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ANTIMICROBIAL ACTIVITY OF OZONE, LOW POWER LASER AND LASER-OZONE ASSOCIATION AGAINST CULTURES OF ORAL BACTERIA FROM CAPTIVE BIG CATS

ATIVIDADE ANTIMICROBIANA DO OZÔNIO, LASER DE BAIXA POTÊNCIA E ASSOCIAÇÃO LASER-OZÔNIO CONTRA CULTURAS DE BACTÉRIAS ORAIS DE GRANDES FELINOS EM CATIVEIRO

ACTIVIDAD ANTIMICROBIANA DEL OZONO, LÁSER DE BAJA POTENCIA Y ASOCIACIÓN LÁSER-OZONO CONTRA CULTIVOS DE BACTERIAS ORALES DE GRANDES FELINOS EN CAUTIVERIO

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ABSTRACT
The goal of the present study was to characterize the antimicrobial and antibiofilm potential of laser therapy, ozone therapy (gas, water and oil presentations), and photo-ozone therapy against bacteria isolated from the oral cavity of captive felines: Puma concolor (n=4), Panthera onca (n=1), and Panthera leo (n=1). The minimum inhibitory and bactericidal concentrations (MIC and CMB, respectively) of the treatments were determined against the planktonic form of the following pathogens: Enterococcus faecalis, Leifsonia aquatica, Oerskovia sp., Serratia marcescens, Corynebacterium sp., Providencia pustigianii and P. rustigianii. The antibiofilm action of a treatment was evaluated whenever the respective therapy was effective in inactivating the planktonic form of the pathogens. Water ozonation (bubbling 500 mL of solvent with 70 µg O3 mL-1 and 125 mL min-1 for 7 minutes) was performed immediately before treatments. The ozonated sunflower oil had a peroxide value >600 mmol-equiv. kg-1. Therapies with O3 gas (70 µg O3 mL-1 and 125 mL min-1) and low-intensity laser (660 nm and 80 J cm2) were performed for 120 and 160 seconds, respectively. Photo-ozone therapy used low-intensity laser and O3 gas sequentially. Regardless of the pathogen, the MIC and MBC of ozonated water, ozonated sunflower oil, and O3 gas against free bacteria were similar to those of their controls. Similarly, both laser therapy and photo-ozone therapy showed MIC and MBC >100%. In contrast, the MIC and MBC of ozonated sunflower oil (0.062 to 40%) and chlorhexidine digluconate (0.46 to 3.69 µg mL-1) confirmed the bactericidal action of both against free bacteria. When evaluated against biofilms, the MIC

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and MBC of ozonated sunflower oil varied between 10 and 60% depending on the pathogen. Under the conditions, only ozonated sunflower oil with a high peroxide index showed germicidal and antibiofilm activity against oral bacteria of captive large cats.

Keywords: Laser Therapy. Ozone Therapy. Integrative Medicine. Panthera Leo. Panthera Onca. Puma Concolor.

RESUMO

O objetivo do presente estudo foi caracterizar o potencial antimicrobiano e antibiofilme da terapia a laser, ozonioterapia (apresentações em gás, água e óleo) e foto-ozônioterapia contra bactérias isoladas da cavidade oral de felinos cativos: Puma concolor (n=4), Panthera onca (n=1) e Panthera leo (n=1). As concentrações inibitórias mínimas e bactericidas (CIM e CMB, respectivamente) dos tratamentos foram determinadas contra a forma planctônica dos seguintes patógenos: Enterococcus faecalis, Leifsonia aquatica, Oerskovia sp., Serratia marcescens, Corynebacterium sp., Providencia pustigianii e P. rustigianii. A ação antibiofilme de um tratamento foi avaliada sempre que a respectiva terapia foi eficaz na inativação da forma planctônica dos patógenos. A ozonização da água (borbulhamento de 500 mL de solvente com 70 μg O3 mL-1 e 125 mL min-1 por 7 minutos) foi realizada imediatamente antes dos tratamentos. O óleo de girassol ozonizado apresentou valor de peróxido >600 mmol-equiv. kg-1. Terapias com gás O3 (70 μg O3 mL-1 e 125 mL min-1) e laser de baixa intensidade (660 nm e 80 J cm2) foram realizadas por 120 e 160 segundos, respectivamente. A foto-ozônioterapia utilizou laser de baixa intensidade e gás O3 sequencialmente. Independentemente do patógeno, a CIM e a CBM da água ozonizada, do óleo de girassol ozonizado e do gás O3 contra bactérias livres foram semelhantes às de seus controles. Da mesma forma, tanto a terapia a laser quanto a foto-ozônioterapia apresentaram CIM e CBM >100%. Em contraste, a CIM e a CBM do óleo de girassol ozonizado (0,062 a 40%) e do digluconato de clorexidina (0,46 a 3,69 μg mL-1) confirmaram a ação bactericida de ambos contra bactérias livres. Quando avaliados em relação a biofilmes, a CIM e a CBM do óleo de girassol ozonizado variaram entre 10 e 60%, dependendo do patógeno. Nessas condições, apenas o óleo de girassol ozonizado com alto índice de peróxido apresentou atividade germicida e antibiofilme contra bactérias orais de grandes felinos em cativeiro.

