Hematological profile of ovines and goatlikes with caseous lymphadenitis treated with ozone.

Perfil hematológico de ovinos y caprinos con linfadenitis caseosa tratados con ozono.

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RESUMEN

La linfadenite caseosa es una enfermedad bacteriana, supurativa que tiene como agente etiológico Corynebacterium pseudotuberculosis, afecta ovinos y caprinos. Dos formas: cutánea externa causando linfadenopatia uni o bilateral, y la otra es la visceral interna con abscesos en los linfonodulos internos, órganos y vísceras. Se utilizaron ocho ovejas y uno reproductor de la raza Santa Inês y una cabra mestiza, todos adultos. Para la realización del hemograma se procedió con el sistema de colección al vacio a través de la punción veno-jugular, se utilizaron tubos estériles conteniendo el anticoagulante ácido etilenodiaminatetracético (EDTA), se obtuvo un volumen total de 2 mL por animal. El conteo diferencial de leucócitos fue realizado en un frotís de sangre con tinción de May-Grünwal-Giemsa. Estos análisis fueron realizados antes y después del tratamiento sistémico (insuflación rectal), local (inyecciones subcutáneas con ozono) y tópico (aguas y aceite ozonizados) aplicado alrededor de los nódulos y/o abscesos. La evaluación del leucograma antes del tratamiento muestra los parámetros dentro de lo normal, hubo la presencia del desvío nuclear de los neutrófilos a la izquierda (DNNE), lo que indica una respuesta inflamatoria. Después del tratamiento, los parámetros leucocitarios continuaron dentro de los valores de referencia, aunque hubo una reducción en el número de leucócitos y neutrófilos totales y el DNNE fue corregido. En general, se puede considerar que hubo una mejora en los índices de las células de la linaje blanca y, de esta forma se puede sugerir que el tratamiento con la mezcla oxígeno-ozono no afectó desfavorablemente los parámetros hematológicos de los animales tratados.

Palabras-clave: Linfadenitis caseosa; Ozono; Hematológico; Ovinos
ABSTRACT

The caseous lymphadenitis is a bacterial disease, suppurative whose etiologic agent *Corynebacterium pseudotuberculosis*, affects ovines and goatlikes. Is presented in two ways: cutaneous external lymphadenopathy causing unilateral or bilateral, and one way is with visceral internal abscesses in internal lymph nodes organs and viscera. Were used eight sheep, one breeder of Santa Ines race, and a mixed goat, all adults. For realization of the hemogram were used a vacuum collection system through jugular venous puncture, were used steriles tubes containing the acid anticoagulant ethylenediaminetetra-acetic (EDTA), obtaining a total volume of 2 mL per animal. The differential counting of leukocytes were accomplishment in bleeding extensions blushing by May-Grünwal-Giemsa. These proceedings were realized before and after the systemic treatment (rectal insufflation), local (subcutaneous injections) and topic (water and ozonized oil) applied around the nodes and/or abscesses. The valuation of WBC before the beginning of the treatment reveals parameters inside of the normality, there was the presence of the nuclear deflection of the neutrophils to the left (DNNE), indicative of one inflammatory answer. After the treatment, the leucocytes parameters persist in the reference values. There was a reduction in the number of total leukocytes and neutrophils and the DNNE was corrected. In general, it can be considered a significant improvement in rates of cells of the white line and thus infer that the treatment with the oxygen-ozone mixture did not affect adversely the hematological parameters of the treated animals.

Keywords: Caseous lymphadenitis; Ozone; Hematological; Ovines

INTRODUCTION

Caseous lymphadenitis is a suppurative disease that happens in sheep and goats, characterized by the appearance of subcutaneous nodules that abscesses and, and make limited the animals entries that are contaminated, in addition the animal get discomfort, pain, and make the production get down. The usual treatment is visual diagnosis, drainage; take out the material infected, chemistry cauterization of injuries, and application of antibiotics. The skin incision of animals is painful and exposes the lesion and the environment to contamination. This disease can be prejudicial to the production oh sheep and goats, it’s because pathology aspect of this illness. At first, surface lymph nodes with abscesses can get worse some normally functions of the animal like graze, chew, regurgitate and suckle. Surface abscesses in animals are responsible by devaluation of skin. The visceral form of the disease can make consequences serious to the body, we can quote debilitation of the animal and spread of the abscesses and this can take to a reproductive problem. Make the carcasses destroyed, and in some cases the death of animals. And in animals that produce wool, can make a decrease in the production. The disease shows a long time of incubation, this make hard separate the healthy and the unhealthy animals.

