

## ANTIBACTERIAL EFFECT OF TOPIC OZONE IN HORSES WITH DISTAL LIMB INFECTED WOUNDS

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**Abstract:** The living way of horses and the environmental factors get these animals in the situation of injuring themselves, especially at the level of their limbs. This usually produces opened traumatic wounds. Some species particularities like lack of elasticity of the skin and low protection at this level, temperamental features, breeding conditions and the category of work they are into, are contributing to a high incidence of wounds. More than this because of contamination, infection and then chronic phase of these wounds, ischemic processes and hypoxia install and they increase the amount of time needed for epithelization.

The biological material of this study consisted in 5 horses of different breed, age, weight and sex. Our aim was to follow the time needed for complete healing and evolution of microbiological activity at the surface of the wounds by taking samples before and after treatment for bacteriological tests. The subjects were treated topically by the ozone bag method at a 50µg/ml dose for 30 minutes every treatment session. No other associated therapies were performed. We obtained a reduction of the wound surface up to 3,3 cm<sup>2</sup>/ day and a reduction of the bacterial population up to 93,81 %. This result confirms us that topic ozone treatment by ozone bag technique has efficient bactericidal properties in the treatment of atonic infected lower limb wounds in horses.

**Keywords:** ozone therapy, microbiology, atonic wounds, Staphylococcus, Streptococcus

### INTRODUCTION

Ozone therapy is the generic name for a category of techniques that use a mixture of oxygen-ozone, which alone or by vectors (water, NaCl, blood, oils, gels, etc.) has healing properties like antimicrobial effects, strengthening the cellular activity and regeneration of damaged tissues (Jani P. et. al., 2012). The advantages of ozone therapy relate to an increased of disponibility and the transfer of oxygen, glucose, ATP in tissue ischemia situations and stimulation of stem cell mobilisation around the site of the lesion, a fact that will lead to angiogenesis, neovascularization and tissue regeneration (Bocci V.A., 2006).

Epithelizing of wounds after ozone exposure is associated with the activation of NF-K B transcription factor which regulates the inflammatory response and eventually the whole healing process of wounds (Bocci V. A., 2005).Lately there has been a considerable interest in treating pathology by alternative means. Used in many purposes during the last 150 years in human medicine. Ozone therapy begins to get the

curiosity of veterinarians as well because it is a less expensive treatment without adverse reactions which can be used by itself or combined with classical therapy.

### MATERIAL AND METHOD

Biological material was represented by 5 equine subjects of different ages (6 months to 13 years), breeds and weight, which were referred to our equine clinic presenting wounds on the lower anterior and posterior limbs. All subjects had similar symptoms: lameness and presence of the wound. A general examination was performed and their status was monitored until the end of therapy. Ozone exposure was made with the ozone bag technique every 3 days, each session lasted for 30 minutes. All subjects were hospitalised until the end of treatment.

Nonbiological materials used for the therapy were: HERRMANN Apparatebau GmbH Medozon Compact normobaric ozone generator, 10 ml syringes, perfusion PVC sets, PVC bags for ozone therapy, syringe filters 4µm porosity, Amies medium tubes, Petri plates, normal agarus and blood bacteriological medium.

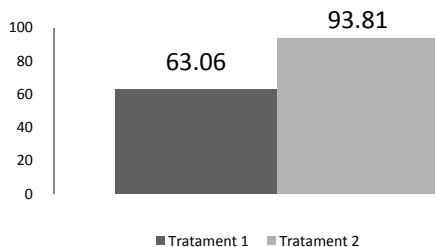
Each subject was restraint into a well ventilated room where the wound was examined before every treatment. Microbiological samples were taken with Amies medium tubes before and after two treatment sessions. Samples were cultivated for 48 hours and bacterial activity was appreciated by counting colonies of all dimensions.

The perilesional area was washed with saline before treatment. A PVC bag was put around the limb and fixed with a tourniquet on the upper and lower ends. A perfusion PVC tube was connected between the upper opening of the bag and the generator. With the generators vacuum option, all air was extracted from the bag and then replaced with ozone at a 50 µg/ml concentration for 30 minutes. Once the treatment ended residual ozone was removed by the vacuum option to avoid atmospheric spreading. During ozone exposure tissues exposed at the level of the lesion absorb the maximum quantity of gas fact observed due to the colour change of granulation tissue from pink to red.

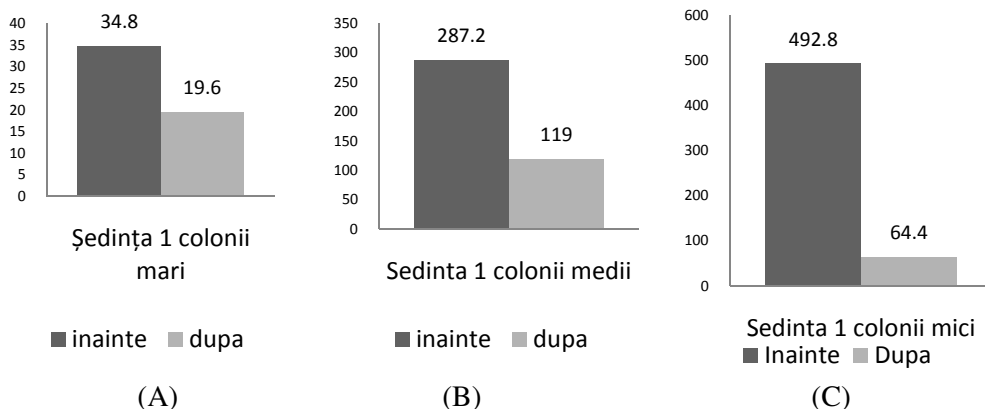
### RESULTS AND DISCUSSIONS

A complete recovery was obtained in 100% of the subjects functionally and aesthetically. The bacterial population consisted in big, medium and small colonies of Staphylococcus and Streptococcus. The after treatment samples showed a bactericidal effect of ozone between 63,06 after the first ozone therapy session and 93,81% after the second session which showed to be statistically relevant with  $p=0,04$  (Fig.1).

