivo de avaliar a eficácia da hemoacupuntura como tratamento complementar da trombocitopenia em cães associada a possível infecção por erliquiose, comparada com o tratamento convencional. Foram utilizados 17 cães, entre machos e fêmeas com contagem de plaquetas abaixo de 200.000/ μ L. Os animais foram aleatorizados e distribuídos em dois grupos: Grupo Controle (CG, n=10) – animais que receberam o tratamento convencional com Cloridrato de Doxiciclina (10 mg/kg, BID); e Grupo Tratamento (TG, n=7) – animais que receberam a terapia convencional associada a auto-hemoterapia com 0,2 a 0,5 ml de sangue autólogo bilateral nos acupontos B17, VB39, BP10 e VG14, bilateralmente. O tratamento foi realizado nos dias 7, 14, 21 e 28 (T1 a T4), com sessões semanais de auto-hemoterapia, e realizado um novo hemograma a fim de avaliar o efeito da terapia; a coleta era feita antes do tratamento. Os dados avaliados não demonstraram diferença entre os tratamentos em nenhum momento avaliado. No entanto, no GC houve aumento dos eritrócitos e hematócrito a partir de T3. No GT, não foi observada diferença significativa ao longo do tempo para nenhum dos parâmetros avaliados, mas todos os animais apresentaram melhora clínica ao final do tratamento. Nas condições deste estudo o uso da auto-hemoterapia em acupontos não apresentou um incremento na terapia da trombocitopenia em cães.

Palavras-chave: Acupuntura, canino, plaquetas, sangue.

Abstract: This study aimed to evaluate the effectiveness of hemoacupuncture as a complementary treatment in dogs with thrombocytopenia, associated with a possible infection by ehrlichiosis, compared to conventional treatment. Seventeen (17) dogs were used, males and females, with a platelet count below 200.000/ μ L. The animals were randomly allocated into two different experimental groups: Control Group (CG, n =10) – animals which received conventional treatment with Doxycycline hydrochloride (10 mg/kg, BID); and Treatment Group (TG, n =7) – animals which received conventional therapy associated with autohemotherapy with 0,2 to 0,5 ml of autologous blood bilaterally in the acupoints BL17, GB39, SP10 and GV14, at day zero, 7, 14 e 21 (T0-T3). The treatment was accomplished in days 7, 14, 21 and 28 (T1 to T4), accounting with autohemotherapy sessions weekly, and a new complete blood count (CBC) was performed to evaluate the effect of the therapy; the collection was performed before the treatment. The data assessment did not demonstrate differences between treatments in any appraised moment. However, in CG there was an increase in erythrocytes and a hematocrit from T3. In the GT, no significant difference was observed over time for any of the parameters evaluated, but all the animals presented clinical improvement at the end of

the treatment. In these conditions, the use of autohemotherapy in acupoints did not present an incremental in the treatment of dogs' thrombocytopenia.

Key-words: Acupuncture, blood, canine, platelets.

INTRODUCTION

Thrombocytopenia is the most common acquired hemostatic disorder in dogs and cats, which mechanism involves the reduction of production, sequestration or increased consumption (TAKAHIRA and MATTOSO, 2015).

The blood parasites infections promote thrombocytopenia as by a reduction in production and by an increase in consumption (TAKAHIRA and MATTOSO, 2015). Hemoparasitosis are common in small animal medicine and can cause serious diseases, sometimes endangering the animal's life (FIGUEIREDO, 2011). These disorders can be caused by protozoa or bacteria transmitted to company animals by ectoparasites, such as fleas and ticks. Frequently, these diseases are associated with anemia, leukopenia, and thrombocytopenia (EVERMANN *et al.*, 2015).

The canine ehrlichiosis is caused by *Ehrlichia* sp., which belongs to a bacteria group known as rickettsia, and transmitted by *Rhipicephalus sanguineus* (CHAVES *et al.*, 2007). This disorder leads to immunosuppression and thrombocytopenia. Although this disease can become severe, the treatment is relatively simple and consists of the administration of antibiotics and support treatment. Doxycycline hydrochloride is the antibiotic chosen, while the support treatment includes fluid therapy, gastric and hepatic protectors, and also blood transfusions in cases of critical anemia and thrombocytopenia (SILVA, 2015). After the resolution of the acute phase of infection, apparent clinical recovery occurs and the disease becomes subclinical (TAKAHIRA and MATTOSO, 2015). The infection can remain as a subclinical form and can persist up to five years in dogs infected naturally. It may also develop the disease's chronic phase, when the host is unable to eliminate the agent, in cases of patients with immunosuppression the diseases scenarios might have an exacerbated relapse (LAPPIN, 2001).