The bacteria’s fatty cellular have pyogenic characteristics and this factor of virulence is associated to formation of granulomas. Phospholipase D is responsible to suppurations, dermonecrose, and the death of many species, of laboratory animals or pets. Beyond that, it’s can be like a factor of permeability, making the dissemination of the pathogenic agent of the infect place to others parts of the animal’s body. Through the lymph way, damage can happen with the lymph nodes of thorax, lungs and after of other organs, it’s can make the increase debilitation of the animal. The lymph
nodes more damaged are submaxillary, pre-scapular, pre-crural, supramammary and popliteal.
The dissemination hematogenous of the microorganism results in abscess in organs, and no
presence of peripheral injuries.\textsuperscript{9}
To control LC is basically by visual diagnostic and incision of seed, which have a lot of
secretion quantity and the agent \textit{Corynebacterium pseudotuberculosis}.
In this work was utilized Ozone therapy, which is a method that uses ozone as therapeutic
agent to many diseases. It’s a natural therapy, with a few contraindications and secondary
effects. It was utilized the rectal insufflation that was introduced by Payr, in 1935, and after
was studied by Aubourg, 1940 and Knock e Klug 1990 and, it’s insufflate slowly in the colon
and recto an oxieng-ozone mixture and this solubilizes up in the water fecal or in the tract
mucoproteins in the mucosa. The reaction is fast because of glycoproteins and
mucoproteins of glycocalyx are goods antioxidants. A lot of part of dosage will be neutralized
but will have the production of $\text{H}_2\text{O}_2$ and products of fatty oxidation (LOPs) which are
absorbed by the mucosa, which is much vascularized, and it doesn’t react with erythrocyte or
the nervous termination, without pain neither reflex contractions. The conclusion of the
author is: the ozone is very reactive and it decompose in the lumen intestinal and the oxygen
due to is absorbed by cells, in the interstitial liquid and in the capillaries
venous and lymph, and it’s can cause a biochemistry effects, and the immune system give a
anti-inflammatory and a bactericidal answer.
Explain that ozone is produced by broking an oxygen molecule, which can join to other
molecule of oxygen, like the reaction:\textsuperscript{10}
\[
\text{O}_2 \rightarrow 2(\text{O}) + 2 \text{O}_2 \rightarrow 2 \text{O}_3
\]
This broke can happens after the passage of oxygen in high voltage electrical discharges or
high or low frequencies at high power radiation, that decompose faster in the air, and like
that produce bactericidal agents.
The ozone attacks first the membrane bacterial by glycolipids or amino acids such as
tryptophan and acts too in the sulfhydryl groups of certain enzymes, resulting in disruption
of normal cellular enzyme activity. The bacterial death is fast and almost always because of the
changes in permeability cell, and after the lyses. The lyses isn’t a primary mechanism of
inactivation, is a high concentrate of oxidant agent. Ozone also promotes action on the
nuclear material of bacteria, by modifying the purine and pyrimidine bases of nucleic acids.
Studies completed in 1980 realize that this gas was effective in destroying bacteria and
viruses at pH 6.0 to 8.5, while other data indicate that ozone destroys bacteria and viruses at
pH 5.6 to 9.8.\textsuperscript{11} Ozone stimulates the endogenous antioxidant systems, being a potent
vasodilator, helping the dissociation curve of hemoglobin with oxygen, which benefits tissue
oxygenation, it also stimulates the release of mediators of immunity such as interferons and
cytokines. This gas can be used in different ways: topically (ozonated water or ozonized oil),
injectable (subcutaneous, intramuscular or intra-articular), mayor and minor
autohemotherapy. The choice of treatment varies according to the local or affected systems,
thus the route of application will fit the case studies.