After the first treatment session large colonies reduced from  $34.8 \pm 3.7$  to  $19.6 \pm 2.05$  with relevant statistics  $p=0.0008$ , medium colonies reduced from  $287.2 \pm 26$  to  $119 \pm 54.07$  with relevant statistics  $p$  values ( $p=0.01$ ) and small colonies diminished from  $492.8 \pm 20.35$  to  $64.4 \pm 48.84$  with relevant statistics  $p=0.0001$  (Fig. 2).



**Fig. 1. Average percentage of bactericidal effect of ozone after first and second treatment sessions**



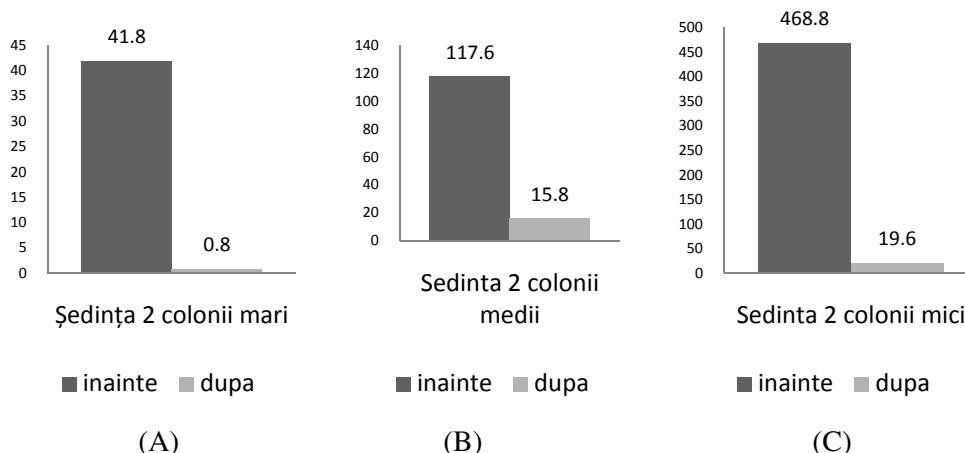
**Fig. 2. Reduction of CFUs after first ozone treatment session; A – Large colonies; B - Medium colonies; C – Small colonies**

After the second ozone therapy session bacteriological exam revealed that the number of large colonies decreased from  $41.8 \pm 14.64$  before treatment (T0), to  $0.4 \pm 0.8$  after treatment (T1) with statistical relevance ( $p=0, 005$ ), also medium colonies decreased from  $117,6 \pm 30.34$  in T0, to  $15.8 \pm 3.7$  in T1, (with relevant statistics values of  $p=0, 003$ ) and small colonies decreased from  $468,8 \pm 56,03$  in T0, la  $19,6 \pm 15,16$  in T1, (with relevant statistics values of  $p=0, 0001$ ), (Figure 3).

The time needed for complete epithelization was between 20 and 49 days depending on the dimensions of the wound. The percentage of reduction of surface bacterial population was between 63,03% and 93,81% after the second session of treatment. The therapy represented a big benefit to the organism by preventing the excessive development of bacteria and stimulating the regenerative process.

Our results can be compared with the ones shown in one study where higher doses of ozone were used to inactivate various bacterial and viral populations (Burlison, G. R., et. al., 1975). The bactericidal effect of ozone might be explained due to chemical and functional alterations that occur at the level of superficial protective layers of exposed bacteria (Doroszkewiaz, W., et. al., 1994). Senol Gulmen et. al. used ozone as a secondary treatment together with vancomycin in septic mediastinitis

caused by staphylococcus aureus and indicated an antimicrobial activity of this gas (Senol Gulmen et. al., 2013).



**Fig.3. Reduction of CFUs after second ozone treatment session;**  
**A – Large colonies; B - Medium colonies; C – Small colonies**

Tanja Boch et. al. appreciate a reduction of endodontic pathogenic germs like *E. Faecalis* after treatment with ozone at a 32µg/ml (Boch, T., et. al., 2016). Luongo C. showed a direct correlation between the volume/frequency of treatments and the bacteriostatic/bactericidal properties of this gas. The bigger the bacterial population (infection), the higher the dose and longer the ozone exposure has to be (Luongo, C., et. al., 2002). In another study high dose ozone exposure (higher values than the therapeutic ones) was performed over contaminated tooth brushes and a good bactericidal effect was observed as well (Bezirtzoglou, E., et. al., 2008). The effect of ozonated saline with a constant concentration of 20µg/ml was tested on bacterial cultures showing the good bactericidal effect (Huth, K. C., et. al., 2011). In our study an exposure of the wounds to a 50µg/ml dose for 30 minutes was enough for a satisfactory bactericidal effect.

### CONCLUSIONS

According to our study, we can assess that topical ozone therapy by the plastic bag technique at a correct dose has a bactericidal effect of up to 93,81% in treating infected lower limb wounds in horses. Also, we observed that there is an increase of bactericidal efficacy if more treatment sessions are performed.

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