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**THE USE OF OZONATED MAJOR AUTOHEMOTHERAPY IN CANINE EHRLICHIOSIS'  
TREATMENT: CASE REPORT**

**EL USO DE LA AUTOHEMOTERAPIA CON OZONO COMO TRATAMIENTO DE LA  
EHRLICHIOSIS CANINA: INFORME DE UN CASO.**

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## THE USE OF OZONATED MAJOR AUTOHEMOTHERAPY IN CANINE EHRLICHIOSIS' TREATMENT: CASE REPORT

### RESUMEN

Ehrlichiosis monocítica canina es una enfermedad transmitida por garrapatas y causada por la *Ehrlichia obliqua* spp parásito intracelular. Las especies que infectan a los perros en general, incluyen *E. canis*, *E. equi*, *E. risticii*, *E. platys* y *E. ewingi*. Es una patología en la que la relación coste-efectividad del tratamiento es alta y la presencia del cuadro clínico grave, a menudo conduce a la muerte del animal. A través de una investigación llevada a cabo en muestras de sangre de la punta de la oreja de un perro de dos años, sin raza conocida y de sexo femenino, se obtuvo el diagnóstico positivo para *Ehrlichia* sp. El protocolo de tratamiento fue consistió en diez sesiones de autohemoterapia con ozono, distribuidas en dos aplicaciones cada semana. Se extrajeron 80 mL de sangre por la vena yugular y se colectó en una bolsa estéril para sangre que contenía 13 mL de citrato de sodio 3,8%, (380/mg). El ozono fue añadido a la bolsa que contiene la sangre en una proporción de 1 : 1. Después de la ozonización y la homogenización constante, la sangre se volvió a introducir en el animal por la vena yugular. En este estudio se evaluó los parámetros hematológicos de línea blanca y roja, la presencia o ausencia de mórulas en sangre periférica y se analizó la orina. Todos los procedimientos fueron realizados antes y después de cada período de sesiones principales autohemoterapia ozonizada. Se concluye que la autohemoterapia con ozono fue eficaz en la reversión de varios parámetros hematológicos y en la cura de la enfermedad renal.

**Palabras clave:** Perro; Autohemoterapia; Ehrlichiosis; Ozono

## ABSTRACT

Canine Monocytic Ehrlichiosis is a disease transmitted by ticks and caused by the obligate intracellular parasite Ehrlichia spp. The species that generally infect the dogs include *E. canis*, *E. equi*, *E. risticii*, *E. platys* and *E. ewingii*. It's a pathology in which the cost-effectiveness of the treatment is high and the presence of severe clinical picture, often leads the animal to death. Through a research conducted in blood samples collected from the tip of the ear of a two years old mongrel female dog, a positive diagnosis for Ehrlichia sp. was obtained. The treatment protocol consisted in ten major autohemotherapy ozonated sessions, divided into two sessions per week. The collection of 80 mL of blood was obtained from the jugular vein, collected in a sterile blood bag containing 13 mL of sodium citrate 3.8%, (380/mg). The ozone was added to the bag containing blood in a ratio of 1:1. After the ozonation and constant homogenization, the blood was reintroduced in the animal by the jugular vein. This study evaluated the hematological parameters of white and red line, presence or absence of morulae in peripheral blood and urinalysis. All procedures were performed in the beginning and after each major autohemotherapy ozonated session. The research has concluded that major autohemotherapy ozonated was effective in reversing several hematological parameters and in the cure of kidney damage.

**Keywords:** Canine; Autohemotherapy; Ehrlichiosis; Ozone

## INTRODUCTION

The ehrlichiosis is caused by a group of microorganisms gramnegativos, obligate intracellular and pleomorphic, that parasitize white circulating cell of various species of domestic and wild animals, including man.<sup>1</sup>

In Belo Horizonte,<sup>2</sup> reported the first once the disease in Brazil. It was later reported involving approximately 20% of dogs treated at hospitals and veterinary clinics in the Northeast, Southeast, South and Midwest States.<sup>3,4</sup>

The cyclical nature of parasitemia and thrombocytopenia decreases with time, it becomes softer, and the thrombocytopenia resolved slowly over association with sporadic occurrences of organisms in the blood.<sup>5</sup>

In some cases, a transient decrease in Total leukocyte counts occurred concomitantly with parasitemia, but usually the values not fall below the reference range for dogs. A mild anemia normocytic normochromic may occur during the first month of infection.<sup>5</sup>

Treatment consists primarily of antibiotics and treatment of support. The cost and possible side effects produced by antibiotics stimulate research that will culminate with the development of new techniques and agents that can minimize these costs and undesirable effects.