Acupuncture is a therapeutic source of Traditional Chinese Medicine (TCM), and involves the stimuli of defined body areas called acupoints (HAYASHI and MATERA, 2005). This technique has been used with success for pain control and as an adjuvant in the treatment of various disorders (SCHOEN, 2001). Recently it was recognized as a specialty by the Brazilian Federal Council of Veterinary Medicine (CFMV, 2014). According to Drummond (2009), western medicine still does not have capable instruments to stimulate blood production, as also other hematologic system cells. However, because it concerns the individual as a whole in an attempt to establish the organism equilibrium, the TCM has an important role in the disease's treatment, which still is without adequate therapy by western medicine (ATHAYDE *et al.*, 2003).

The acupuncture points communicate with internal organs through meridian's system (SHOEN, 2008), and it can be stimulated by several techniques, for example, electroacupuncture, pharmacopuncture (medicament's injection in the points), laserpuncture, moxibustion (heat application through a moxibustion stick made of *Artemisia vulgaris*), massages, suckers and others (FOGANHOLLI *et al.*, 2007). Even though anecdotal reports about the use of autologous blood for the same purpose, studies with control groups were not found in scientific databases. One single case study has been found and used as sole treatment the acupoints Bladder 23, Bladder 60, Governador Vessel 1 and Immunity Point, in eight horses after orchiectomy. According to the authors were observed no complications resulting from surgery, and all animals showed good recovery (ESCODRO, 2012).

Autohemotherapy consists of venous blood withdrawal from a patient, followed by immediate intramuscular administration to the same individual. The technique has been described in the early twentieth century, but it still lacks proof of its safety and efficacy (LEITE *et al.*, 2008). A systematic review concluded that the technique appears to be somewhat more effective than traditional treatment to urticaria and eczema (BREWER, 2014). However, a recent study in chronic urticarial patients

showed that administration of autologous serum resulted in clinical improvement at 9 to 21 weeks, and reduction of IgE and IL24 autoantibodies (YU et al., 2019). Nonetheless controlled studies on autohemotherapy, especially in veterinary medicine, are scarce.

This study set out to evaluate the efficacy of hemoacupuncture as a complementary treatment in dogs with thrombocytopenia associated with a possible infection by ehrlichiosis compared to conventional treatment.

MATERIAL AND METHODS

This study was approved by the Animal Ethical Committee of Universidade Estadual de Maringá (CEUA/UEM – 031/2014). Seventeen animals (12 females and 5 males) with 11.3 ± 6.4 kg and 2.08 ± 0.9 years, from the Small Animals Medical Clinic's routine, of the Universidade Estadual de Maringá and also from Non-Governmental Organizations (NGOs), were used in this study. The dogs were selected based on a compatible historic diagnosis of ehrlichiosis, as in CBC (hematocrit, erythrocyte count, hemoglobin, and total leukocytes), in a manner of including only animals with platelet account inferior to $200.000/\mu\text{L}$. The platelet count was performed shortly after the collection, and although there was no counter-test with a citrate sample or serial samples, the laboratory data were countable with the clinical presentation of the patient. For inclusion, owners were informed of all procedures realized and signed an Informed Consenting Term. The presence of severe systemic diseases (acute or chronic renal disease, cardiopathies or hepatic diseases) and the necessity of blood transfusions were considered as exclusion criteria.

The animals were distributed by an aleatory form in two groups: Control Group (CG) and Treatment Group (TG). The CG animals received conventional treatment with Doxycycline from the magisterial pharmacy 10mg/kg, two times per day (BID), by oral via (OV), during 28 days (NEER *et al.*, 2002). This group included 10 animals (4 females and 6 males). The TG animals received conventional therapy as did the CG animals, associated to hemoacupuncture in the acupoints BL17, GB39, SP10, and GV14, in weekly sessions, in days zero, 7, 14 e 21 (T0, T1, T2 e T3). This group included 7 animals (1 male and 6 female). For the hemoacupuncture we used 0.2 to 0.5 mL of autologous blood in each acupoint, collected in the injection moment. The treatment with doxycycline was instituted empirically, based on the clinical history and the compatible blood count, due to the impossibility of confirmatory tests. The autohemotherapy applications on acupoints started on the same day as doxycycline treatment.