Palavras-chave: Terapia a Laser. Terapia com Ozônio. Medicina Integrativa. Panthera Leo. Panthera Onca. Puma Concolor.

RESUMEN

El objetivo del presente estudio fue caracterizar el potencial antimicrobiano y antibiofilm de la terapia láser, ozonoterapia (presentaciones de gas, agua y aceite) y fotoozonoterapia contra bacterias aisladas de la cavidad oral de felinos cautivos: Puma concolor (n=4), Panthera onca (n=1) y Panthera leo (n=1). Se determinaron las concentraciones mínimas inhibitorias y bactericidas (CMI y CMB, respectivamente) de los tratamientos contra la forma planctónica de los siguientes patógenos: Enterococcus faecalis, Leifsonia aquatica, Oerskovia sp., Serratia marcescens, Corynebacterium sp., Providencia pustigianii y P. rustigianii. La acción antibiofilm de un tratamiento se evaluó siempre que la terapia respectiva fuera efectiva en la inactivación de la forma planctónica de los patógenos. La ozonización del agua (burbujeo de 500 mL de disolvente con 70 μg O3 mL-1 y 125 mL min-1 durante 7 minutos) se realizó inmediatamente antes de los tratamientos. El aceite de girasol ozonizado tuvo un valor de peróxido >600 mmol-equiv. kg-1. Las terapias con gas O3 (70 μg O3 mL-1 y 125 mL min-1) y láser de baja intensidad (660 nm y 80 J cm2) se realizaron durante 120 y

160 segundos, respectivamente. La fotoozonoterapia utilizó láser de baja intensidad y gas O3 secuencialmente. Independientemente del patógeno, la MIC y MBC del agua ozonizada, el aceite de girasol ozonizado y el gas O3 contra bacterias libres fueron similares a las de sus controles. De igual manera, tanto la terapia láser como la fotoozonoterapia mostraron una MIC y MBC >100%. Por el contrario, la CMI y la CMB del aceite de girasol ozonizado (0,062-40%) y del digluconato de clorhexidina (0,46-3,69 µg mL-1) confirmaron la acción bactericida de ambos frente a bacterias libres. Al evaluarse frente a biopelículas, la CMI y la CMB del aceite de girasol ozonizado variaron entre el 10 y el 60%, dependiendo del patógeno. En estas condiciones, solo el aceite de girasol ozonizado con un alto índice de peróxido mostró actividad germicida y antibiopelícula frente a bacterias orales de grandes felinos en cautiverio.

Palabras clave: Terapia Láser. Ozonoterapia. Medicina Integrativa. Panthera Leo. Panthera Onca. Puma Concolor.

1 INTRODUCTION

The feline oral cavity has a diverse, uniform and unique microbiota. The open and dynamic ecosystem of the oral microbiome is essential for preventing the colonization of pathogenic microbes and maintaining oral health (STURGEON et al., 2014). In domestic cats, the oral microbial population is directly influenced by breed and environment (OLDER et al., 2019), and imbalances in this microbial community with a predominance of Gram-negative bacteria have been reported in animals affected by gingivostomatitis (NAKANISHI et al., 2019).