According to<sup>6</sup> the specific indications for topical use ozone are skin infections by: viruses as Herpes simplex and zoster, infections bacterial infections such as impetigo, ecthyma contagiosum *B-hemolytic streptococcus* and *Staphylococcus aureus*; fungal infections including tinea by *Trichophyton spp*, Candidiasis and tinea versicolor; protozoan infections especially Leishmaniasis, a parasitic infections including scabies *Sarcoptes scab*, Pediculosis and cutaneous larva migrans *Ancylostoma brasiliensis*; conditions multifactorial skin as acne, psoriasis, rashes, pemphigus and dermatitis herpetiformis, and inflammatory skin conditions such as dermatitis, eczema and hives.

Ozone can be used in the veterinary clinic for treatment of following situations: conchectomy (prewash with ozone and application of ozonized oil in the surgical wound); return of anesthesia in 30 seconds (20 mL ozone concentration in 20 µg/mL via rectal insufflation), trauma (oil ozonized for topical use and subsequent washing with ozonated water); disorders not parasitic gastrointestinal (ingestion of 20 to 50 ml of ozonated water on 1<sup>st</sup> day, 2 to 3

mL/day of ozonized oil) in otitis media (oil application ozonized followed by small autohemotherapy done with 2 to 3 mL ozonized blood mixed with 5 ml of ozone concentration 20 µg/mL for 3 to 4 days). It is observed reduction of pain: how adjuvant therapy in laminitis (20. 000 g of ozone in 1 000 mL of blood before anesthesia and after surgery, repeat 5 to 6 times), in postpartum paralysis in cattle (30 mg ozone/1.500 mL of blood) in the postpartum metritis (500 mL of ozonated water followed by application site for 2 days 1 to 2 mL of ozonized oil).<sup>7</sup>

The aim of this study was to evaluate the efficiency of autohemotherapy with ozone in the treatment of canine Ehrlichiosis.

## MATERIALS AND METHODS

A female dog about two years old, without a set breed, with positive diagnosed for *Ehrlichia* sp. obtained by viewing of morulae in an optical microscope from the tip of the blood of the animal's ear, Underwent a higher autohemotherapy ozonated. In each session of therapy were used 80 mL of blood from the jugular vein, collected in sterile bag of transfusion blood containing 13 mL of sodium citrate to 3.8% (380mg/ml.) The volume of blood (80 mL) was calculated based on the weight of animal (assuming that the total volume represents 8 % of body weight), and corresponded to 5% of the total volume of blood is seen that the animal had 20 kg body weight. The oxygen-ozone mixture (OOM) was added to the bag containing blood at a ratio of 1:1. MOO was produced by a generator with a production capacity of 0.00023 g/min, powered by ampoule of oxygen with 99.5% purity at a pressure of about 250 kgf/cm<sup>2</sup> with a flow of 3 L/min. After ozonation and constant homogenization, the blood was returned to the animal through the jugular or radial vein. This process was performed two or three times per week. Before the sessions of autohemotherapy, blood samples were collected to perform complete cells count in blood and measurement of alanine aminotransferase (ALT). It was also gathered samples of urine, via urethral probe, to perform urinalysis and a drop of blood via venipuncture with a needle at the tip of ear of the animal, in order to search for blood parasites.

## RESULTS AND DISCUSSION

Table 1 - Series red blood cell counts before and after treatment with higher autohemotherapy ozonated.

Red Serie	Before	After	Normal Values
Erythrocytes x 10 <sup>6</sup> / mm <sup>3</sup>	5.0	7.51	5.5 a 8.5
Hemoglobin (g%)	12.1	15.8	12 a 18
VG (%)	38.0	49.2	37 a 55
VGM (µm <sup>3</sup> )	69	66	60 a 77
HGM (pg)	22,0	21.0	19 a 23
CAGH (%)	31.8	32.1	31 a 34
RDW (%)	14.9	13.7	14 a 17

Table 2 - Values of white blood cell counts before and after treatment with higher autohemotherapy ozonated.