To evaluate the treatment's evolution all animals were reevaluated at day 7, 14, 21 and 28 (T1 a T4) by CBC. Blood samples were obtained weekly by jugular puncture, immediately before treatment application. The results were evaluated by the Shapiro-Wilk test, to assess the data normality. To test the difference between groups, the ANOVA Test to parametric data and the Wilcoxon to nonparametric data were used. To compare differences between times inside the group, the Tukey Test to parametric data and Friedman to nonparametric were utilized.

RESULTS AND DISCUSSION

All animals presented clinical recovery, however the statistical analyses of parameters – CBC, total plasmatic protein (TPP), platelets, erythrocytes, hemoglobin, and total leukocytes; did not present differences between the studied treatments (board 1 and 2).

Board 1: Average standard deviation of hematocrit, total plasmatic proteins, platelets and erythrocytes parameters, over time to Control and Treatment Group.

	T0	T1	T2	T3	T4
Hematocrit (%)					
CG	31,4 ± 3,0aA	36,6 ± 4,4aA	38,8 ± 6,0aA	41,1 ± 6,1aB	40,9 ± 8,1aB
TG	30,7 ± 5,6aA	34,6 ± 6,4aA	35,2 ± 3,6aA	36,7 ± 4,7aA	36,0 ± 3,5aA
TPP (g/dL)					
CG	7,6 ± 1,4aA	8,0 ± 1,3aA	7,2 ± 1,5aA	7,1 ± 1,3aA	7,8 ± 0,8aA
TG	8,3 ± 1,8aA	8,5 ± 1,7aA	8,4 ± 1,8aA	8,1 ± 1,5aA	7,9 ± 1,6aA
Platelets (10³/μl)					
CG	66,5 ± 31,4aA	179,7 ± 75,1aA	170,3 ± 120,2aA	181,0 ± 65,9aA	208,3 ± 36,8aA
TG	99,8 ± 25,4aA	141,9 ± 53,7aA	175,5 ± 67,8aA	162,6 ± 40,9aA	219,4 ± 47,5aA
Erythrocytes (10⁶/μl)					
CG	4,6 ± 0,4aA	5,3 ± 0,5aA	5,5 ± 0,8aA	5,8 ± 0,9aA	5,8 ± 1,1aA
TG	4,7 ± 0,9aA	5,1 ± 0,8aA	5,2 ± 0,5aA	5,4 ± 0,7aA	5,3 ± 0,5aA

CG: Control Group; TG: Treatment Group; TPP: total plasmatic proteins. T0: 1st blood collection/triage's moment; T1: seven days after starting treatment; T2: 14 days after beginning treatment; T3: 21 days after commencing treatment; Q4: 28 days after starting treatment and final evaluation. Different lowercase letters in the column identify differences between treatments. Different capital letters on the line identify differences between the moments.

When it compared the times about initial values (T0), in the CG observed an increase in the hematocrit and erythrocytes count in T3 and T4 ($P < 0.05$). In the hemoglobin case, the increase was only significant in the T4 ($P < 0.05$). Although, after the beginning of the treatment the platelet counts were major in all stages, there was no statistical difference between the moments for platelet count and the other parameters evaluated for TG, there were no differences to any studied parameter between the assessed times.

Clinically it has been observed that both groups presented normal values to leukocytes and TPP to the species in all times. In the CG, these values were obtained to hemoglobin from the T1, and to erythrocytes and hematocrit from the T2. In the TG, the hemoglobin values were normalized to the species from the T2, though erythrocytes and hematocrit remained below expected even after T4. Platelets presented, to both groups, values over 200.000/μl in the T4.

Board 2: Median (maximum and minimum) to hemoglobin and leukocytes, over time to Control and Treatment Group.