Large wild felines are vitally important for the establishment of most ecosystems as they occupy the top of the food chain (AZEVEDO et al., 2013; MORATO et al., 2013). In wild felines, oral dysbiosis often acts as a precursor agent of various local illnesses like dental caries, gingivitis, and periodontitis (ROSSI JR, et al., 2007). In addition, emerging researches show suggest links between the oral microbiome and systemic diseases, including gastrointestinal, cardiovascular, and neurological conditions, as well as cancer (PENG et al., 2022; RAJASEKARAN et al., 2024; AZZOLINOET al., 2025).

Ozone (O3) is a highly unstable and energy-rich triatomic oxygen molecule (BOCCI, 2006) widely used in the medical disinfection (IRIE et al., 2022). Due to its high oxidizing power (SCIORSCI et al., 2020), O3 has been used in the treatment of infectious disorders caused by bacteria (BOCCI et al., 2011), fungi (DE ALENCAR et al., 2012) and virus (SATO et al., 1990). In human dentistry, O3 is an alternative for integrative treatment in periodontology (NOGALES et al., 2008), endodontics (AZARPAZHOOH and LIMEBACK, 2008) and maxillofacial surgeries (STÜBINGER et al., 2006). However, its use in the treatment of oral cavity infections deserves caution because it is a toxic gas for the respiratory system, in addition to potentiating asthma attacks and bronchoalveolar damage (MENZEL, 1984).

The enrichment of aqueous solutions and water and vegetable oils with O3 offers a broad-spectrum antimicrobial property against the oral microbiota (NAGAYOSHI et al., 2004; KHATRI et al., 2015; BRITO JR et al., 2022). Both ozonated water and oil are less toxic to oral epithelial cells and gingival fibroblasts compared to the gaseous form of ozone therapy (HUTH et al., 2006).

Laser therapy is a non-invasive therapeutic modality (DOĞAN et al., 2017) that activates cells through the irradiation with a beam of monochromatic light (RIEGEL and GODBOLD, 2017). The absorption of photons by endogenous microbial chromophores exposure to photobiomodulation triggers the production of reactive and cytotoxic molecules (KARU, 1999).

Low-level laser therapy may be a promising alternative to conventional antibiotics and antiseptics in eliminating cariogenic and periodontopathogenic bacteria from oral lesions (MARTU et al., 2023). However, various studies still question the antimicrobial effectiveness of the photobiomodulation (PERCIVAL et al., 2015; ANDRAUS et al., 1999). However, the combination of laser therapy and O3 was effective in inactivating fungal biofilms (CINTRA et al., 2022) and oomycetes (TREVISANI, 2022).

At this moment, there are no reports on the efficacy of O3 and low-level laser in inactivating oral bacteria in wild felines. Therefore, the objective of this study was to characterize the antimicrobial potential of ozone therapy (ozonated water, ozonated sunflower oil, and an O2-O3 gas mixture), laser therapy and photo-ozone therapy against planktonic and adherent forms of oral bacteria in captive large felines.

2 METHODOLOGY

This study was conducted at the Applied Microbiology Research Laboratory (LaPeMA) of the University of Franca which complies with all standard licensing requirements for the care and maintenance of large wild cats in captivity (IN 07/2015). The research protocol was approved by the University of Franca's Animal Use Ethics Committee (process no 9615071020).

2.1 CAPTIVE BIG CATS

Bacteria from the oral cavity were isolated from six big cats from the Dr. Fábio Barreto Municipal Forest, Ribeirão Preto, São Paulo: Puma concolor (two females and two males), Panthera onca (one male), and Panthera leo (one male). Only healthy, adult animals (between 2 and 18 years of age) weighing between 45 and 180 kg were used. All animals had lived in captivity for more than a year and were kept in individual enclosures with environmental enrichment. The cats were fed daily in the afternoon with bones, beef, and chicken, as well as water ad libitum. The cats had never undergone prior dental treatment, and their integrity was confirmed by laboratory tests performed by the research team immediately after the material was collected for this study.

2.2 BIOLOGICAL MATERIAL COLLECTION

After a 12-hour fast, all animals were anesthetized using darts and dissociative medication (midazolam and a combination of ketamine, dexmedetomidine, and butorphanol). Doses were calculated by interspecific allometric extrapolation.