White Serie	Before	After	Normal Values
Leukocytes total / mm <sup>3</sup>	5 900	11 000	6 000 a 18 000
Young Neutrophils / mm <sup>3</sup>	118	770	0 a 540
Segmented Neutrophils / mm <sup>3</sup>	3 717	4 730	3 600 a 13 860
Neutrophils total / mm <sup>3</sup>	3 835	5 500	3 600 a 14 400
Eosinóphils / mm <sup>3</sup>	59	2 200	120 a 1 800
Monocytes / mm <sup>3</sup>	177	550	180 a 1 800
Lymphocytes / mm <sup>3</sup>	1 829	2 750	720 a 5 400

As shown in Table 2, the number of monocytes before the treatment was slightly down and, observation in cases of ehrlichiosis, which causes monocytosis. There was a significant increase after the beginning of ozone therapy, which can be attributed to the treatment and remained within the reference values cited.<sup>8</sup>

The eosinopenia observed before treatment is a leukocyte change attributed to the action of glucocorticoids and catecholamines released by the adrenal gland in the stress period of the acute infection. Although the mechanism responsible for eosinopenia is not yet well understood, has been proposal to justify its occurrence intravascular lysis of eosinophils, kidnapping reversible monocytic phagocytic system organs and severe migration to the tissues.<sup>9</sup>

After the eighth, ninth and tenth sessions of treatment the numbers of young neutrophils were found above the reference values. This may be assigned to the case of phlebitis of the jugular vein suffered by animals given the successive punctures, of the same, in a short space of time, which became necessary in the process of ozone therapy.

The low value of monocytes found after the sixth session of ozone therapy has not clinical relevance.

Table 3 - Values of platelet count before and after higher autohemotherapy ozonated.

Platelet	Before	After	Normal Values
Platelet Count /mm <sup>3</sup>	26.000	198.000	200.00 a 500.00
VPM (µm <sup>3</sup> )	7.8	7.7	6.7 a 11.1

Note: mean platelet volume (MPV).

As shown in Table 3, there was a significant increase in platelet count. According to, the infectious cause more common of thrombocytopenia in dogs is ehrlichiosis.<sup>10</sup> Thrombocytopenia is commonly described in dogs with ehrlichiosis being important for diagnosis of the disease. It is a consistent finding in the acute stage of the disease.<sup>10</sup>

Some mechanisms are proposed to explain this change. Besides the aplasia of the bone marrow and/or deletion of its erythropoietic activities, leucopoietics and trombopoietics, can be attributed to decreased half-life circulating platelets during the acute phase of infection, reduced aggregation platelet due to antiplatelet antibodies, direct effect of the ehrlichia on circulating platelets or due to endothelial damage and platelet aggregation.<sup>9</sup> The bactericidal action and modulator of the immune system of ozone<sup>7</sup> may explain the return of platelets to physiological levels.

Evaluating the Table 4 can realize on the research results of hemoparasites after starting the treatment. Before the treatment were found morulae of *Ehrlichia sp.* in monocytes and platelets. After start of ozone therapy, all results were negative for the above finding. These negative results may be attributed both to the bactericidal action of ozone on Ehrlichia about the fact that the presence of morulae occurs usually only the first two weeks post-infection and less than 1% of infected cells, so the absence of morulae not exclude the possibility of infection.<sup>11</sup>

Table 4 - Presence or absence of haemoparasite in morulae before and after treatment with higher autohemoterapia ozonated in a dog.

Variable studied	Before	After									
		1 <sup>a</sup>	2 <sup>a</sup>	3 <sup>a</sup>	4 <sup>a</sup>	5 <sup>a</sup>	6 <sup>a</sup>	7 <sup>a</sup>	8 <sup>a</sup>	9 <sup>a</sup>	10 <sup>a</sup>
Morulae	+	-	-	-	-	-	-	-	-	-	-

According to, renal proteinuria may be of physiological origin and transitory character or for kidney damage, constant and characterized by the appearance of cylinders in the sediment.<sup>12</sup> Table 5 shows that the animal suffered from a kidney injury that was reversed after the second session treatment and could be confirmed by the presence of proteinuria combined with presence of granular cylinders.

Table 5 - Results of urinalysis before and after treatment of higher autohemotherapy ozonated.

Element Abnormal	Before	After	Normal Values
Albumin	P <sup>++</sup>	-	-
Acetone	-	-	-
Glucose	-	-	-
Hemoglobin	-	-	-
Bile Salts	-	-	-
Bile Pigments	P <sup>++</sup>	P <sup>+++</sup>	-
Urobilinogen	-	-	-
Cylinders	granular +	-	-

The presence of bile pigments shown in Table 5 is a nonspecific finding. Normally, the presence of bile pigments in urine is indicative of liver damage, but this could not be confirmed, because the levels of ALT and alkaline phosphatase remained within normal limits.

## CONCLUSION

It can be concluded that the higher autohemotherapy ozonated was effective in reversal of clinical and laboratory findings of Ehrlichiosis in a dog.

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