	T0	T1	T2	T3	T4
Hemoglobin (g/dL)					
CG	10,9 (14,7 - 10,2) aA	12,6 (16 - 10,8) aA	13,35 (16,7 - 9,1) aA	12,95 (17,8 - 10,4) aA	14,00 (17,5 - 9,9) B
TG	11,1 (14,9 - 7,3) aA	11,2 (16,3 - 9,5) aA	12,5 (14 - 10,2) aA	12,7 (14,4 - 10,1) aA	12,1 (13,7 - 10,2) aA
Leukocytes (10³cells/μL)					
CG	9,0 (18,0 - 5,4) aA	7,6 (18,3 - 5,2) aA	7,2 (17,4 - 5,0) aA	8,1 (16,7 - 5,6) aA	7,7 (17,7 - 5,1) aA
TG	11,8 (18,0 - 5,7) aA	12,3 (21,0 - 9,9) aA	13,5 (26,7 - 6,2) aA	11,4 (16,7 - 8,2) aA	13,1 (17,7 - 10,5) aA

CG: Control Group; TG: Treatment Group. T0: 1st blood collection/triage's moment; T1: seven days after starting treatment; T2: 14 days after beginning treatment; T3: 21 days after commencing treatment; Q4: 28 days after starting treatment and final evaluation. Different lowercase letters in the column identify differences between treatments. Different capital letters on the line identify differences between the moments.

According to a consensus of the American College of Veterinary Internal Medicine, the recommended treatment is doxycycline administered in doses of 10mg/kg twice a day, during 28 days (NEER et al., 2002). Even though this therapy was adopted and both groups presented normal values of platelets (over of 200.000 / μ l) by the treatment's end, a significant increase of this value happened only in the T4 to the CG, and has not been observed in the TG.

Furthermore, evaluating the leukogram results, no statistical differences were observed over time in both groups comparing total leukocyte, lymphocyte and neutrophil values. Likewise, no differences occurred between the administered treatments. Besides, these parameters were within those considered normal for the species since T0 (TRALL, 2007).

The acupoints have been chosen based on their indications, such as hemorrhagic chronic diseases, sanguine dyscrasias, and stimulus of the immunologic system (SHOEN, 2006). In consonance with Traditional Chinese Medicine, anemia is related to Xue (Blood) deficiency, which may be a consequence of Qi deficiency from Spleen, Heart, or Kidney (SHOEN, 2001). Thus, the acupoints BL17 and GB39 strengthen and maintain the blood in the sanguine vessels, and stimulate the bone marrow in the blood cells production (DRUMMOND, 2009). In contrast, the Spleen and Stomach Meridians belong to the Earth Element and have the function of toning up Qi and Xue. Therefore, the acupoint SP10 has the function of moving, cooling and invigorating Xue (Ross, 2003).

The absence of an additive effect of hemoacupuncture to the conventional treatment could be due to the inefficacy of this technique, or the acupoints choice. Besides, we must consider the interference caused by the owner, as it was a domiciliary treatment. A limiting factor of this study was the small number of evaluated animals. As it was a clinical study, with a minimum period of 28 days and with weekly assessments, many owners refused to participate. Moreover, the presence of comorbidities and the necessity of blood transfusion were exclusion criteria, what limited, even more, the number of available cases, as many times the animals were referred here with advanced stages of the disease.

In conditions of the present study, the association of hemoacupuncture to conventional treatment of animals with thrombocytopenia does not result in an incremental change in the treatment's response.