Biological material was collected from the oral cavity after weighing and physical examinations. All oral microbiological samples were obtained by the same veterinary dentist using a sterile swab (K41-0102, Olen®, China). Materials were collected from the gingiva, mucous membranes, soft and hard palate, tongue, and teeth.

The samples were stored individually in Stuart medium and sent to the laboratory, where they were preserved at -80°C until antimicrobial analyses. After thawing, the bacteria were previously inoculated in Brain-Heart Infusion Agar culture medium in order to confirm the purity of the strains. A total of six bacteria were identified: Enterococcus faecalis, Leifsonia aquatica, Oerskovia sp., Serratia marcescens, Corynebacterium sp., Providencia pustigianii, and P. rustigianii.

2.3 MICROBIAL CULTURE

2.3.1 Planktonic Inoculum

To prepare the planktonic culture inoculum, the colonies of each bacterium were initially transferred to a test tube containing 3 mL of 0.9% saline using a sterile inoculation loop. After standardization to 1.5 x 108 CFU mL-1, 500 µL of the solution was transferred to a tube containing 4.5 mL of 0.9% saline (concentration of 1.5 x 107 CFU mL-1). Then, 2 mL of this solution was transferred to a tube containing 10 mL of MH broth, obtaining a concentration of 2.5 x 106 CFU mL-1. A concentration of 5 x 105 CFU mL-1 was obtained in a final volume of 100 µL by transferring 20 µL of this solution to each microplate well.

2.3.2 Bacterial Biofilm

Biofilm production was performed according to methodology previously described by Ramage et al. (2001). After cultivation and washing with phosphate-buffered saline (PBS - 10 mM potassium phosphate; 0.15 M NaCl, pH 7.0), the inoculum was adjusted to a turbidity equivalent to 0.5 on the MacFarland scale in RPMI 1640 medium, corresponding to a concentration of 1.0 to 2.5 x 106 colony-forming units per milliliter (CFU mL-1). Subsequently, 200 µL of each suspension was dispensed into 96-well microtiter plates (TPP, BIOGEN, Europe) and incubated at 37°C for 90 min under shaking (100 rpm). The wells were aspirated, washed with PBS, and a new 200 µL of RPMI 1640 medium was added. An additional incubation was performed for 48 h at 37 °C in a bacteriological incubator. Subsequently, the supernatant medium was aspirated, and the biofilms were washed with PBS.

Biofilm biomass was quantified using the crystal violet method. After biofilm cultivation at 37 °C for 24 h, non-adherent cells were removed by washing with PBS. The biofilms were then fixed with 90% methanol and stained with crystal violet. Biofilm biomass was quantified by measuring optical density at 570 nm.

3 TREATMENTS AND EXPERIMENTAL GROUPS

The microorganisms were distributed among nine experimental groups according to the treatments: non-ozonated distilled water (H2O group), ozonated distilled water (H2O-O3 group), non-ozonated sunflower oil (oil group), ozonated sunflower oil (oil-O3 group), O2-O3 gas mixture (gas-O3 group), pure oxygen (gas-O2 group), low-intensity laser (laser group), photo-ozone therapy (laser-O3 group), and chlorhexidine digluconate (Chlorhex group). The treatments were performed in triplicate.

The ozonation of distilled water was performed by bubbling 500 mL of refrigerated distilled water (5 °C) for 7 minutes with an O2-O3 gas mixture containing 70 µg O3 mL-1 and a continuous flow of 125 mL gas min-1. Ozonation was performed immediately before treatments using a portable O3 generator, model O&L Smart (Ozone&Life, São José dos Campos, SP) and a ozonation tower (Ozone&Life, São José dos Campos, SP).

In the Oil-O3 and Oil groups, pathogens were treated with ozonated sunflower oil with high and low peroxide values (>600 and <15 mmol-equiv. kg-1, respectively). Both oils were supplied by the same commercial company (Ozone & Life®, São José, Brazil).

In the Gas-O3 and Gas-O2 groups, bacteria were exposed to an O2-O3 atmosphere containing, respectively, 38 and Ø µg O3 mL-1 for 120 minutes. Gas treatments were performed using a portable ozone generator (Ozone & Life, São José, Brazil) attached to a glass suction cup.

