TITLE: Germicidal effect of the photodynamic therapy associated associated to ozone against canine and equine *Pythium insidiosum*.

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ABSTRACT:
The *Pythium insidiosum*, the etiologic agent of pythiosis in mammals, is highly resistant to conventional antimicrobial agents. However, the photodynamic therapy (PDT) with photosensitizers and the exposure to high concentrations of ozone (O_3) have been recently used to inactivate the pathogen. The aim of this study was to describe the antimicrobial ability of the PDT-O_3 association against canine and equine *P. insidiosum*. A total of 48 *P. insidiosum* hyphae plugs obtained from three horses and one dog were distributed into four experimental groups according to the treatment: PDT, O_3, PDT-O_3 and control (n=12 plugs/group). Samples from PDT group were irradiated for 160 seconds, in the absence of a photosensitizer, with an iodine laser with light absorption wavelength of 660nm and final fluence of 80 J cm^2. In the O_3 group, the hyphae were exposed to an atmosphere containing 50µg O_3 mL^{-1} for 15 minutes. Fragments of the PDT-O_3 group were subjected to both treatments. Hyphae plugs were cultivated in Petri dishes with 4% Sabourad dextrose Agar (ASD) during the first 8 days post-treatment. Petri dishes with no growth were recultured in a new dish containing 4% ASD for additional 14 days to determine the germistic or germicidal action of the respective therapy. In the first eight days post-treatment, growing areas were detected in all cultures of the PDT and control groups. Similarly, 10 hyphae plugs showed growth area after being submitted to ozone therapy alone (group O_3). In the opposite, *P. insidiosum* growth was not detected in 11 of the 12 hyphae plugs in the PDT-O3 group. As in the control group, pathogen growth was detected 24 hours post-treatment in all light-irradiated samples (PDT group). Plugs of *P. insidiosum* group O_3 grew from 45.6±6.7 h after treatment. In the only culture in which PDT-O_3 treatment was ineffective, microbial growth was detected within 76 h. The recultured *P. insidiosum* plugs from the PDT-O_3 group did not show growth after 14 days, confirming the germicidal ability of the therapy with 91.7% of efficacy. The present study demonstrated, for the first time, the effectiveness of the PDT-O_3 association in the inactivation of canine and equine *P. insidiosum*.

Keywords: Light, integrative medicine, ozone therapy, pythiosis

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