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REFERENCES

- ATHAYDE, I.B.; CHAVES, N.S.T.; LUNA, S.P.L.; VIEIRA, D.; OLIVEIRA, L.F.; ALMEIDA, R.L.; AZEVEDO, E.M.R.; FARIA, B.C.F. Avaluation of Reticulocyte Activity on Healthy Dogs After Eletroacupuncture Stimulation, In: **29th International Congress on Veterinary Acupuncture in Santos**, São Paulo, 2003. p.273-280.
- BREWER, D.D. A Systematic review of autohemotherapy as a treatment for urticaria and eczema. **Cureus** 6(12): e233. doi:10.7759/cureus.233. 2014.
- CFMV. **Acupuntura Veterinária: nova especialidade reconhecida pelo CFMV**. Disponível em: <<http://portal.cfmv.gov.br/noticia/index/id/3552/secao/6>>. Acesso em: 1 abr. 2019.
- DRUMMOND, B. L. **Acupuntura na modulação da produção sanguínea**. Belo Horizonte: Instituto Homeopático Jacqueline Peker, 2009.
- EVERMANN, J.F.; SELLON, R.K.; SYKES, J.E. Infecções por *Erlichia* e *Anaplasma*. In: GREEE, C. E. **Doenças infecciosas de cães e gatos**. Rio de Janeiro: Guanabara Koogan, 2015.
- ESCODRO, P.B.; JOAQUIM, J.G.F.; MARIZ, T.M.A.; OLIVEIRA, A.S.; ESCODRO, L.O.; SANTOS FILHO, E. N.; FERREIRA JUNIOR, J.V.T.; BERNARDO, J.O. Autohemotherapy at acupuncture points post orchiectomy surgery in cart horses- eight cases report. **Veterinária e Zootecnia**, 2012; 19(4): 502-506.
- FELDMAN, B.F.; REBAR, A.H.; MACWILLIAMS, P.S.; METZGER, F.L.; POLLOCK, R.V.H.; ROCHE, J. **Guia de hematologia para cães e gatos**. 1.ed., São Paulo: Roca, 2003. p. 133-156.
- FIGUEIREDO, M.R. 2011. **Babesiose e Erliquiose Caninas**. Monografia em especialização em Clínica Médica de Pequenos Animais, Qualittas Instituto de Pós-Graduação, Rio de Janeiro.
- FOGANHOLLI, J.N.; RODRIGUES, R.V.; PROCÓPIO, V.A.; FILADELPHO, A.L. A Utilização da Acupuntura no Tratamento de Patologias na Medicina Veterinária. **Revista Científica Eletrônica de Medicina Veterinária**, São Paulo, 2007. Disponível em: <http://faef.revista.inf.br/imagens_arquivos_arquivos_destaque/uaSZjdxJxwMMJbT_2013-5-27-15-50-36.pdf>. Acesso em: 28 de maio de 2015.
- LAPPIN, M.R. Doenças riquetsianas polissistêmicas. In: Nelson R.W. & Couto C.G. **Medicina Interna de Pequenos Animais**. 2.ed. Rio de Janeiro: Guanabara Koogan, 2001. p. 1006 – 1011.
- LEITE, D.F.; BARBOSA, P.F.T.; GARRAFA, V. Auto-hemoterapia, intervenção do estado e bioética. **Revista da Associação Médica Brasileira**, 2008; 54(2): 183-8. DOI: 10.1590/S0104-42302008000200026.
- NEER, M.T.; BREITSCHWERDT, E.B.; GREENE, R.T.; LAPPIN, M.R. Consensus Statement on Ehrlichial Disease of Small Animals from the Infectious Disease Study Group of the ACVIM. **Journal of Veterinary Internal Medicine**, p. 309–315, 2002. DOI: 10.1892/0891-6640(2002)016<0309:csoded>2.3.co;2
- ROSS, J. **Combinações de pontos de acupuntura: a chave do êxito clínico**. São Paulo: Roca, 2003.
- SILVA, I.P.M. Erlichiose Canina – Revisão de Literatura. **Revista Científica de Medicina Veterinária**, São Paulo, 2015.
- SCHOEN, A. M. **Veterinary acupuncture : ancient art to modern medicine**. 2nd. ed. [s.l.] Mosby, 2001.
- TAKAHIRA, R. K.; MATTOSO, C. R. Defeitos hemostáticos primários. In: JERICÓ, M. M.; ANDRADE NETO, J. P. .; KOGIKA, M. M. (Eds.). **Tratado de medicina interna de cães e gatos**. 1ed. ed. Rio de Janeiro: Roca, 2015. p. 1895–1902.
- THRALL, M. **Hematologia e Bioquímica Clínica Veterinária**. 1.ed. Roca: São Paulo, 2007. p. 181.
- YU, L.; BUTTGEREIT, T.; SKOV, S.P.; SCHMETZER, O.; SCHEFFEL, J.; KOCATURK, E.; ZAWAR, V.; MAGERL, M.; MAURER, M. Immunological effects and potential mechanisms of action of autologous serum therapy in chronic spontaneous urticaria. **Journal of the European Academy**

of Dermatology and Venereology. DOI:
10.1111/jdv.15640. 2019