In the Laser group, pathogens were irradiated with an iodine laser with a light absorption wavelength of 660 nm and a final fluence of 80 J cm2 (Laserpulso, IBRAMED®, Amparo, SP, Brazil) for 160 seconds. A single light irradiation was directed individually at each hyphal plug in the absence of photosensitizer.

Samples in the Laser-O3 group underwent the treatments described for the Laser and Gas-O3 groups. Treatment with an O2-O3 gas mixture was performed immediately after irradiation of the pathogens with the low-intensity laser.

4 ANTIMICROBIAL AND ANTIBIOFILM ASSAYS

Dilutions ranging from 0.08 to 100% were used to determine the minimum inhibitory concentration (MIC) of aqueous and oily solutions (H2O-O3, H2O, Oil-O3, Oil, and Chlorhex groups) against the planktonic form of bacteria. The methodology used was previously described by the Clinical and Laboratory Standards Institute (CLSI, 2012), with adaptations to use resazurin as a microbial activity indicator (SARKER et al., 2007).

The minimum bactericidal concentration (MBC) was used to indicate the bactericidal or bacteriostatic effect of treatments against the respective pathogens (CLSI, 2012). Similar MIC and MBC values indicated that the treatment had bactericidal action against the free form of the bacteria analyzed. Different MIC and MBC results indicated that the treatment in question had bacteriostatic action.

Treatments with gas and photobiomodulation (Gas-O3, Gas-O2, Laser, and Laser-O3 groups) were performed after 72 hours of culture of the planktonic bacteria in Petri dishes containing agar medium. Immediately after each treatment, bacterial samples were recultured in sterile Petri dishes containing agar. The absence of microbial growth after 72 hours of reculture indicated the antimicrobial action of the respective treatment.

The anti-biofilm potential was evaluated only for those therapies that demonstrated antimicrobial (germicidal or germistatic) action against the planktonic form of the bacteria investigated. The method used was microplate microdilution and followed the methodology recommended by CLSI (2012), with modifications. The procedures were performed in triplicate.

5 RESULTS

Both aqueous solutions (H2O-O3 and H2O groups) showed MICs and MBCs greater than 100% against the planktonic forms of all bacteria (Table 1). In the same antimicrobial assay, the MIC and MBC for chlorhexidine were ≤3.69 ug mL-1.

The MICs and MBCs of ozonated sunflower oil (Oil-O3 group) against the planktonic form of oral bacteria ranged from 40.00 to 0.62% (Table 2). In addition, ozonated sunflower oil concentrations ≤60% inhibited microbial activity against bacterial biofilms (Table 3).

Regardless of the pathogen, non-ozonated sunflower oil (Oil group) showed MICs and MBCs >100% for the planktonic and adherent forms (Tables 2 and 3, respectively).

Exposure to a gas mixture containing 38 μg O3 mL-1 for 120 seconds (Gas-O3 group) did not affect the growth of CFUs of free forms of oral bacteria from large felines (Figure 4). Similarly, the re-culture of pathogens previously treated with low-level laser therapy or photo-ozone therapy (Laser and Laser-O3 groups, respectively) showed similar growth to those exposed to a pure O2 atmosphere (Figure 4).

6 DISCUSSION

This study reported for the first time the antimicrobial effectiveness of ozonated sunflower oil against bacteria isolated from the oral cavity of big cats. Oral bacterial infections are important causes of morbidity in domestic (STURGEON et al., 2014; NAKANISHI, et al., 2019; THOMAS et al., 2021) and wild (KAPOOR et al., 2016) felines. Considering the ability of ozonated vegetable oils to reestablish surface microbial diversity (ZENG et al., 2020), our findings suggest ozone therapy as a potential approach for preventive and clinical treatments of oral diseases in big cats.

The respiratory system is sensitive to oxidative damage induced by the gaseous presentation of ozone (SCHWARTZ, 1996). The inhalation of O3 causes a drastic increase in nitric oxide production by alveolar and interstitial macrophages (LASKIN et al., 1994). Additionally, contact with O3 gas compromises the synthesis of interferon by the tracheal epithelium (IBRAHIM et al., 1976) and superoxide by pulmonary alveolar macrophages (RYER-POWDER et al., 1988), which predisposes to opportunistic infectious diseases (RUBIN, 2001). Finally, chronic exposure of the respiratory system to O3 has been associated with pulmonary metaplasia (NTP, 1994) and liver damage (LASKIN et al., 1994). Considering all the potential side effects of O3 inhalation, the topical use of ozonated vegetable oils in the oral cavity is a promising option for the treatment of local infections.