\textbf{MATERIALS AND METHODS}

The present search was realized at the Veterinary Hospital and at the Experimental Farm of
the Capim Branco, both properties belongs to the Federal University of Uberlândia – Minas
Gerais – Brazil.
Were used eight sheeps and one breeder of Santa Inês race and one mixed sheep, all
adults. The visual clinical diagnosis was the method used to corroborate this pathology,
since these animals were selected to be subjected to the treatment with oxygen-ozone
mixture.
For the ozone production, was used an ozone generator Ozone & Life O&L3.0RM®,
adjusted to the concentration of 60 mg-O³/L, powered by an oxygen ampulle with 99.5%
purity, with pressure of about 250 kg/cm² in a flow of 1.0 L/min. Five liters of distilled
deionizer water, previously chilled, were ozonized for 20 min. and put in a cold box
containing ice packs, to be used immediately.
For the production of the ozonized oil were used 200 mL of sunflower oil, purchased in local
shops and subjected to the same protocol used for water ozonation, but the ozonation time
was restricted to five minutes and set to the concentration of 2.5 mg-O³/L. The ozonized oil
was packaged in a cold box containing recycled ice packs and transported as quickly as
possible to the search location.
In the local of the search, the ozonized water was poured in the lymph nodes that were
suppurated, being done the topic treatment this way. Then the ozonized oil was applied with
the help of gauze pads over the suppurated abscess, this procedure was performed once a
day, every day, until clinical cure of the animal.
The subcutaneous injections of small doses of the oxygen-ozone mixture were applied
around the nodules or lesions with a syringe of 20 mL, siliconized and disposable, charged
by coupling the hose silicone of the ozone generator set to the concentration of 20 mg-O³/L.
For the rectal insufflations of the oxygen-ozone mixture was used four syringes with the
capacity of 20 mL each, which are attached by silicone hose to an ozone generator of the
brand Ozone & Life O&L3.0RM®, powered by an oxygen ampulle with 99.5% purity, with a
pressure of about 250 kg/cm² in a flow of 1.0 L/min, adjusted to a concentration of 32
mg-O³/L, the oxygen-ozone mixture (OOM) was rectal administered with the help of a probe
in the animals that needed to be treated. This procedure was performed after the topic
application of the ozonized water and oil and subcutaneous injections also realized once a
day, every day, until the clinical cure of the animals.
The blood collection was performed before the start of the treatment (1st day) and the end of
the treatment (56th day), there was jugular venous-puncture in collection tubes, vacuum, for
hematological containing anticoagulant ethylenediaminetetra-acetic acid (EDTA), resulting
in a total volume of 2 mL of blood per animal. The tubes with EDTA were identified before the
collection with the number of animals and their species, then the samples were sent to the
Laboratory of Clinical Pathology, Faculty of Veterinary Medicine, Federal University of
Uberlândia, for hematologic analysis. The making of the blade to the differential count of
leukocytes was performed at the moment of blood collection, bringing the same to the
laboratory to be stained by May-Giemsa-Grünewal.
The globular volume, hemoglobin, red blood cells, leukocyte, platelet, and absolute RBC
indices, average corpuscular volume, average corpuscular hemoglobin and average
concentration of corpuscular hemoglobin were obtained by electronic method using the
apparatus ABC Vet 16 p.

RESULTS AND DISCUSSION
In this work was evaluated the hematological parameters before and after the mixture
oxygen-ozone treatment. The animals, being eight sheeps and one breeder of Santa Inês
race and one mixed sheep, all adults, showed before the beginning of the treatment
parameters of the red series inside of the normal values.
About the white line, are also inside the references values, but with the end of the treatment
was observed that happened a reduction of the leukocytes, the nuclear deflection of the left
neutrophils (DNNE) disappeared. It was also observed a reduction of the total neutrophils. Signaling in this way the action of ozone in relation of the antibacterial activity, so, factors like: appropriate dose, route of application, treatment duration, which might be evaluated and determined according to pathology results in one correct dose allowing that the OOM be one inducer almost ideal of Interferon-gamma, Tumor Necrosis Factor-alpha, Interleukin-2 (IL-2) and Interleukin-6 (IL-6) and produce minimum cellular damage. Is important to stand out that the WBC changes in the ruminants are often subtle and not so drastic like in the carnivorous species or in the equines, so the few changes observed in the CBC of the animals in question, can be explained, as this is a ruminant specie that sets the framework reported above, the own characteristic of the disease can be also mentioned, as this is a encapsulated process, in which there is agent isolation.

CONCLUSIONS

In general, the systemic and topic use of the OOM in the concentrations mentioned above was satisfactory. It was observe also an improvement in the hematological parameters, in the case of the white line.

BIBLIOGRAPHY REFERENCES