The high peroxide index of the ozonated vegetable oil used in the present study is directly related to the germicidal action against planktonic (GÜNAYDIN et al., 2018; FERREIRA et al., 2021) and adhered (HIGA et al., 2021) pathogens. The peroxidic compounds present in the ozonated oil degrade the protein thiol groups in the cell envelope (CURTIELLAS et al., 2005), lipids and unsaturated proteins (KOMANAPALLI et al., 1997; CHEVRIER et al., 1988). The damage associated with reactive oxygen species leads to the progressive dysfunction of biomolecules essential for microbial viability (CELENZA et al., 2020). Consequently, the topical application of ozonated oils with a high peroxide index is efficient in inactivating oomycetes (CARRIJO et al., 2021), bacteria (ZENG et al., 2020), fungi (HIGA et al., 2021) and multidrug-resistant microorganisms (GRAND et al., 2022).

When performed alone, gaseous ozone therapy (Gas-O3 group) showed no antimicrobial action against pathogens isolated from the oral cavity of large felines. It is known that the high levels of reactive species produced by ozonation significantly reduce cell viability by compromising the metabolism of lipids, proteins, and microbial DNA (RANGEL et al., 2022). O3 can also react directly with constituents of the bacterial membrane (VON GUNTEN, 2003), increasing its permeability (CLAVO et al., 2019; THANOMSUB et al., 2002). Considering that nebulization with a low dose of O3 (20 μg O3 mL-1 for five minutes) induces complete bacterial inhibition (FONTES et al., 2012), the ineffectiveness of the proposed treatment (38 μg O3 mL-1 for two minutes) was possible due to the short period of exposure.

The lack of antimicrobial action of the Laser group (660 nm and 80 J cm2) corroborates previous reports. According to Andraus and collaborators (2014), low-intensity laser irradiation (660 nm) did not produce bactericidal or bacteriostatic effects. Similarly, the in vitro growth of S. aureus was not affected by laser therapy with a wavelength of 904 nm and a dose of 3 J cm2 (PEREIRA et al., 2014). In addition, a supposed proliferative effect of biostimulation on bacterial cultures has also been described (KARU, 1999; BAXTER, 2003). Controversially, recent studies describe the benefit of laser therapy in the healing of infected wounds (RANJBAR and TAKHTFOOLADI, 2016) and in the degradation of periodontal biofilm (TONIN et al., 2022). Therefore, the use of low-power laser as an antimicrobial agent deserves further studies.

Similar to the described for oomycetes (TREVISANI, 2022), prior low-intensity laser irradiation was expected to enhance the antimicrobial effect of O3 gas treatment. However, photo-ozone therapy was not effective in controlling the growth of the planktonic form of the evaluated pathogens. Considering all the therapies evaluated in this study, only the treatment with ozonated sunflower oil inactivated the oral bacteria of large felines. However, it is important to emphasize that ozone therapy is still considered an adjunctive treatment and should be administered alongside, not as a replacement for, allopathic medication (SCHWARTZ et al., 2020).

7 CONCLUSION

Under the current conditions of the study, the ozonated sunflower oil with a high peroxide index demonstrated germicidal action against planktonic and adherent forms of bacteria isolated from the oral cavity of captive large cats. Treatments with ozonated water, an O2-O3 gas mixture, low-intensity laser, and photo-ozone therapy did not demonstrate antimicrobial or antibiofilm action.

DISCLOSURE STATEMENT

The authors declare that they have no conflicts of interest with respect to the authorship or publication of this paper.

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APPENDIX

Table 1

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (MIC and MBC, respectively; 0.078 to 100%) of ozonated and non-ozonated waters (H_2O-O_3 and H_2O groups) against the planktonic form of oral bacteria isolated from captive large felids. Treatment with Chlorhexidine digluconate (Cholrex group; 0.12 to 59 µg mL^{-1}) was used as control

		H_2O-O_3		H_2O		Cholrex	
Bacteria	**Felids**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Enterococcus faecalis	*Puma concolor*	>100	>100	>100	>100	3.69	3.69
Leifsonia aquática	*Panthera leo*	>100	>100	>100	>100	1.84	1.84
Oerskovia sp.	*Panthera leo*	>100	>100	>100	>100	1.84	1.84
Serratia marcescens	*Panthera onca*	>100	>100	>100	>100	3.69	3.69
Corynebacterium sp.	*Puma concolor*	>100	>100	>100	>100	0.92	0.92
Providencia pustigianii	*Puma concolor*	>100	>100	>100	>100	0.46	0.46
Providencia rustigianii	*Puma concolor*	>100	>100	>100	>100	0.92	0.92

Table 2

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (MIC and MBC, respectively; 0.078 to 100%) of ozonated and non-ozonated sunflower oils (Oil-O_3 and Oil groups, respectively) against the planktonic form of oral bacteria isolated from captive large felids. Treatment with Chlorhexidine digluconate (Cholrex group; 0.12 to 59 µg mL^{-1}) was used as control

		Oil-O_3		Oil		Cholrex	
Bacteria	**Felids**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Enterococcus faecalis	*Puma concolor*	40.00	40.00	>100	>100	3.69	3.69
Leifsonia aquática	*Panthera leo*	10.00	10.00	>100	>100	1.84	1.84
Oerskovia sp.	*Panthera leo*	10.00	10.00	>100	>100	1.84	1.84

Serratia marcescens	*Panthera onca*	5.00	5.00	>100	>100	3.69	3.69
Corynebacterium sp.	*Puma concolor*	1.25	5.00	>100	>100	0.92	0.92
Providencia pustigianii	*Puma concolor*	1.25	1.25	>100	>100	0.46	0.46
Providencia rustigianii	*Puma concolor*	0.62	0.62	>100	>100	0.92	0.92

Table 3

Minimum Inhibitory Concentration and Minimum Bactericidal Concentration (MIC and MBC, respectively; 0.078 to 100%) of ozonated and non-ozonated sunflower oils (O_3-Oil and Oil groups, respectively) against biofilm of oral bacteria isolated from captive large felids. Treatment with Chlorhexidine digluconate (Cholrex group; 0.12 to 59 μg mL^{-1}) was used as control

		Oil-O_3		Oil		Cholrex	
Bacteria	**Felids**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Enterococcus faecalis	*Puma concolor*	10.00	10,00	>100	>100	3.69	3.69
Leifsonia aquática	*Panthera leo*	--	--	>100	>100	1.84	1.84
Oerskovia sp.	*Panthera leo*	20.00	20.00	>100	>100	1.84	1.84
Serratia marcescens	*Panthera onca*	40.00	40.00	>100	>100	3.69	3.69
Corynebacterium sp.	*Puma concolor*	40.00	40.00	>100	>100	0.92	0.92
Providencia pustigianii	*Puma concolor*	40.00	40.00	>100	>100	0.46	0.46
Providencia rustigianii	*Puma concolor*	60.00	60.00	>100	>100	0.92	0.92

Table 4

Colony-forming units (CFUs) of oral bacteria isolated from captive large felids re-cultured after treatment with gas mixture containing 0 or 38 μg mL^{-1} O_3 (Gas-O_2 and Gas-O_3 groups, respectively; 2 min exposure), low-level laser (660 nm 80 J cm^2 ; 160 sec exposure) or a combination of laser and O_3 gas (Laser-O_3 group; 660 nm 80 J cm^2 for 160 seconds followed by 38 μg O_3 mL^{-1} for 2 min)

	Treatments	

Bacteria	Felid	Gas-O_2	Gas-O_3	Laser	Laser-O_3
Enterococcus faecalis	*Puma concolor*	countless	Countless	countless	countless
Leifsonia aquática	*Panthera leo*	countless	Countless	countless	countless
Oerskovia sp.	*Panthera leo*	countless	Countless	countless	countless
Serratia marcescens	*Panthera onca*	countless	Countless	countless	countless
Corynebacterium sp.	*Puma concolor*	countless	Countless	countless	countless
Providencia pustigianii	*Puma concolor*	countless	Countless	countless	countless
Providencia rustigianii	*Puma concolor*	countless	Countless	countless	countless